Frank G. R. Taylor Tim J. Brazil Mark H. Hillyer

Diagnostic Techniques in Equine Medicine

SECOND EDITION



Dedication

To my wife Sabine and our children Anna, James and Max, for their forbearance during the preparation of this book.

Frank Taylor

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ISBN 978-0-7020-2792-5

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

Library of Congress Cataloging in Publication Data

A catalog record for this book is available from the Library of Congress

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PREFACE

Diagnosis is fundamental to the appropriate treatment and wellbeing of the equine patient. Despite the many excellent clinical texts that are available, few seem to explain in sufficiently precise terms which clinicopathological tests are appropriate or how particular techniques should be performed. The first edition of this book was designed to provide an illustrated practical guide to the various diagnostic techniques employed in equine medicine. This second edition is an update by international experts in the field. Once again, it predominantly covers the adult horse and is intended for students, recent graduates and those veterinarians who do not specialize in equine work and may therefore be unfamiliar with some of the diagnostic approaches. Some of the more specialized techniques made possible by recent advances, notably ultrasound, are now available to practitioners and figure more prominently in this edition.

We have tried to ensure that the instructions are sufficiently detailed to allow completion of a procedure by following the text. Where appropriate, the advantages and disadvantages of a technique receive brief comment, together with a guide to the interpretation of results. For the purpose of practicality the techniques are again grouped by chapter on an organ system basis. In addition, a number of chapters have appendices that indicate applications of the described techniques to a given set of clinical circumstances such as anaemia, polyuria/polydipsia, nasal discharge, etc. The importance of recognizing clinical signs is paramount and these are given when relevant.

We hope that this book will prove useful to practitioners, and beneficial to their patients.

Bristol 2009

FGR Taylor TJ Brazil MH Hillyer

Submission of laboratory samples and interpretation of results

Chapter contents

I. Submission of laboratory samples 1 Choice of test 2 Suitability of the sample for the intended test 2 Haematology samples 3 Biochemistry samples 4 Urine samples 6 Faecal samples 7 Microbiology samples 7 Cytopathology samples 8 Histopathology samples 8 Information that should accompany the sample 8 Packaging for postal or other delivery 8 II. Interpretation of results 10 Laboratory reference ranges 10 Interpretation of haematological results 11 Erythrocyte parameters 11 Leukocyte parameters 12 Plasma fibrinogen concentration 14

Bile acids 19 Cardiac troponin (cTnl) 19 Blood urea and creatinine 19 Blood glucose 19 Serum bilirubin 19 Electrolytes 19 Triglycerides 21 Serum biochemistry profiles 21 Interpretation of endocrinological test results 21 Pregnancy tests 21 Cryptorchidism 22 Thyroid function 23 Pituitary function 23 Interpretation of urine analysis results 23 Interpretation of parasitological test results 23 Faecal worm egg counts 23

Serum enzymes 17

Further reading 24

APPENDIX 1.1 25

Haematological and biochemical reference ranges for adult non-Thoroughbred horses

I. SUBMISSION OF LABORATORY SAMPLES

Serum proteins 14

Clinical pathology should be used to help narrow a differential diagnosis, to confirm a diagnosis or to

Interpretation of blood biochemical results 14

assist in the systematic deduction of a diagnosis. Laboratory investigations are no substitute for a thorough consideration of the history and clinical examination; they are complementary in that they provide further information. However, laboratory screening may play a part in preventive medicine and performance assessment programmes.

Routine clinicopathological investigations include the following:

- Haematology
- Biochemistry of serum/plasma or other fluids
- Endocrinology
- Parasitology
- Microbiology
- Cytopathology
- Histopathology.

Many practices have or are developing their own laboratory facilities but in many cases it will be necessary to forward samples to a more specialized equine clinical pathology laboratory. One of the major limitations to test quality is the suitability of the sample that is received by the laboratory. Before submitting material, several factors should be considered:

- The choice of test
- The suitability of the sample for the intended test
- The information that should accompany the sample
- The suitability of packaging for postal or other delivery.

Choice of test

Tests must be relevant to and provide information about the implicated organ system or the clinical presentation. One of the purposes of this book is to indicate the range of clinicopathological tests that can be applied to the different organ systems of the horse. From these guidelines the clinician must select the laboratory tests most likely to confirm or refute a diagnosis based upon the history and clinical examination. A batch of ill-chosen tests will provide little or no information at considerable expense. If in any doubt, test selection should be discussed with a clinical pathologist by telephone. Communication between clinician and clinical pathologist will only enhance the end result of the investigation.

Suitability of the sample for the intended test

An adequate sample volume must be collected into an appropriate container and submitted to the laboratory as quickly as possible. Commercial laboratories recommend 5 ml anticoagulated samples for haematological analyses and 10 ml clotted blood samples for biochemical analyses. Blood samples that are haemolysed or lipaemic are unsuitable for analysis and those taken from dehydrated horses must be interpreted carefully, as haematological and serum biochemical parameters may be raised for that reason alone.

Table 1.1 shows the samples and containers that are appropriate to particular tests, but the specific requirements of individual laboratories should be checked. Some will supply their own preferred containers, packaging and labels on request. Two blood collection systems are currently in common veterinary use: the Vacutainer (Becton Dickinson) and the Monovette (Sarstedt) systems (Fig. 1.1 (Plate 1)),

Test	Anticoagulant	Monovette (Sarstedt)	Vacutainer (BD)		
Haematology	EDTA	4.5 ml (blue)	10 ml (mauve)		
Serum biochemistry, endocrinology	None or clot separation beads or gel	9 ml (brown)	10 ml (red)		
Clotting function/plasma fibrinogen	Sodium citrate	3 ml (green)	4.5 ml (blue)		
Glucose	Fluoride oxalate	5.5 ml (yellow)	4.5 ml (grey)		
Plasma biochemistry, endocrinology	Lithium heparin	9 ml (orange)	10 ml (green)		

Table 1.1 The two most commonly used blood sampling systems for equine clinical pathology sampling

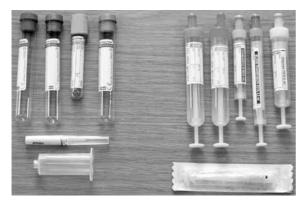


Figure 1.1 (Plate 1 in colour plate section) Various tubes suitable for collecting specific blood samples from horses (see Table 1.1). (Left) Becton Dickinson's Vacutainers. (Right) Sarstedt's Monovettes.

with individual clinicians and laboratories having their own preferences.

Haematology samples

The most suitable anticoagulant for haematological investigations is ethylenediamine tetra-acetic acid (EDTA). Heparin may cause 'clumping' of leukocytes and alter their staining properties. Plasma fibrinogen estimation can be undertaken using an EDTA sample, but only if the laboratory employs a heat precipitation technique. The more accurate thrombin coagulation estimation requires blood to be submitted in sodium citrate anticoagulant. Blood coagulation studies (e.g. prothrombin time; partial thromboplastin time) require whole blood to be submitted in sodium citrate. It is wise to collect blood samples into three tubes for general equine clinical pathology purposes:

- EDTA for haematological studies
- Sodium citrate for plasma fibrinogen estimation
- Empty or clot separation bead tube for serum biochemical studies.

If blood glucose estimation is required then an additional sample should be collected into fluoride oxalate anticoagulant.

Blood samples should be collected at rest from the jugular vein. If possible, the horse should not be excited, but if this seems likely the first sample taken should be the one submitted for haematological examination, in order to minimize the effect of splenic contraction. If the horse is clearly excited or has recently been exercised, this should be noted on the request form to the laboratory. Blood tubes should be filled to capacity and gently mixed by several inversions.

If a needle and syringe are used to collect blood, the following precautions must be observed:

- Blood must not be kept in the syringe for more than 90 seconds, otherwise clots form
- The needle must be removed from the syringe before transferring blood into the sample tube, otherwise haemolysis may occur
- The sample tube must be filled to the indicated line to maintain the working concentration of EDTA. An increased concentration causes changes in red cell size and inaccurate results, whereas a decrease predisposes clot formation
- The blood must be mixed with the anticoagulant by immediate, gentle inversion.

Haematology samples are best processed immediately but for short-term storage the tube should be kept cool. Refrigeration at 4°C is not recommended for equine blood samples. An air-dried smear should be prepared soon after sampling, because prolonged contact with EDTA can alter cell morphology and leukocytes can become difficult to identify. The smear can be dispatched to the laboratory in the unstained state, together with the parent blood sample. Special slide holders can be supplied for this purpose (Fig. 1.2). However, in most cases wellpackaged equine blood samples that have been carefully collected into EDTA and properly mixed will travel well for next-day delivery to the laboratory. Most problems occur in hot weather and when samples are delayed for more than 24 hours in the post.

Preparation of a blood smear

The glass slides used for smear preparation must be scrupulously clean. Ideally, they should be stored in spirit and wiped dry with a tissue before use. The sample is well mixed by gentle inversion and a drop of blood is placed towards the end of a horizontal



Figure 1.2 Polypropylene slide holders suitable for transporting blood smears.

slide by pipette. The short edge of a second slide is used as a spreader and is placed in front of the drop of blood at an angle of about 40° (Fig. 1.3). It is first drawn gently backwards to make contact with the drop, which is immediately distributed along the spreading edge by capillary action. Once evenly distributed along this edge, the blood is then smeared along the length of the slide by a single, steady, forward movement of the spreader. The prepared smear is then dried quickly by waving it rapidly in air. The slide can be identified by writing across the frosted end or the centre of the dried smear with a pencil; this will not interfere with subsequent staining or the differential count.

The technique of smear preparation is easily acquired but requires a little practice. Poor smears are produced by one or more of the following mistakes:

- Using dirty slides and/or a chipped spreader
- Using a drop of blood that is too large
- Using a spreader angle that is insufficiently acute
- Using a forward movement that is too fast
- Using a slow, jerky forward movement.

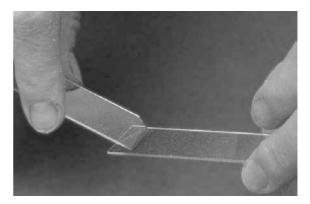


Figure 1.3 Preparing a blood smear.

Biochemistry samples

Samples submitted for biochemical and endocrinological testing may be of serum, plasma or other fluid. Serum is preferred by most laboratories for blood biochemical and endocrinological testing and is essential for certain tests such as serological tests (antibody titration), protein electrophoresis and equine chorionic gonadotrophin (eCG) testing. Although a perceived advantage of plasma is that it is easily separated from whole blood by standing or centrifuging prior to dispatch, it is unsuitable for some electrolyte and enzyme estimations and does not store satisfactorily. Always send a clotted blood sample if possible. Where plasma is acceptable, the blood should be collected into lithium heparin anticoagulant. Common container requirements are shown in Table 1.2.

Whether clotted or heparinized samples are used, the serum or plasma should be separated from the clot or red cells as soon as possible to avoid interactions between the two. Haemolysis may interfere with the measurement of enzymes, electrolytes and minerals. Haemolysis can be minimized by using clean dry equipment, avoiding perivascular blood sampling and not traumatizing the sample during or after collection. Whole blood samples sent by post during extremes of hot or cold weather are particularly prone to haemolysis.

Serum separation

An optimal serum yield can be obtained by collecting blood into a plain Monovette or Vacutainer tube, Table 1.2 Appropriate samples and containers for clinicopathological tests

Test	Sample	Container/medium
Haematology		
Blood count ± differential	Whole blood	EDTA
Plasma fibrinogen	Labs vary:	
	Whole blood (heat precipitation)	EDTA or heparin
	Plasma (thrombin coagulation)	Sodium citrate
Coagulation tests PT/PTT	Whole blood	Sodium citrate
Blood enzymes		
Most enzymes	Labs vary:	
	Serum usually preferred	Plain glass
	Plasma possible	Heparin
Glutathione peroxidase	Whole blood	Heparin
LDH	Serum	Plain glass
Blood electrolytes		
Serum electrolytes	Serum preferred	Plain glass
	Plasma electrolytes possible	Heparin
Other biochemistry		
Urea	Serum (preferred) or plasma	Plain glass or heparin
Creatinine	Serum (preferred) or plasma	Plain glass or heparin
Total protein	Serum	Plain glass
Albumin (and globulin)	Serum	Plain glass
Protein electrophoresis	Serum	Plain glass
Glucose	Plasma	Oxalate-fluoride
Total bilirubin	Serum (preferred) or plasma	Plain glass or heparin
Total serum bile acids	Serum	Plain glass
Serum triglycerides	Serum	Plain glass
Blood hormones		
Cortisol	Serum (preferred) or plasma	Plain glass or heparin
Thyroxine	Serum (preferred) or plasma	Plain glass or heparin
Triiodothyronine	Serum (preferred) or plasma	Plain glass or heparin
Progesterone	Serum (preferred) or plasma	Plain glass or heparin
Testosterone	Serum (preferred) or plasma	Plain glass or heparin
Oestradiol	Serum (preferred) or plasma	Plain glass or heparin
Oestrone sulphate	Serum (preferred) or plasma	Plain glass or heparin
eCG	Serum	Plain glass

Continued

1

Test	Sample	Container/medium		
Blood culture				
Aerobic/anaerobic	Whole blood	Aerobic and anaerobic bottles or single system		
Serology				
Bacterial/viral antibody	Serum	Plain glass		
Urine				
Urine analysis	Urine	Clean non-leak container		
Urinary fractional excretion of electrolytes	Urine plus serum (preferred) or plasma	Clean non-leak container plus plain glass or heparin		
Culture	Midstream	Sterile non-leak container		
Oestrogens (Cuboni test)	Urine	Clean non-leak container		
Body fluids				
Cytology	Fluid	EDTA		
Biochemistry	Fluid	Plain glass		
Culture	Fluid	Plain sterile container		
Faeces				
Faecal egg count	Faeces	Clean non-leak container		
Larval count	Faeces	Clean non-leak container		
Culture	Faeces	Clean non-leak container		

Table 1.2 Appropriate samples and containers for clinicopathological tests-cont'd

or one containing clot separation beads, and transporting it in a warm pocket to stand in a warm room, or a 37°C incubator, to allow optimal clot formation. Once the clot has formed, it can be freed from the sides of the container with a length of sterile swab stick and left to retract fully from the glass or plastic surface. Using tubes with clot separation beads or gel facilitates simple decanting of the serum after centrifugation. The serum is either decanted into a clean container or centrifuged to sediment the clot and cells, depending on the system used. Many referral laboratories now recommend the use of unbreakable polypropylene tubes for safe transit of samples in the post.

If separation is not possible, the sample should be kept cool (4°C) until dispatch, in order to decrease the rate at which enzymes, metabolites, electrolytes and minerals are exchanged between the cells and fluid. However, in most cases well packaged equine blood samples will travel well for nextday delivery to the laboratory.

Urine samples

Urine analysis is useful to help detect renal or bladder pathology and to investigate cases of septic nephritis, cystitis or urethritis. Midstream samples should be collected without the use of diuretics or alpha-2 agonist sedatives (which alter urine composition) into a sterile, empty universal container. Beware of owners collecting samples into used jam jars or milk bottles before pouring the urine into a universal container, since spurious glucosuria and bacterial culture may result. For fractional urinary electrolyte and mineral clearance ratio measurements, paired urine and serum samples should be collected simultaneously or within 30 minutes of each other (see Ch. 6: 'Urinary diseases').

Faecal samples

Faecal analysis is helpful in providing worm egg counts to help monitor parasite control programmes and to investigate cases of diarrhoea and septic enterocolitis. Freshly produced or rectal faecal samples should be collected into a clean, inverted rectal sleeve so that environmental contamination and alteration is minimized and there is no doubt about the identity of the horse that produced the sample. Fluid diarrhoea samples should be submitted in sterile universal containers with screw-on caps and on sterile swabs immersed in Amies charcoal transport medium. In cases of suspected bacterial enterocolitis, sampling of the more solid faecal components may be of greater diagnostic value.

Microbiology samples

Where possible, samples should be collected before the use of antibiotics and due care should be taken to avoid contamination. Appropriate precautions are given in the relevant sections of this book.

Sufficient quantities of material should be submitted in sterile containers. Sample volume and transport conditions directly influence the prospect of obtaining positive results. In general, the ideal samples for culture are aseptically collected pus, exudate, faeces, urine or tissue fluid collected into sterile containers with airtight screw caps. Fluids that are normally sterile, such as blood and pleural, peritoneal and synovial fluids, should be collected under sterile conditions. These fluids should be added, in a sterile manner, into a Bloodgrow medium bottle (Medical Wire & Equipment Co.) in order to maximize the laboratory's chances of isolating a pathogen. Blood samples for cultures should always be collected by sterile venepuncture into Bloodgrow medium. The identification and interpretation of culture results can be helped by: 1) preparing and fixing a smear at the time of sampling (for subsequent Gram stain); 2) submitting fluid samples in EDTA for a total nucleated cell count; and 3) submitting samples in an equal volume of cytological fixative (e.g. Cytospin collection fluid (Shandon)) for cytopathological assessment.

Bacteriological swabs may provide an insufficient sample for culture and unless submitted fully submerged in an appropriate transport medium they will certainly dry out and the microorganisms will die. Swabs can be used to obtain specimens from the conjunctivae, freshly ruptured skin pustules, deep wounds and soft tissue infections. A suitable transport medium for bacteriological screening is the Amies charcoal transport medium swab (Medical Wire & Equipment Co.). As an example, these are required for swabbing stallions and mares in screening for potential venereal infection for the Horserace Betting Levy Board's Code of Practice scheme (UK). Transport media considerations are particularly important to the successful isolation of viruses from nasopharyngeal swabs and clinicians should seek advice from an appropriate laboratory.

For the culture of anaerobes, samples must be protected from air because most clinically important obligate anaerobes cannot survive more than a brief exposure to atmospheric oxygen tensions. This can be achieved by placing a swab, fully submerged, in a suitable transport medium, or filling a container with the sample in order to minimize the air gap.

Antibiotic sensitivity tests

It is usually necessary to begin antibiotic treatment before the results of sensitivity testing are available. In such cases antibiotic choice is dictated by clinical judgement based on experience. However, if possible, a sample for isolation of the causative organism should be taken before treatment begins. In the laboratory, some bacteria that are recognized by Gram stain and culture may have predictable sensitivity patterns and therefore testing is not always necessary. Others, such as Gram-negative facultative aerobes (*Escherichia coli, Salmonella* spp., etc.), do not have predictable sensitivity patterns and warrant testing.

Most laboratories employ direct antibiotic sensitivity testing, in which an antibiotic-impregnated disc is placed on the surface of a plate that has been cultured or subcultured from the original bacterial isolate. Although this technique offers a relatively rapid result, the information obtained is empirical and less useful than the more sophisticated and expensive dilution techniques that provide information on the minimum inhibitory concentration (MIC) of an appropriate antibiotic. The likely significance of an isolate and its apparent sensitivity pattern should be discussed with the microbiologist if it is reported.

Cytopathology samples

Specimens for cytopathology (smears or fluid samples) should be handled carefully as recommended by the referral laboratory. Smears should be carefully made by direct impression or by rolling a swab (e.g. endometrial swab) on to a clean or gelatin-coated slide (gelatin helps to avoid loss of cells during processing). The slide is then fixed with a proprietary cytological fixative (e.g. Cytological Fixative (non-aerosol) or Spray Fix (Surgipath)) and sent in a proprietary slide container. Slides with ground glass label ends should be used so that the smear can be properly labelled in pencil on the side on which the smear is made.

Fluid samples (e.g. synovial, peritoneal, pleural, tracheal aspiration, bronchoalveolar lavage) should, in general, be submitted in EDTA for a nucleated cell count and fixed with a suitable fixative (e.g. Cytospin fixation fluid (Shandon)) for specific cytological processing. Another undiluted and unfixed sample should be submitted in a sterile container or on a sterile swab in transport medium, or in blood culture medium (particularly for synovial fluid samples), for concurrent bacteriological culture. Special fixatives may sometimes be required for specialized procedures. These should be discussed with the referral laboratory, which should be able to supply them.

Histopathology samples

Specimens for histopathological assessment of suspected tumours (biopsy or necropsy tissues) should be representative of the tissue sampled, or of the lesion found, and should include the junction between normal and abnormal tissue if appropriate. For skin or subcutaneous lumps, full-thickness wedge biopsies or complete lesions should be taken as these are more representative of the primary pathology than aspirates or needle biopsies. Needle biopsies are appropriate for sampling internal organs, e.g. liver, lung and kidney. Here, ultrasound guidance is vital, both in terms of sampling technique and the provision of additional diagnostic information.

Samples should be fixed in 10% formol saline and be of a sufficiently small size to allow rapid penetration of the fixative. As a guide, a diameter of no more than 1 cm and a thickness of no more than 5 mm are ideal dimensions, but not all specimens will permit this. The volume of tissue to fixative should be no more than 1:10 and both should be placed in a sturdy, wide-necked container, which can be sealed. Special fixatives are required for certain tissues such as endometrial biopsy because reproductive tissues have a higher water content than other tissues and suffer less artefactual shrinkage when fixed with Bouin's fluid, rather than in 10% formol saline.

Information that should accompany the sample

Most laboratories supply their own request forms indicating the information that they need to process and interpret the sample optimally. Some detailed clinical history is essential for investigations that are expected to produce a diagnosis, particularly histopathology. The clinical differential diagnosis may be useful to the laboratory as it helps with the interpretation of findings and/or suggests further tests.

Packaging for postal or other delivery

In general, most tests are not significantly affected by a postal transmission period of up to 48 hours, but next-day delivery is preferable and weekends should be avoided. Some samples are of sufficient bulk or urgency to warrant a courier delivery service. If the laboratory is within travelling distance, the client may be willing to deliver the specimen personally, by arrangement with the laboratory concerned. However, discussion of the results and their implications will, in the first instance, be between

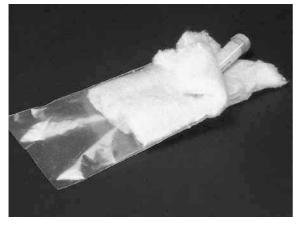


Figure 1.4 Primary package. The sample tube is wrapped in absorbent material and sealed in a leakproof plastic bag.

the referral laboratory and the referring veterinary surgeon.

The sender must ensure that the packaging complies with legal requirements and that the sample will not expose anyone to danger. In the UK, the Royal Mail's conditions for sending samples must be observed, otherwise packages may be destroyed and the sender made liable to prosecution. For packaging requirements in other countries, check with the appropriate postal service. As a guide to packaging, the Royal Mail approves the following procedure for the UK:

- Primary containers. A sealed container, such as an evacuated glass or polypropylene blood tube, should be wrapped in sufficient absorbent material to contain all possible leakage. This is then sealed in a leakproof plastic bag. Any container must not exceed 50 ml capacity, but special multi-specimen packs are approved; providing that each primary container is separated from the next by sufficient absorbent packing (Fig. 1.4)
- Secondary containers. The primary package must be placed in one of: a strong cardboard box with a full-depth lid; a grooved two-piece polystyrene box sealed with self-adhesive tape; a cylindrical light metal container with a screw-top lid; or a polypropylene clip-down container (Fig. 1.5)



Figure 1.5 The primary package is placed in a secondary container - in this case a cardboard box with a full-depth lid.



Figure 1.6 The complete package is placed in a padded bag bearing a clear hazard warning address label.

- Outer packaging. The complete package is then placed in a padded bag of appropriate size (Fig. 1.6)
- Labelling. The label must clearly declare that the package is a 'PATHOLOGICAL SPECIMEN' and must bear the warning 'FRAGILE - WITH CARE' (Fig. 1.6). As well as the laboratory address, the package must bear the name and address of the sender.

In the USA, regulations governing packaging and labelling of interstate shipments of aetiological agents are in Part 72, Title 42 of the Code of Federal Regulations. This contains the definitions of biological products, diagnostic specimens and aetiological agents, and provides requirements for packaging



Figure 1.7 Smashed blood tube and soiled packaging due to inadequate packing precautions.

and labelling these materials for transportation in interstate commerce.

Figure 1.7 shows an example of unsatisfactory packaging in which the primary container has no absorbent wrap or secondary container. The padded bag failed to protect the sample from destruction and exposed those handling it to pathological material.

II. INTERPRETATION OF RESULTS

A disease process is dynamic and has a beginning, a middle and an end. However, a solitary test result obtained somewhere along this time course can only reflect the situation at a fixed point, and this limits its interpretation. By analogy, it is like attempting to uncover the plot of a movie from a single frame. It is often more informative to have the results of several sequential samples. This section should act as a guide to the interpretation of haematological and blood biochemical reports.

In many instances the clinical history and examination that led to the selection of a test will lend weight to its interpretation. Where a marginal abnormality is reported, a repeat submission at a later time will confirm or refute a significant trend. One of the traps to avoid in evaluating laboratory data is over-interpretation of scant or inconclusive information. Pathological situations are most usually associated with dramatic and recognizable changes but smaller variations may indicate an early stage of disease and the need for repeated examinations.

Laboratory reference ranges

Before any interpretation can be made, the laboratory reference range for a parameter must be considered. It is common for a laboratory to provide 'normal' ranges. However, the word 'normal' is difficult to define meaningfully in the context of animal health and disease and should be avoided, as health and disease is a dynamic equilibrium between various challenges and responses, most of which are clinically inapparent. For clinical pathology data, 'reference' ranges for individual laboratories are preferred and are derived from the mean values (±2 standard deviations) of a healthy horse population. However, this concept excludes 5% of samples from the 'normal horse' reference range; i.e. 2.5% of 'normal' samples will predictably be above the upper reference range and 2.5% will be below the lower reference range. This method also requires a standard distribution of results on either side of the mean in the population, which is seldom the case, and so 95th percentiles are often a preferred method of producing reference ranges. Therefore, it is difficult to be certain that the result of a single sample, obtained from an unfamiliar patient, is a reliable indicator of disease unless the parameter value is extreme. Repeat sampling inevitably aids interpretation.

In general terms, the laboratory will report a result as 'below', 'within' or 'above' its accepted range. However, reference ranges invariably differ between laboratories, because of inherent differences in analytical procedure and of different horse populations used to produce the reference ranges. The difference is most marked in the quantification of serum enzyme activity and in this instance the same tests on the same sample will produce different results in different laboratories. *In consequence, haematological and biochemical results must always be interpreted in the context of the reference range given by an individual laboratory*.

Parameter	Thoroughbred	Hunter	Pony	
PCV%	40–46	35–40	33–37	
RBC × 10 ¹² /I	7.2–9.6	6.2–8.9	6.0–7.5	
Hb g/dl	13.3–16.5	12.0–14.6	11.0–13.4	
MCHC g/dl	34–36	34–36	33–36	
MCV fl	48–58	45–57	44–55	
MCH pg	14.1–18.1	15.1–19.3	16.7–19.3	

Table 1.3 Typical erythrocyte parameter ranges* for different groups of adult horse

*Adapted from data supplied by the Clinical Pathology Diagnostic Service, Department of Clinical Veterinary Science, University of Bristol.

Interpretation of haematological results

Haematological profiles may display marked differences between breeds and animals at different stages of training (see below). Some differences will occur between laboratories as a result of laboratory technique and differences in the settings of automated cell counters. Automated haematological counters must be calibrated properly for equine blood samples or spurious results will be obtained.

Erythrocyte parameters

It is important to realize that adult equine erythrocyte parameters are subject to a number of physiological variables that will influence laboratory results. These include breed, current fitness, and activity or excitement at the time of sampling.

- *Breed.* The 'hot-blooded' breeds (light horses, Arabians and Thoroughbreds) have higher erythrocyte parameters in terms of packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentration (Hb), than 'coldblooded' breeds (native ponies and draught horses). Interbreeds, such as hunter types, lie somewhere in between. Table 1.3 illustrates this point by showing typical erythrocyte ranges for the different groups.
- *Fitness*. Fit horses show a higher PCV, RBC and Hb than those that are resting or unfit. Fit racing Thoroughbreds therefore have the highest

values. Erythrocyte results in a fit horse that are at the low end of the reference range should be suspected as abnormal.

• Activity or excitement. Recent exercise, or excitement at the time of sampling, will significantly increase the PCV, RBC and Hb as a result of splenic contraction. Clinicians need to adopt quiet, calm techniques for sampling horses (particularly race and performance horses). This may require special visits to stables at quiet times.

In a healthy horse it is usual to find day-to-day variations in red cell parameters, but these should all be within the reference range indicated for the breed. In an unhealthy, non-excited horse, an increase in parameters above the range suggests dehydration (haemoconcentration). Decreases below the reference range suggest anaemia (see 'Diagnosis of anaemia' in Ch. 8: 'Blood disorders'). However, in anaemic horses the low erythrocyte parameters may be masked by splenic contraction due to excitement at sample collection.

In addition to variations in breed, type and management, reference ranges for haematological parameters differ with age. Because of this, clinicians who regularly monitor specific groups of horses (e.g. Thoroughbred foals, 2-year-olds in training, etc.) are advised to develop their own sets of reference ranges for these groups, either at their own laboratory or in collaboration with their referral laboratory.

Packed cell volume (PCV)

The PCV is a measure of the volume percentage of red cells in whole blood. It is easily determined by centrifuging a column of whole blood to separate the cellular elements from the plasma. The volume occupied by packed cells is then expressed as a percentage of the total volume (PCV%). Being easily determined, it is the most useful indicator of dehydration (haemoconcentration) during a disease process.

Red blood cell count (RBC)

The red cell count is expressed as the number of red cells per litre of whole blood (RBC $\times 10^{12}$ /l). Increases over the reference range are consistent with haemo-concentration (in the non-excited horse), whereas decreases are consistent with anaemia.

Haemoglobin (Hb)

The haemoglobin content of whole blood is expressed as the concentration in grams per decilitre (100 ml) (Hb g/dl). Increases over the reference range are consistent with haemoconcentration, whereas decreases are consistent with anaemia.

Mean corpuscular haemoglobin concentration (MCHC)

The MCHC is an index of the haemoglobin concentration per 100 ml of *packed red cells* expressed as g/dl. It is obtained by multiplying the haemoglobin concentration of whole blood (Hb g/dl) by the packed cell factor ($100 \div PCV\%$).

Mean corpuscular volume (MCV)

MCV is an index giving the average volume of each erythrocyte in femtolitres (fl). It is calculated by dividing the volume of red cells per litre (PCV% × 10) by the number of red cells per litre (RBC × 10^{12} /l). Depending upon the reported volume, the cells may be variously described as microcytic, normocytic or macrocytic. However, these features are not useful in interpreting regenerative or non-regenerative types of anaemia in the horse in contrast to other species, since equine erythrocytes mature within the bone marrow rather than in the

circulation, even during intense erythropoiesis. In consequence, the MCV is seen to progressively increase or decrease over time, but it usually remains within a 'normal' reference range. That said, macrocytic anaemia is most commonly seen in haemorrhagic conditions, including intestinal parasitism; normocytic anaemia is most commonly seen with viral infections and challenges, and microcytic anaemia is sometimes seen in chronic inflammatory and degenerative conditions. The best indicator in the horse of whether an anaemia is regenerative or non-regenerative is a bone marrow aspirate or biopsy (see Ch. 8: 'Blood disorders').

Mean corpuscular haemoglobin (MCH)

This index is an expression of the average haemoglobin content of a single cell in picograms (pg). It is obtained by dividing the concentration of haemoglobin in a litre (Hb g/dl \times 10) by the number of red cells in a litre (RBC \times 10¹²/l). An increase above the normal range is consistent with haemolysis.

Leukocyte parameters

As with erythrocytes, the white blood cell parameters are also subject to physiological variables. These usually take the form of a leukocytosis, which can be induced by apprehension, stress or recent exercise.

White blood cell count (WBC)

In the healthy adult, the total white cell count is usually between 6.0 and 12.0×10^{9} /l and the resting ratio of neutrophils to lymphocytes is about 60:40. Small numbers of monocytes and/or eosinophils may be present, but each will not usually exceed 5% of the total count. However, knowledge of 'normal' reference ranges for the automatic analyser used is important to the interpretation of monocyte counts.

Leukopenia is a depression of the WBC below normal limits and is most commonly seen in horses as a feature of acute stage infection or inflammatory challenge (subclinical infection), endotoxaemia and/or septicaemia. It therefore occurs in intestinal catastrophes associated with toxaemia (e.g. colitis/ typhlitis), or in the early stages of any severe bacte-

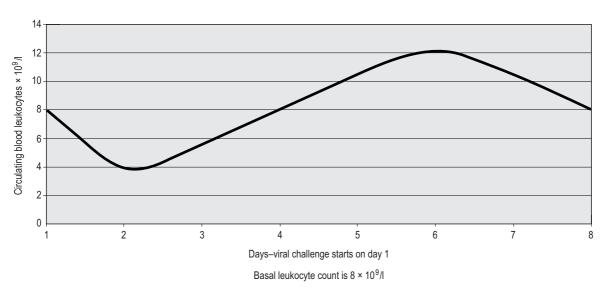


Figure 1.8 Example of the equine blood leukocyte kinetics in response to a viral challenge.

rial disease (e.g. pleuropneumonia; peritonitis; salmonellosis). Toxic neutrophils (seen on stained smear examination) may be a feature in these cases. Leukopenia invariably progresses to a state of leukocytosis over 2–3 days. The dynamics of the equine leukocyte response to, for example, viral infection/ challenge is shown in Figure 1.8 and these temporal changes are important to understand when making interpretations.

Leukocytosis is an elevation of the WBC above reference limits and is a feature of acute and chronic inflammatory disease.

Neutrophils

Neutrophilia occurs in response to inflammation (often, but not invariably, associated with infection), stress or the concurrent use of corticosteroids. Acute inflammatory leukocytosis features a neutrophilia and, if severe, juvenile 'band' forms appear ('left shift'). In protracted states of toxaemia, the cytoplasm fails to complete its maturation and is reported as 'foamy'.

Lymphocytes

Lymphopenia and neutropenia occur during the early stages of viral infection and the former is attributed to lymphocyte sequestration in lymphoid tissues. The numbers recover within a few days and then often become relatively raised (Fig. 1.8). When the total leukocyte count returns to within the 'normal' reference range it displays a relative lymphocytosis. The lymphocyte count can also be depressed by stress and the concurrent use of corticosteroids.

Monocytes

In health, monocytes hardly feature in the differential count and they are depressed in acute disease. However, chronic inflammatory leukocytosis is usually accompanied by a monocytosis.

Eosinophils

In health, the eosinophil portion of the differential count is low. Eosinophilia is most commonly seen in horses in association with leukopenia in earlyphase viral infections/challenges. Eosinophilia may also be provoked by hypersensitivity responses and in some instances this could be associated with active parasite migration. However, eosinophilia cannot be interpreted as pathognomonic of hypersensitivity or parasitism; neither do heavily parasitized horses necessarily show an eosinophilia. Idiopathic eosinophilia is occasionally seen in horses.

Basophils

Basophils rarely feature in the differential count of healthy horses. In other species they are regarded as circulating mast cells but the role associated with their appearance in the circulation of sick horses is undefined. Basophils are occasionally a feature of hyperlipidaemia and in some horses that are recovering from colic.

Platelets

The normal platelet count of horses is low compared with other domesticated species. Thrombocytosis is sometimes seen in association with chronic bacterial infections, particularly in foals. Thrombocytopenia (low platelet count) may reflect decreased production, increased use or destruction, or association with various spurious factors, e.g. drug administration, the presence of cold agglutinins in the sample or platelet clumping in EDTA. Where the latter is suspected, platelet counts should be measured on two blood samples collected at the same time, one into EDTA and the other into sodium citrate anticoagulant. If the platelet count in the sodium citrate sample, after correction for dilution, is considerably higher than the EDTA sample, then the 'thrombocytopenia' is likely to be an artefact of EDTA collection. Decreased platelet production may be associated with neoplasia or a toxic insult to the bone marrow. Thrombocytopenia is seen in horses with disseminated intravascular coagulopathy (DIC), a serious complication of endotoxaemia. Idiopathic thrombocytopenia is occasionally seen in horses and may be an immune-mediated condition.

Plasma fibrinogen concentration

Some laboratories employ a heat precipitation technique using whole blood in EDTA, so that it may be convenient to report it with a haematology profile. However, most laboratories use a thrombin coagulation technique, which gives more accurate and repeatable results, but the blood sample must be submitted in sodium citrate.

Plasma fibrinogen is an acute phase protein, the circulating concentration of which increases to a

peak within 48–72 hours of the onset of an inflammatory process. It is a sensitive indicator of septic inflammation in the horse and when used with serum amyloid A (SAA) measurements may be a more reliable monitor of changes in disease progression than blood leukocyte counts (Fig. 1.9). These acute phase protein measurements are particularly useful in monitoring the response to antibiotic treatment. Persistently raised fibrinogen and SAA concentrations are consistent with an ongoing bacterial infection and inflammation, even if there is an apparently normal WBC and differential count.

Interpretation of blood biochemical results

Serum enzyme concentrations (international units per litre) are estimated using commercial kits, which are optimized for different reaction temperatures. Different laboratories may use different kits, and the results and reference ranges may differ accordingly. It is therefore essential for the clinician to interpret the significance of an enzyme result against the reference range for the individual laboratory. It is the responsibility of that laboratory to ensure, through quality control procedures, that the results accurately reflect a comparison with their own reference ranges. Non-enzymic blood constituents that have absolute concentrations, such as g/l or mmol/l, are relatively unaffected by analytical conditions but, even so, variations occur. When communicating or discussing test results, the clinician should always be prepared to quote the laboratory's reference ranges.

The various chapters in this book that deal with the different organ systems give specific indications for clinical biochemistry, together with the interpretation of results. The notes below serve as a brief collective reference to the interpretation of blood biochemistry in the horse.

Serum proteins

Total serum protein (g/l) is a measure of the combined concentration of albumin and globulins in the serum. A gradual increase in the total protein over days/weeks usually reflects an increase in the globulin component as a result of a response to

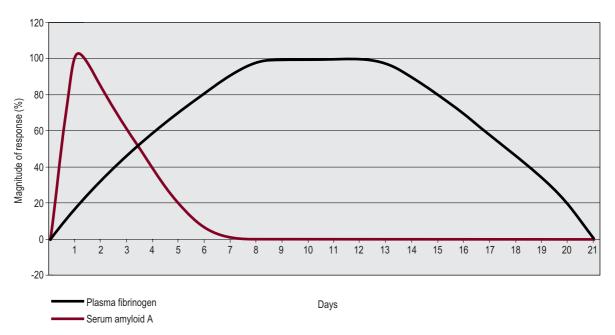


Figure 1.9 Schematic diagram showing equine acute-phase protein kinetics following an inflammatory challenge.

infection and/or inflammation. Sudden increases probably reflect dehydration and excited/exerted horses have temporarily raised albumin and globulin levels. However, many diseases associated with progressive dehydration may also be accompanied by albumin loss (e.g. gastrointestinal, liver and renal crises), and in these instances total protein is not a sensitive indicator of dehydration. Because of this, concurrent sequential PCV determinations may aid assessment of patient dehydration.

Albumin

Albumin is synthesized in the liver. Increases in serum concentration may be associated with dehydration, but decreases are most usually associated with a *protein-losing enteropathy* and therefore reflect alimentary disease. Less common causes of hypoalbuminaemia in the horse include loss to effusion (e.g. peritonitis; pleuritis), and least likely are glomerulonephropathy or liver failure.

Globulin

Apart from dehydration, total globulin concentrations may also be increased by:

- Acute inflammatory processes causing increases in acute phase protein (alpha-2 globulin) concentrations
- Chronic inflammatory processes causing increases in immunoglobulin (gamma globulin) concentrations
- Large strongyle parasitism causing increases in the beta-1 globulin concentration. Some veterinary laboratories offer an electrophoretic assay of beta-1 globulin concentration (see below). If raised above the reference range it can suggest active large or mixed strongyle migration, but intestinal parasitism cannot be ruled out on the basis of 'normal' beta-1 globulin levels. The sensitivity and specificity of this test for the presence of strongyles are low
- Liver failure associated with increases in beta-2 and gamma globulins.

Albumin: globulin ratios

In health, the albumin: globulin (A/G) ratio approximates to 1.0 or more. Shifts in the ratio may occur in a number of pathological states, but the informa-

Disease	Albumin	Alpha-2	Beta-1	Beta-2	Gamma
Acute infection	Normal	++ (APPs)	Normal	Normal	Normal
Chronic infection	Normal	+ (APPs)	+ (IgG(T))	+ (lgs)	++ (lgs)
Viral infection	Normal	Normal	+ (lgG(T))	+ (lgs)	++ (lgs)
Intestinal parasitism	Low (PLE)	++ (APPs)	++ (IgG(T))	Normal	Normal
Hepatic failure	Low	Normal	Normal	+++ (lgs)	+++ (lgs)

Table 1.4 Empiric interpretation of serum protein shifts as revealed by electrophoresis

APPs, acute phase proteins; IgG_m), immunoglobulin G (subclass T); Igs, immunoglobulins; PLE, protein losing enteropathy.

tion lacks specificity. A fall in the ratio, due to a decrease in albumin and an increase in globulin, may be a feature of either inflammatory bowel disease, exudative effusion (e.g. peritonitis; pleuritis), strongyle parasitism or liver failure. Any chronic inflammatory process in which the globulin concentration increases will also cause the ratio to fall, even if the albumin concentration remains normal. To differentiate these possibilities the clinician requires, in addition to the clinical findings, the results of serum protein electrophoresis and serum enzyme analysis.

Serum protein electrophoresis

Agarose gel electrophoresis separates equine serum proteins into four fundamental bands, which are characterized in order of their molecular weight and hence electrophoretic mobility. These bands are stained and identified as albumin with subdivisions of alpha, beta and gamma globulins. Once the total protein concentration is known, the laboratory can determine the individual protein concentrations within each band by densitometry. The results of electrophoresis of horse serum are not always comparable between laboratories because of differences in the separative technique. As a result there are conflicting data regarding the 'normal' concentration ranges of the various protein fractions. Once again, clinicians are advised to interpret protein shifts in relation to the reference ranges supplied by the individual laboratory. Table 1.4 shows an empiric interpretation of protein shifts.

Most laboratories identify electrophoretic elevations in specific globulin fractions as:

- Alpha-2 globulin reflecting acute phase inflammatory protein production
- **Beta-1 globulin** possibly reflecting large (*Strongylus vulgaris*) and mixed strongyle larval activity
- Beta-2 globulin reflecting hepatopathy and (in lithium heparin plasma samples) fibrinogen responses
- Gamma globulin reflecting immunoglobulin (antibody) responses to bacterial or viral infections.

Small strongyle disease (cyathostominosis) often results in low albumin and raised alpha-2 globulin (acute-phase inflammatory protein) concentrations. Horses with abscesses will often show characteristic alpha-2 and gamma globulin responses.

Occasionally, horses with generalized lymphosarcoma or plasma cell myeloma have massively increased total protein and globulin levels, the serum protein electrophoresis of which shows a massively raised, discrete, 'skyrocket' peak, usually in the beta-2 globulin range, suggesting monoclonal lymphoma protein production.

Serum amyloid A (SAA)

This is a highly sensitive, rapidly reacting acute phase inflammatory protein, which can be very helpful in monitoring early responses to infection and their response to treatment. Most normal horses have no measurable levels and, in the face of acute inflammation, particularly septic inflammation, the serum concentration increases quickly (within 24 hours) to over 20 mg/l and sometimes more than 100 mg/l. Concentrations peak and fall similarly quickly with subsidence of inflammation when the infection responds to antibiotic therapy (Fig. 1.9).

Immunoglobulin G (IgG)

Serum IgG assays are used in neonatal foals to assess the adequacy of passive transfer of maternal immunoglobulins via the colostrum. Assays are ideally performed at 12–36 hours after birth, the results denoting a satisfactory absorption of colostral immunoglobulin (>4 g/l), partial failure (2–4 g/l), or complete failure (<2 g/l). Foals that are considered at higher risk of susceptibility to infection may then be transfused with donor hyperimmune plasma at an early age and the IgG assay is repeated 24 hours later to assess the response. In cases where IgG levels have not risen or have fallen following transfusion, septicaemia should be suspected and further investigation undertaken.

Serum enzymes

In health, the equine circulation contains low levels of most intracellular enzymes derived from normal cell turnover. In disease, there is a release of enzymes from damaged cells, which increases their circulating concentration. Depending upon organ specificity, these enzymes may be used diagnostically to identify the diseased organ or cell type. The major drawbacks to interpretation are the ubiquitous nature of some enzymes and the poor stability of others. Poor stability results in a rapid loss of activity between collection and assay and it is therefore best to avoid referring highly labile samples for assay, e.g. sorbitol dehydrogenase (SDH). Where delays in transport are inevitable, referring the separated serum may help to improve the quality of results.

Alkaline phosphatase (SAP or ALP)

ALP may be released following damage to the intestinal epithelium, the hepatobiliary tract or bone. Many laboratories can narrow these differentials by estimating the concentration of the isoenzyme *intestinal alkaline phosphatase* (IAP). Increases in ALP concentration are either associated with intestinal damage (parasites or other inflammation), biliary obstruction or increased bone metabolism. Circulating SAP concentrations vary with age, being high in foals and skeletally immature horses before stabilizing in mature horses. Serum ALP has good stability in transit.

Amylase and lipase

In health, serum amylase and lipase concentrations are very low. Concentrations may increase significantly in serum, peritoneal fluid and urine during pancreatic necrosis. This is a very rare condition in the horse, which presents as acute intractable colic. However, because of its rarity, it is unlikely that a differential of pancreatitis would be pursued in cases of acute colic and the diagnosis usually follows post-mortem examination. Amylase and lipase are very stable in serum.

Aspartate aminotransferase (AST or AAT)

This was formerly designated glutamine oxaloacetate transaminase (GOT) and is occasionally found as such in older literature. The enzyme is released following cell disruption in a number of soft tissues including the liver, skeletal muscle and cardiac muscle. When the concentration is found to be increased, a cross-check on the serum concentration of a muscle-specific enzyme, most conveniently creatine phosphokinase (CK), will indicate whether or not the likely origin is muscle. A slight increase above normal range is usual after exercise. Following myopathy, AST levels peak at 24-48 hours and return to baseline by 10-21 days, assuming that no further damage occurs (see Ch. 13: 'Musculoskeletal diseases'). In transit serum AST will lose some 10% of its activity over 3 days at ambient temperature.

Creatine phosphokinase (CPK or CK)

The highest concentrations of this enzyme are found in skeletal muscle, heart muscle and brain tissue. Modest increases follow hard exercise, but massive increases in the circulation are invariably associated with muscle damage (*rhabdomyolysis or myopathy*). CK levels peak at 4–6 hours and return to baseline by 3–4 days, assuming that no further myopathy occurs. When measured alongside AST (see above), which takes longer to rise, peak and return to normal, the timing and response to treatment of myopathy can be usefully monitored. Serum CPK has good stability in transit.

Gamma glutamyl transferase (GGT or γ GT)

This enzyme is found in the biliary tract, renal tubules and pancreas of the horse. An increased serum concentration is almost invariably associated with liver disease. Elevations usually indicate biliary or cholestatic disease. Nephropathy in horses associated with tubular pathology is rare and does not usually result in significantly raised serum GGT concentrations, although urine GGT: creatinine ratios are elevated (>4.0). Pancreatitis in horses is extremely rare. Chronic pyrrolizidine alkaloid toxicity (ragwort poisoning) causes bile duct hyperplasia and biliary stasis and therefore typically results in raised serum GGT and serum alkaline phosphatase (SAP) concentrations.

Idiopathic GGT elevations are not uncommonly seen in horses in training that appear otherwise healthy but perform poorly. The cause of these abnormal concentrations has not yet been defined, although plant and fungal hepatotoxins have been suspected. In most cases, other liver enzymes are within reference ranges, as are urea and creatinine levels, and liver biopsy reveals insignificant histopathological findings. Most cases respond (i.e. GGT levels return to normal) following a period of rest from exercise. Serum GGT has excellent stability in transit.

Glutamate dehydrogenase (GLDH)

GLDH is liver-specific and increases in the serum concentration reflect acute or ongoing hepatocellular damage. This is a mitochondrial enzyme found mainly in liver, heart muscle and kidney. It is a relatively stable enzyme (it will lose some 15% of its activity over 3 days at ambient temperature) and is a suitable replacement for the more labile sorbitol dehydrogenase (SDH) in transported samples (see below).

Glutathione peroxidase (GSH-Px)

GSH-Px is a red cell enzyme isolated from heparinized whole blood but it is considered here

under 'Serum enzymes' for convenience. It is a sensitive indicator of dietary selenium. GSH-Px activity will vary between stables (i.e. different feeding regimens) but should remain constant throughout the year.

Lactate dehydrogenase (LDH)

LDH is widely distributed in all tissues (including muscle, liver and intestine) and an increase in the circulating concentration is therefore of little specific diagnostic value unless interpreted alongside other liver and muscle enzyme results. Subsequent estimation of the relative concentrations of its five isoenzymes is more useful, since each of these is more organ-specific.

- LD isoenzyme 1 most dramatically increased by intravascular haemolysis
- LD isoenzyme 2 elevated in some cases of cardiac pathology (an indication for cardiac troponin assay; see below)
- LD isoenzyme 3 no known disease association in the horse
- LD isoenzyme 4 most commonly elevated by intestinal pathology
- LD isoenzyme 5 rises seen with skeletal myopathy and hepatopathy, requiring further differentiation with CK and liver enzyme assays (see above).

Serum LDH has good transit stability for up to 3 days.

Sorbitol dehydrogenase (SDH)

SDH is substantially liver-specific and is used to detect acute or ongoing liver damage. It has a short half-life and therefore declines to the reference range once the hepatic insult ceases to be progressive. Unfortunately, it is not stable in blood and the assay must be undertaken as soon as possible after sampling and certainly within 24 hours. Serum SDH will lose well over 50% of its activity within 3 days at ambient temperature. It is therefore not suitable for samples referred by post and most commercial clinical pathology laboratories now offer GLDH estimation as the most appropriate alternative (see above).

This is a much better guide to functional hepatobiliary status than bilirubin assays (see below). High bile acid concentrations occur with impaired hepatic function and are a useful diagnostic indicator of liver dysfunction in horses (see Ch. 4: 'Liver diseases').

Cardiac troponin (cTnl)

Cardiac troponin I concentrations in the serum of clinically normal horses are less than 0.2 ng/ml. Experience so far suggests that greater than 0.3 ng/ml is abnormal, reflecting myocardial pathology. Concentrations of 0.9–5.4 ng/ml have been estimated in horses with cardiomyopathy confirmed by echocardiography.

Blood urea and creatinine

An increase in the circulating concentrations of urea and creatinine (azotaemia) is consistent with a state of renal failure. However, some 75% of glomerular function is lost before azotaemia becomes apparent and it is therefore an insensitive indicator of the onset of failure. Nevertheless, once raised, urea and creatinine concentrations reflect improvements or deteriorations in the glomerular filtration rate and become useful monitors of disease progress.

Small increases in blood urea concentration alone (i.e. up to twofold, with creatinine remaining within its normal range), frequently accompany dehydration and/or wasting diseases associated with increased tissue catabolism. Feeds that are high in protein may also raise blood urea slightly. Urine analysis is therefore indicated to investigate the significance of increased blood urea concentrations in horses.

Blood glucose

A blood sample collected into fluoride oxalate preservative is essential for the measurement of glucose. Increases above the reference range are often transient and relatively common. Causes of hyperglycaemia include insulin resistance (stress, pregnancy and/or obesity) and corticosteroid or alpha-2 agonist administration. Persistent hyperglycaemia is uncommon in the horse and is most commonly the result of pituitary pars intermedia dysfunction (PPID), often referred to as 'equine Cushing's disease', or hyperadrenocorticism (see also Ch. 5: 'Endocrine diseases'). Hypoglycaemia is very uncommon in adult horses but can be associated with anorexia or liver failure. Screening for and monitoring the correction of hypoglycaemia is an essential part of equine neonatal critical care.

Serum bilirubin

An increase in total bilirubin may cause jaundice of the mucous membranes and may be noted in a variety of equine diseases including haemolysis, liver disease, impaction colic and any condition associated with a reduction in food intake. However, it is unusual for serum bilirubin to be elevated in equine liver disease; an increase may be diagnostically useful but normal values do not discount liver disease. In fasting (or inappetence) there is a physiological decrease in the removal of bilirubin by hepatocellular transport. Anorexia, for whatever reason, is probably the commonest cause of hyperbilirubinaemia (with increased serum indirect bilirubin) and jaundiced mucous membranes in the horse.

Electrolytes

Sodium, potassium, chloride, calcium, magnesium and phosphorus can be measured in either serum or plasma. However, whole blood samples should be separated soon after collection because any tendency to haemolysis will alter electrolyte concentrations in both serum and plasma. Rapid 'patient-side' electrolyte analysers are now an essential part of equine anaesthesia and intensive care monitoring for adult and neonatal horses and for the monitoring of endurance horses during training and competition. Serial sampling over time with immediate results provides much more useful data than single sampling and 'historical' results. A more useful measure of a horse's whole body electrolyte and mineral status than single blood tests is the *urinary*

fractional clearance ratio (see 'Fractional excretion of electrolytes' in Ch. 6: 'Urinary diseases'). However, this assessment assumes that renal function is normal.

Sodium

Sodium is the major cation within the extracellular fluid and is largely responsible for maintaining the osmotic forces that regulate this compartment's fluid volume. However, the laboratory estimation of serum or plasma sodium should not be interpreted in absolute terms as a blood deficit or excess. This is because its concentration at any one time depends upon the exchangeable body stores of water, sodium and potassium, which are able to move in and out of the circulation and produce changes in the serum or plasma sodium concentration.

Hyponatraemic states (<135 mmol/l) usually occur in diarrhoeic diseases where massive losses of fluid and electrolyte are followed by the oral intake of water and partial replacement of lost fluid. Hyponatraemia therefore indicates a relative water excess. Hypernatraemic states (>145 mmol/l) are rare but may be associated with acute dehydration in which water loss exceeds that of electrolytes. Excess sodium replacement during fluid therapy will also cause hypernatraemia.

Potassium

Potassium is the major cation of the intracellular fluid and estimations of its serum or plasma concentration are of very limited value in inferring total body potassium.

Hypokalaemia (<3.3 mmol/l) is often associated with increased intestinal loss (diarrhoea) or, importantly, decreased food intake. Large amounts of potassium are excreted by the normal equine kidney, so that deficits soon occur when the horse's feed intake is reduced. Marked hypokalaemia is usually indicative of an alkalotic state, which causes cells to take up potassium and release hydrogen.

Hyperkalaemia (>5 mmol/l) is unusual in the horse unless associated with haemolysis, impaired renal function, muscle necrosis or severe acidosis. In acidotic states potassium leaves cells in exchange for hydrogen ions, so that the circulating potassium

concentration increases. Spurious hyperkalaemia may follow blood sample spoilage by haemolysis or leakage of potassium out of red cells. If possible, hyperkalaemia should be confirmed by a second blood sample. For obvious reasons, whole blood samples are unsatisfactory if laboratory processing is delayed.

Chloride

Chloride is largely located in the extracellular fluid, so that changes in its serum or plasma concentration tend to reflect changes in its whole body status.

Hypochloraemia (<93 mmol/l) is usually the result of increased loss to the gastrointestinal tract (diarrhoea or high obstruction) or, alternatively, prolonged heavy sweating. In the extracellular fluid, chloride concentrations are inversely related to bicarbonate concentrations, so that hypochloraemia is usually accompanied by metabolic alkalosis.

Hyperchloraemia (>103 mmol/l) may be associated with acute dehydration (when water is lost in excess of electrolytes) or metabolic acidosis.

Calcium

Calcium exists in the blood in three states: ionized, chelated and protein-bound. Routinely, calcium estimation in serum or plasma measures the total of all three, but only the ionized portion is biologically active and it should be requested specifically.

Hypocalcaemia (<2.86 mmol/l) is most usually associated with reduced feed intake. Clinical hypocalcaemia develops only when the circulating concentration of ionized calcium falls below the homeostatic requirement. In these circumstances the patient may show a low total serum or plasma calcium concentration, but this is not a reliable measure of the biologically available (ionized) calcium.

Persistent *hypercalcaemia* is a rare disorder of horses, which usually reflects a regulatory problem (see 'Hypercalcaemia' in Ch. 5: 'Endocrine diseases').

Blood calcium concentrations may be influenced by renal disease, but the effect is not consistent in horses. Acute or chronic renal disease may be accompanied by low, normal or high blood calcium levels.

Magnesium

Abnormalities in serum or plasma magnesium are uncommon. Low concentrations may accompany hypocalcaemia and as such may be associated with neuromuscular irritability and muscle stiffness (tetany).

Inorganic phosphorus

The estimation of phosphorus in serum or plasma may be of little diagnostic value. With the exception of acute conditions, such as clinical hypocalcaemia, the circulatory concentrations of these minerals are often within 'normal' reference ranges, despite whole body abnormalities. This is because complex homeostatic mechanisms act to sustain their blood concentrations.

Triglycerides

In healthy horses receiving adequate nutrition, the serum triglyceride concentration is usually less than 1 mmol/l. Short-term fasting may produce a physiological hyperlipidaemia, which is reversible and without clinical sequel. In hyperlipaemic states the value exceeds 5 mmol/l and the serum or plasma may develop a visible opacity. In extreme cases a cloudy, milk-like appearance develops, which renders the sample unfit for any biochemical or haematological analyses.

Serum biochemistry profiles

Most clinical pathology laboratories offer equine biochemistry profiles at a cost advantage over the individual tests. The clinician should always aim to select laboratory investigations that are complementary to a thorough history and clinical examination, rather than 'trawling' a profile. However, when the clinical examination is inconclusive, such profiles can be useful in identifying further routes of investigation. A typical profile is shown in Table 1.5, together with a tentative interpretation of abnormalities and suggestions for further investigations.

Interpretation of endocrinological test results

Pregnancy tests

Serum gonadotrophins

Equine chorionic gonadotrophin (eCG) may be detected in mares where functional endometrial cups are present. For accurate results, serum samples should be collected between 45 and 95 days since the last date of mating. False-negative results are unusual inside this period but can occur in rare cases where eCG levels are below test 'threshold'. False-positive tests are more common and may occur when early fetal death has left residual functional cups. In such cases the mare's serum may remain eCG-positive for the functional life of the cups, sometimes up to 100 days.

Oestrone sulphate

Oestrone sulphate may be detected in the serum/ plasma of mares beyond 120 days of gestation. At that time, levels of >100 ng/ml are usually found (0–25 ng/ml in non-pregnant mares). Most of the oestrone sulphate peak originates from the fetal gonads, so this may be a useful test of fetal viability as well as a pregnancy test. Levels fall during the last few weeks of pregnancy.

Urinary oestrogens

Urinary oestrogens of placental origin may be detected in mares after 150 days of gestation. However, the test is less reliable than the serum oestrone sulphate test.

Progestogens

The analysis of plasma progesterone levels is a useful guide to diagnosis and treatment in the acyclic or irregularly cyclic mare. In the non-pregnant mare, levels >2 ng/l (6.3 nmol/l) indicate functional luteal tissue and suggest that prostaglandin treatment should induce luteolysis, providing that the corpus luteum is more than 4 days old.

In the pregnant mare, there is no proven relationship between progesterone concentrations and the integrity of pregnancy. Mares with levels <2 ng/ml

Test	Result	Possible cause and investigation
Urea Uraemia		Dehydration: check PCV and total serum protein
		Tissue catabolism: check indicators of inflammation
		High protein diet
		Renal failure: compare serum creatinine concentration
Total protein	High	Dehydration: check PCV
		High globulin: check SPE:
		Raised alpha-2 globulin: acute inflammation
		Raised beta-1 globulin: parasitism
		Raised gamma globulin: chronic inflammation
Albumin	Low	Protein-losing enteropathy
		If diarrhoeic: check faecal culture, enterotoxin assay, faecal larvae and SPE (cyathostominosis); rectal biopsy
		No diarrhoea: check strongylosis (FEC and SPE); SAP (or IAP) activity; oral glucose absorption test (see Ch. 2: 'Alimentary diseases')
		Loss to inflammatory effusion: check abdominal and thoracic paracentesis
		Renal disease: check urea and creatinine; urinalysis
		Liver failure: check liver enzymes and bile acids
Globulin	High	Parasitism: check SPE
		Chronic inflammation: check SPE
		Liver failure: check liver enzymes and bile acids
AST	High	Soft tissue damage: check indicators of inflammation
		Acute or progressive liver disease: check liver-specific enzymes and bile acids
		Myopathy: check CPK activity
GGT	High	Liver disease: check other liver enzymes and bile acids
SAP	High	Hepatobiliary disease: check liver-specific enzymes; function tests
		Gut damage: check SAP (or IAP) activity; serum albumin concentration
		Bone damage

Table 1.5 Tentative interpretation of a serum biochemistry profile with suggestions for further investigations

AST, aspartate aminotransferase; CPK, creatine phosphokinase; FEC, faecal egg count; GGT, gamma glutamyltransferase; IAP, intestinal alkaline phosphatase; PCV, packed cell volume; SAP, serum alkaline phosphatase; SPE, serum protein electrophoresis.

are unlikely to be pregnant. Most normally pregnant mares have levels >4 ng/ml, but there is considerable daily and individual variation. The progesterone assay is in no way an accurate pregnancy test, but may be helpful in monitoring individual mares with histories of repeated pregnancy failure.

Cryptorchidism

The *oestrone sulphate assay* (Table 1.6) may be used as a cryptorchid or 'rig' test for horses that are over 3 years old (but not for donkeys). The *testosterone stimulation test* (Table 1.6) should be used for

of tests for cryptorchidism			
	Normal male	Cryptorchid	'False rig'

Oestrone sulphate assay		10–50 ng/ml	0.1–10 ng/ml	0–0.02 ng/ml
Testosterone stimulation test	1st measurement	5–30 nmol/l	0.3-4.3 nmol/l	0.03-0.15 nmol/l
	2nd measurement	5–30 nmol/l	1.0–12.9 nmol/l	0.05–0.19 nmol/l

horses under 3 years old and for donkeys. Testosterone levels are measured in serum or heparinized plasma samples taken before and then 30–120 minutes after the intravenous injection of 6000 IU human chorionic gonadotrophin (hCG). A 'false rig' is a castrated gelding that continues to display typical stallion- or rig-like behaviour.

Thyroid function

Table 1.6 Results

Test

The thyroid hormones, *thyroxine* (T_4) and *triiodothyronine* (T_3), are measured in equine serum samples. As diurnal rhythms are involved, two samples should be collected: early in the morning and late in the afternoon. Low levels ($T_4 < 7.7 \text{ nmol/l}$, $T_3 < 0.48 \text{ nmol/l}$ in adult horses) may indicate hypothyroidism, which is sometimes seen in overweight, lethargic horses and ponies that may be prone to laminitis, but the results of single tests are very non-specific (see Ch. 5: 'Endocrine diseases').

Pituitary function

The most common indication to assess pituitary gland function is equine 'Cushing's syndrome' (PPID), associated with adenoma formation in the pars intermedia of the pituitary gland (see Ch. 5: 'Endocrine diseases').

Interpretation of urine analysis results

Urine samples should be examined grossly for colour, consistency, the presence of blood (either fresh or changed), pus or excessive crystalline material. Horse urine is highly variable in colour from near colourless to golden or brownish and in its thickness, turbidity and mucoid content. Specific gravity (1.008–1.040 in adult horses, 1.001–1.025

in foals) should be measured with a refractometer. Dipsticks are commonly used to measure pH (normally 7.5-8.5 in adult horses, 5.5-8.0 in foals) and to detect other abnormalities. Urine pH reflects diet, and horses grazing pasture will normally have alkaline urine whereas those on a cereal-based performance horse diet will normally have acid urine. Proteinuria may occur in rare cases of renal tubular pathology. Glucosuria may be seen in PPID in older horses and ponies. Haematuria and sometimes haemoglobinuria may occur following traumatic injury, or with renal or cystic calculus formation. Haemoglobinuria may occur with haemolytic conditions. Myoglobinuria is seen with myopathies. Microscopic examination of spun sediment should be used to detect casts (protein and cellular masses), which suggest renal tubular pathology; leukocytes, which suggest inflammation/infection; bacteria, which if seen in association with leukocytes following Gram's stain may indicate infection; and erythrocytes, which indicate haemorrhage.

Horse urine is fundamentally a supersaturated solution of calcium carbonate and will normally contain variable amounts of predominantly calcium carbonate crystals. Excessive quantities indicate the need for further investigations, including bladder and kidney palpation, ultrasound scan and cystoscopic examinations (see also Ch. 4: 'Urinary diseases').

Interpretation of parasitological test results

Faecal worm egg counts

These remain the basis of equine intestinal parasite surveillance and of monitoring the efficacy of control

programmes, but they are not always a reliable means of assessing the parasite burden of an individual horse.

Testing methods vary between laboratories. The Ovatec[®] Plus test (SynBiotics Corporation, San Diego, CA) is a flotation method that is particularly suitable for use in horse samples as it is extremely sensitive at detecting low numbers of eggs of *strong-yle* and related species. *Ascarid* and *Strongyloides* spp. eggs are also detected. Well-managed horses under good endoparasite control regimens consistently have zero strongyle eggs per gram of faeces, using the Ovassay technique.

Tapeworm segments may sometimes be seen in the faeces by gross examination and are often seen at surgery in the caecal content of patients with tapeworm-related intussusception. A serological (enzyme-linked immunosorbent assay (ELISA)) test is available for detecting tapeworm infestations in horses. The concentration of specific antibody against *Anoplocephala perfoliata* correlates well with the intestinal burden of this parasite.

Faecal lungworm larval counts

Dictyocaulus arnfieldi larvae may be detected in the faeces of infested horses using the Baermann funnel gravitation method, but this is rare. An associated and often severe eosinophilic bronchitis may be detected by cytological examination of tracheal wash samples (see 'Cytology of tracheal aspirates and BAL fluid' in Ch. 12: 'Respiratory diseases').

FURTHER READING

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APPENDIX 1.1 HAEMATOLOGICAL AND BIOCHEMICAL REFERENCE RANGES FOR ADULT NON-THOROUGHBRED HORSES

Test	Abbreviation	Units	Mean	Ref. range
Total erythrocytes	RBC	×10 ¹² /I	8.2	6.2–10.2
Packed cell volume	PCV	I/I	0.37	0.31-0.43
Haemoglobin	Hb	g/dl	13.5	11.1–15.9
Mean cell volume	MCV	fl	46.0	40.0–50.0
Mean cell haemoglobin concentration	McHc	g/dl	36.1	33.5–38.7
Mean cell haemoglobin	McH	pg	16.6	15.2–19.0
Total leukocytes	WBC	×10 ⁹ /I	7.5	6.0–10.0
Segmented neutrophils	Segs	×10 ⁹ /I	4.4	3.4–5.4
	Segs	%	58	51–65
Lymphocytes	Lymphs	×10 ⁹ /I	2.6	2.0–3.2
	Lymphs	%	35	29–41
Monocytes	Monos	×10 ⁹ /I	0.3	0.2–0.4
	Monos	%	4	2–6
Eosinophils	Eos	×10 ⁹ /I	0.2	0–0.4
	Eos	%	2	1–3
Platelets	Plts	×10 ⁹ /I	156	100–250
Plasma viscosity	PV	mPa	1.50	1.45–1.55
Total protein	TSP	g/l	63	53–73
Albumin	Alb	g/l	35	29–41
Globulin	Glob	g/l	28	18–38
Alpha-1 globulin	α_1 glob	g/l	1.2	0.4–2.0
Alpha-2 globulin	α_2 glob	g/l	5.8	3.2–8.4
Beta-1 globulin	β_1 glob	g/l	7.4	4.0–10.8
Beta-2 globulin	β_2 glob	g/l	5.3	1.7–8.9
Gamma globulin	γ glob	g/l	9.0	4.6–13.4
Plasma fibrinogen	Fib	g/l	2.1	0.3–3.9
Serum amyloid A	SAA	mg/l	1.3	0–20
Aspartate amino transferase	AST	IU/I	263	102–350
Creatinine kinase	CK	IU/I	186	110–250
Lactate dehydrogenase	LD	IU/I	525	225–700
LD isoenzyme 1	LD1	% total LD	14	10–18
LD isoenzyme 2	LD2	% total LD	26	22–30
LD isoenzyme 3	LD3	% total LD	38	34–42
LD isoenzyme 4	LD4	% total LD	18	13–23

Continued

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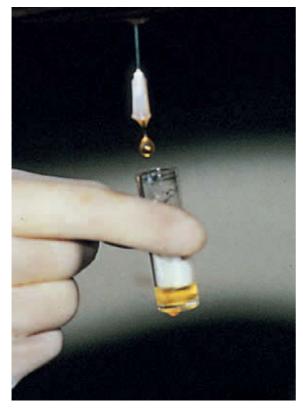
APPENDIX 1.1 HAEMATOLOGICAL AND BIOCHEMICAL REFERENCE RANGES FOR ADULT NON-THOROUGHBRED HORSES—cont'd

Abbreviation	Units	Mean	Ref. range
LD5	% total LD	4	1–7
GGT	IU/I	16	1–40
GLDH	IU/I	3	1–10
SAP	IU/I	204	147–261
IAP	IU/I	47	13–87
IAP	% total SAP	22.6	10–34
Urea	mmol/l	5.2	2.5–10.0
Creat	µmol/l	125	85–165
Glu	mmol/l	4.9	4.3–5.5
TBili	µmol/l	20	13–34
DBili	µmol/l	9	4–16
BAcids	µmol/l	5.1	1–8.5
Trigs	mmol/l	0.7	0.2-1.2
Lip	mmol/l	30	8–50
Amyl	IU/I	9	3–15
Ca	mmol/l	3.1	2.9–3.3
Ca	%	6.2	2.6–15.5
PO ₄	mmol/l	1.4	0.9–1.9
PO ₄	%	0.3	0.02–0.53
Mg	mmol/l	0.8	0.6–1.0
Mg	%	11.7	3.8–21.9
Cu	µmol/l	18.2	14.0–22.0
Cu	µmol/l	23.0	18.0–28.0
Zn	µmol/l	12.7	10.0–15.0
Na	mmol/l	138	134–142
Na	%	0.09	0.02-1.0
K	mmol/l	4.0	3.0–5.0
K	%	32.8	15–65
CI	mmol/l	99	95–103
CI	%	0.72	0.04–1.6
T ₃	nmol/l	1.0	0.48–1.46
T ₄	nmol/l	22.7	7.7–42.8
cTnl	ng/ml	0.1	0.05-0.2
	LD5 GGT GLDH SAP IAP IAP Urea Creat Glu TBili DBili BAcids Trigs Lip Amyl Ca Ca Ca Ca PO ₄ PO ₄ PO ₄ PO ₄ PO ₄ Mg Mg Cu Ca Ca Ca Ca Ca Ca Ca Ca Ca Ca	LD5% total LDGGTIU/IGLDHIU/ISAPIU/IIAPIU/IIAP% total SAPUreammol/IGlurmmol/IGlummol/IBli μ mol/IBAcids μ mol/ILipmmol/ICa%PO4mmol/ICu μ mol/IPO4%Mg%Cu μ mol/INa%Cu μ mol/ITrigsmmol/ICa%Ca%Cu μ mol/ICu μ mol/ICu μ mol/IK%Cl m mol/INa%CI%T_3nmol/IT_4nmol/I	LD5 % total LD 4 GGT IU/I 16 GLDH IU/I 3 SAP IU/I 204 IAP IU/I 47 IAP % total SAP 22.6 Urea mmol/I 5.2 Creat µmol/I 125 Glu mmol/I 4.9 TBili µmol/I 20 DBili µmol/I 9 BAcids µmol/I 9 Ca mmol/I 30 Amyl IU/I 9 Ca % 6.2 PO4 Mmol/I 3.1 Ca % 0.3 Mg mmol/I 1.4 PO4 % 0.3 Mg mmol/I 1.8.2 Cu µmol/I 1.8.2 Cu µmol/I 1.38 Na % 0.09 K mmol/I 1.38 Na

Courtesy of Beaufort Cottage Laboratories, Newmarket, UK.



Plate 1 (Fig. 1.1) Various tubes suitable for collecting specific blood samples from horses (see Table 1.1). (Left) Becton Dickinson's Vacutainers. (Right) Sarstedt's Monovettes.



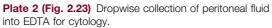




Plate 3 (Fig. 2.24) Huge volume of cellular deposit in the peritoneal fluid obtained from a patient with peritonitis.

CHAPTER

Alimentary diseases

Chapter contents

I. Practical techniques 28 Examination of the mouth 28 Restraint 28 External examination 28 Internal examination 29 Radiography of the upper alimentary tract 31 Radiography of the teeth 31 Radiography of the oropharynx 31 Radiography of the oesophagus 32 Oesophageal transit time 33 Endoscopy of the upper alimentary tract 33 Endoscopy of the nasopharynx 33 Endoscopy of the oesophagus 34 Abdominal auscultation 35 Normal sounds 35 Anomalies of sound 35 Examination of the alimentary tract per rectum 36 Restraint 36 Technique 36 Normal structures 37 Abnormal structures 38 Other abnormal masses 44 Gut rupture 44 Abdominal paracentesis (peritoneal tap) 45 Technique 45 Gross appearance of peritoneal fluid 47 Complications of abdominal paracentesis 47

Interpretation of peritoneal fluid analysis 48 Nasogastric intubation 50 Restraint 50 Passing the tube 50 Checking the tube position 51 Aspiration of reflux 52 Tube withdrawal 52 Potential problems 52 Rectal biopsy in the horse 53 Technique 53 Ultrasonography of the alimentary tract 54 Equipment 54 Appearance of organs 54 II. Clinical evaluation of the colic patient 55 Clinical parameters of colic 55 Behavioural signs 55

Clinical parameters of colic 55 Behavioural signs 55 Abdominal enlargement 56 Respiration 56 Muscle tremors 56 Cessation of defaecation 56 Heart and pulse rate 56 Rectal temperature 57 Mucous membrane colour and capillary refill time 57 Gut sounds 57 Rectal examination 57 Stomach intubation 57 Abdominal paracentesis 58 Laboratory aids 58 Indications for surgical exploration (laparotomy) 58 A strategic approach to the colic case 58

III. Clinical pathology 60

Serum biochemistry 60 Total protein 60 Albumin 61 Globulins 61 Albumin: globulin ratios 61 Serum protein electrophoresis 61 Serum alkaline phosphatase 62 Pancreatic enzymes 62 Fluid, electrolyte and acid-base balance 62 Fluid balance 62 Electrolyte balance 62 Acid-base balance 63 Haematology 63 Erythrocyte parameters 63 Leukocyte parameters 63 Plasma fibrinogen concentration 64 Tests of intestinal malabsorption 64 The oral glucose absorption test 64 The D-xylose absorption test 65 Faecal analysis 66 Intestinal parasites 66 Bacterial culture of faeces 67 Faecal leukocytes 68 Faecal blood 68 Faecal sand 68

Further reading 69

APPENDIX 2.1 70

Some applications of diagnostic techniques for the investigation of dysphagia

APPENDIX 2.2 71

Some applications of diagnostic techniques for the investigation of diarrhoea

I. PRACTICAL TECHNIQUES

Examination of the mouth

The most usual indication for examination of the mouth is dental disease, frequently associated with difficulty in chewing or inflammatory conditions of the gums, palate or cheeks. Additional indications for examination of the mouth are headshaking or refractory behaviour while being ridden, excessive salivation and difficulty in swallowing (dysphagia). Furthermore, any horse suffering reduced food intake and/or weight loss should have its mouth examined.

Restraint

Optimal restraint consists of a set of stocks, but realistically most horses are examined in the stable. One way to restrain a horse in a stable is to reverse the horse diagonally into a corner to prevent backward movement. A competent handler should stand close to the side of the head, facing forwards. A strong head collar should be used, but the nose and jowl pieces should be sufficiently slack to allow the mouth to open wide. Some horses resent manipulation within the mouth to the extent that sedation may be necessary. If strong sedation is to be used, the best approach is to have a set of stocks or an overhanging structure where the noseband of the head collar can be attached to keep the horse's head at a suitable level. The clinician should always be aware that he/she will be examining directly in front of the animal and will therefore be vulnerable to rearing and striking.

External examination

An external examination of the mouth is made by standing to the side of the animal, facing forwards,

and separating the lips with both hands to reveal the labial mucosa and incisor teeth. The colour of the mucous membranes is noted together with the presence of abnormal features such as petechial or ecchymotic haemorrhages. Inspection of the incisors may reveal abnormalities in bite (e.g. parrot mouth), deciduous teeth, supernumerary teeth or undue wear due to crib-biting or poor grazing (chronic close cropping at the soil surface). Sharp edges to the upper cheek teeth can be palpated through the cheeks, where associated regional pain may also be found.

Internal examination

The mouth may be held open for more detailed examination by using the patient's tongue as a gag or, alternatively, using a gag manufactured for the purpose.

Use of the tongue

Standing to one side or other of the horse, the clinician introduces his/her hand into the interdental space to grasp the free end of the tongue. This is brought out through the interdental space and gently lifted to a position between the cheek teeth to hold the mouth open (Fig. 2.1). Care should be taken not to injure the ventral frenulum by pulling the tongue too hard. The clinician should also beware of impaling the tongue on a canine tooth during manipulation. The hold is more assured if the little finger of the hand grasping the tongue is hooked around the halter or head collar.

The clinician's free hand may now direct a pen torch to view the teeth and soft structures on the opposite side of the mouth. The teeth on this side may also be palpated by careful insertion of the fingers inside the cheek. In general, providing the tongue is held between the horse's teeth on the opposite side, it will not attempt to bite the examiner's hand for fear of biting its own tongue. However, a gag and torch or a headlamp designed to be worn by the veterinarian are highly recommended to adequately and safely examine all the teeth without injury to the clinician or horse.



Figure 2.1 Examination of the mouth using the tongue as a gag.

The other half of the mouth may then be examined by releasing the tongue, taking hold of it again through the opposite interdental space and repeating the procedure. The tongue itself should not be overlooked during examination; lack of normal tone during manipulation suggests paralysis.

Use of a mouth wedge

A mouth wedge acts by holding apart the cheek teeth on one side of the mouth, allowing the clinician to examine the opposite side. It is available in a range of sizes and is simple and relatively safe to use (Fig. 2.2).

The clinician opens the mouth by moving the tongue to one side as described above and inserting the gag between the teeth of the opposite side (Fig. 2.3). The handle of the wedge may be held by



Figure 2.2 A wedge gag (Swale type).



Figure 2.4 A mouth speculum (Haussman gag).



Figure 2.3 The gag in position.

the assistant standing at that side or fastened to a ring on the head collar, depending upon the design.

Use of a full dental speculum

This is a more sophisticated instrument, which is applied over the head collar (Fig. 2.4). Two plates rest on the incisor tables and are moved apart by a ratchet system, which prises the jaws to the required distance and then locks the position (Fig. 2.5).

The speculum allows the maximum amount of room for examination and/or manipulation within the mouth and can be released readily. Its disadvantage is the danger to personnel if the horse becomes fractious during adjustment and the speculum is not fully in place. This can be avoided by sedation of the horse and correct placement. The latter is achieved by close positioning of the plates on the incisors and fully tightening the straps that hold the instrument in place.

Comments

- Examination should include an assessment of mouth odour a foul odour suggests dental necrosis, or severe soft tissue injury that has not been attended to.
- On occasion a satisfactory examination of the mouth is only possible under short-term general anaesthesia.



Figure 2.5 The mouth speculum applied.

Radiography of the upper alimentary tract

Comprehensive radiography and fluoroscopy of the upper alimentary tract are restricted to centres with specialized equipment. However, good-quality diagnostic radiographs of the teeth, pharynx and oesophagus of adult horses are possible using portable units with settings ranging from 60 kV and 30 mA to 90 kV and 15 mA. *This of course presumes that stringent safety precautions are possible in line with current ionizing radiation regulations.* The use of digital radiography enables diagnostic films at lower exposures.

Radiography of the teeth

Radiographs are useful in determining the extent to which dental disease involves periodontal tissues.

Diagnostic radiographs are best obtained under deep sedation or short-term general anaesthesia, which also facilitates detailed inspection. Lateral and oblique views are obtained with the jaws held apart by an incisor gag and the horse positioned with the diseased side next to the cassette. An isolated image of the diseased root is best obtained using a 45° beam, which prevents superimposition of the images formed by the diseased and normal arcades. The X-ray beam should be directed from the nasal side for the maxillary cheek teeth and from the mandibular side for the mandibular cheek teeth. Where possible, referral to a centre capable of performing computed tomography (CT) is advantageous because this will allow higher-resolution images without superimposition, so that the correct tooth can be isolated. This is particularly useful if a tooth extraction is ultimately planned.

Radiography of the oropharynx

Plain lateral radiographic views of the pharynx reveal gross lesions, such as a retropharyngeal mass or guttural pouch enlargements (blood, pus or gas), which can compress the pharynx. On rare occasions fracture of the hyoid bone is diagnosed in this way. Dorsoventral and oblique images, as described for the teeth, can be very helpful when guttural pouch disease is suspected, as it can aid in the determination of which side is affected.

Technique

Lateral radiographs of the pharynx are usually obtained in the conscious, standing animal. Sedation may be needed in fractious animals but can also be useful in others to keep the head and neck in a lowered position.

The horse should be held in a rope halter with no metal attachments. The handler should stand directly in front of the horse and it may be useful for the horse's head to be supported under the chin by a lead-gloved hand. This also allows the height of the horse's head to be controlled and positioned within the primary beam. No part of the handler's body should encroach on the primary beam, even if shielded. The cassette should be placed in a mechanical support and not held manually. A practical support can be contrived from a bag, into which the cassette is inserted, suspended from a drip stand. The cassette is then positioned against the side of the horse's head and aligned with the tube, which is centred on the area of interest. Standard radiographs of the oropharynx are obtained by centring on the caudal edge of the vertical ramus of the mandible immediately dorsal to the larynx.

The presence of air in the nasopharynx, larynx, trachea and guttural pouches provides good contrast with the adjacent soft tissues. A short focus-to-film distance (1.0-1.3 m) allows a short exposure time to be used and thereby minimizes any movement blur on the radiograph. kV/mA settings will vary with the size of the horse.

Although gross lesions are identifiable using plain films, the more subtle soft tissue problems require contrast radiography. Barium sulphate suspension given orally by catheter syringe (60 ml) will outline the oropharynx, lateral food channels and cranial oesophagus. Obstructions such as a subepiglottic mass or oesophageal diverticulum/stricture may then be identified easily. In cases of dysphagia (e.g. pharyngeal paralysis), attempts at swallowing usually disperse barium sulphate into the nasopharynx, larynx and trachea. In cases of complete pharyngeal stasis no contrast medium is seen to reach the oesophagus.

Radiography of the oesophagus

The radiographic appearance of the normal, empty oesophagus is indistinct because of its collapsed state. An outline is only apparent when air, fluid, food material or a radiopaque foreign body are present. Oesophageal radiography may therefore be of diagnostic use in the dysphagic patient, or in patients with oesophageal obstruction or strictures.

Radiographs of the cervical oesophagus are possible in most adults using portable equipment, but investigations at the level of the thoracic inlet and shoulders require the use of more high-powered equipment. However, views of the thoracic oesophagus behind the shoulder are also possible with portable equipment.

Technique

Radiographs of the oesophagus can be obtained in the conscious standing animal. Sedation may be used if necessary, although this will tend to alter oesophageal motility. The cassette is suspended against the side of the horse as described above for oropharyngeal radiography.

Multiple exposures are needed to cover the length of the oesophagus. The exposure factors will also vary along the length of the oesophagus according to the amount of soft tissue present at the various levels. For each exposure the beam is centred on the known path of the oesophagus down the neck and through the thorax. As a rough guide the oesophagus would be included in standard views of the cervical vertebral column and the dorsal lung fields.

The normal oesophagus is a collapsed soft tissue tube and as such is not visible on plain radiographs because of other soft tissue density structures in the field. However, the lumen may be delineated in pathological conditions or by the use of contrast agents. Impacted ingesta will be visible along a length of oesophagus in cases of choke and air will be visible in cases of mega-oesophagus.

Greater clarification of oesophageal anomalies is obtained using contrast radiography. This is achieved by giving 60–180 ml barium sulphate suspension per os by catheter syringe or via a nasogastric tube that is passed as far as the upper cervical oesophagus. In either case the suspension may be given neat or in warm water. Investigation of the mid- to lower cervical oesophagus requires the larger volume given by a nasogastric tube.

Using contrast medium, the normal oesophagus appears collapsed and its longitudinal mucosal folds may be outlined. Any obstruction will disrupt the flow of medium, thus producing a radiolucent outline of the foreign body. Oesophagitis, e.g. following the relief of an obstruction, may be associated with thickening of the longitudinal folds and pooling of contrast medium owing to a motility disturbance. Oesophageal diverticula are also demonstrable using contrast radiography.

2

The narrowing of a column of contrast is consistent with either stricture, neoplasia or external compression by a perioesophageal mass, whereas an 'hourglass' shape indicates pre- and post-stenotic dilatation. NB: A normal peristaltic contraction 'caught' at exposure may be confused with stenosis. This possibility can be investigated by taking a second radiograph of the same area.

Extensive dilatation and pooling of contrast media suggests mega-oesophagus, which often accompanies the intestinal stasis and gastric distension of grass sickness – but it should not be regarded as a pathognomonic sign.

Double-contrast studies (media and air) may be indicated to diagnose some oesophageal problems. This can be achieved by administering contrast media via a stomach tube, followed by a bolus of air. Double-contrast studies are particularly helpful in horses with suspected disease of the wall of the oesophagus because the air will dilate the oesophagus and the positive contrast media will line the mucosa.

Oesophageal transit time

An assessment of oesophageal transit time can be achieved by serial radiography of the passage of contrast suspension. This must be given by catheter syringe, not by nasogastric tube. Alternatively, the passage of a food bolus, such as mash mixed with contrast agent, can be followed in the same manner.

In normal horses the passage of a fluid bolus from the cricopharynx to the stomach is rapid, occupying some 5–10 seconds, and solid boluses are only a few seconds slower. Liquid contrast does not pool in the normal oesophagus and little contrast residue is left after the passage of a treated food bolus. In contrast, most oesophageal lesions are associated with significantly longer clearance times, minutes to hours. Postobstructional oesophagitis, stricture and all motility disorders are associated with impaired transit times, so that serial radiographs reveal minimal movement of a bolus.

Comments

• Normal oesophageal peristalsis will give the appearance of a 'false' stricture or dilatation when

caught at exposure. In contrast studies it should be remembered that swallowing (of medium) is the trigger for a peristaltic wave. If the interpretation is in doubt, repeat the exposure.

- Dynamic studies using digital radiographic equipment are more reliable and informative in assessing the swallow reflex and the speed of passage of a bolus through the oesophagus to the stomach. However, these techniques are only available at specialist centres.
- In dyspnoeic animals free gas may be seen in the oesophagus. This is usually secondary to an increased respiratory effort.

Endoscopy of the upper alimentary tract

Endoscopy should be used routinely to examine the nasal, pharyngeal, laryngeal and upper oesophageal regions in cases of dysphagia. Direct observation of swallowing enables assessment of pharyngeal function. In addition, foreign bodies, inflamed tissues and defects of the palate, pharynx and larynx are usually easy to identify.

Endoscopy of the nasopharynx

Most young horses show a dense aggregation of lymphoid tissue in the dorsal pharyngeal recess (lymphoid hyperplasia). On occasion plaques of lymphoid tissue extend down to the nasopharyngeal roof. In horses above 6 years of age the nasopharyngeal mucosa is relatively smooth (see also 'Endoscopy' in Ch. 12: 'Respiratory diseases').

Defects of the soft palate are readily recognized. The most common is a midline cleft extending through the soft palate. Touching the nasopharynx of a healthy horse with a catheter or forceps wire (protruded through the biopsy channel) usually stimulates a swallow in which the elevation of the soft palate is seen, together with the opening of both guttural pouch flaps. In cases of laryngeal hemiplegia, one side of the larynx (typically the horse's left side) will show varying degrees of immobility during the swallow. In addition, the pharynx may be affected by paralysis; with complete paralysis there are no

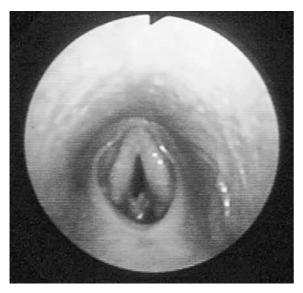


Figure 2.6 Endoscopic view of displacement of the soft palate above the epiglottis in a case of pharyngeal paralysis.

pharyngeal movements and the soft palate remains permanently displaced above the epiglottis (Fig. 2.6). In such cases food material is often seen on the soft palate and within, and around, the larynx.

Comment

• In some healthy horses there may be a reluctance to swallow. In addition, the soft palate may be displaced above the epiglottis during endoscopic examination of a normal animal. A diagnosis of pharyngeal paralysis must therefore be made with care, after repeated attempts to stimulate swallowing. It should be emphasized that the diagnosis and extent of the problem are best assessed on a treadmill (with an endoscope in place) at a referral centre.

Endoscopy of the oesophagus

The endoscope may be directed towards the oesophageal entrance and introduced into the oesophagus in the same way as a stomach tube. This can be achieved in the conscious horse with minimal restraint. Oesophagoscopy may complement oesophageal radiography, but it is of unique value for inspection of the mucosal lining – especially after the relief of an obstruction. Transendoscopic procedures such as biopsy, foreign body retrieval and laser surgery are restricted to specialist centres.

Examination of the entire oesophagus of an adult horse requires an endoscope of at least 2 m length. Ideally, gastroscopic examination should accompany oesophagoscopy as gastric lesions are often associated with oesophageal disorders. However, the common endoscope lengths preclude this, allowing examination of the proximal oesophagus only. An endoscope of 3 m in length is required to fully explore the stomach.

Because normal oesophageal tone causes the wall to collapse on the endoscope, it is necessary to use inflation with air (insufflation) during examination. Observations are best made by starting with the endoscope fully inserted and then examining the mucosa during withdrawal under insufflation.

Normal appearance

The mucosal surface is pink and thrown into longitudinal folds, which become more prominent in the distal oesophagus. There is a natural narrowing of the lumen in the postpharyngeal area, the thoracic inlet, the heart base and the terminal oesophagus.

Anomalies of appearance

Inflammation (oesophagitis) is readily apparent, with or without ulceration or perforation of the luminal wall. An inability to dilate the lumen at some point suggests a stricture or external compression of the oesophagus. The appearance of transverse mucosal folds under insufflation is pathognomonic of a mural lesion, but these must be distinguished from the folds that are inevitably produced by the forward passage of the endoscope in a collapsed oesophagus. Linear mucosal ulcers in the distal oesophagus, which are progressively more abundant towards the cardia, are consistent with reflux oesophagitis and are typically associated with grass sickness. In some of these cases a retrograde movement of gastric fluid may be seen.

Comments

• The cranial cervical oesophagus is difficult to examine adequately by endoscope because repeated stimulation of the swallow reflex causes

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the tip of the endoscope to be directed dorsally. Structural anomalies in this region may be outlined on a radiograph following oral administration of a barium suspension by catheter syringe.

- In obstructive lesions of the oesophagus, food material may be packed down above the lesion so that endoscopy cannot identify the underlying cause of the problem. In these cases food deprivation for 24 hours may allow identification of a partial obstruction, such as a twig. If not, radiography may be more helpful.
- Oesophageal disorders are often best assessed by radiography or endoscopy and in many instances both techniques are required for a complete examination.

Abdominal auscultation

Gut sounds reflect gut activity and the greatest value of abdominal auscultation is in the assessment of colic. As a routine, at least four sites are auscultated: both paralumbar fossae and both sides of the lower abdomen.

Normal sounds

Abdominal sounds are for the most part generated by the caecum and large colon. There are two components: weak sounds associated with localized bowel contractions (mixing the ingesta), and louder fluid sounds or borborygmi associated with peristalsis (propelling the ingesta onwards). One or both of these sounds should be audible during a minute's auscultation at each site.

Sounds heard in the right paralumbar fossa reflect ileocaecal (and possibly caecocolic) valve activity and differ from the other sites. Here, a period of silence is broken once or twice a minute by a sudden rush of fluid rumbling as secretions from one compartment pass through the valve and hit the gas/ fluid interface of the next.

Anomalies of sound

A simple obstruction in an otherwise healthy gut provokes hyperperistalsis in adjacent gut segments.

The best example is spasmodic colic, in which continuous sounds, of greater than usual intensity, are heard at all sites.

In contrast, reflex movement is reduced by inflammation and ischaemia. An absence of sound, or infrequent sounds of reduced intensity, may therefore be associated with peritonitis or the development of gut hypoperfusion. However, in the case of strangulating obstruction causing ischaemia, an initial increase in the frequency of gut sounds may be detected as the intestine reflexively attempts to push contents beyond the site of obstruction. A horse in colic with deteriorating clinical signs (see later), and progressively diminishing gut sounds, suggests the development of a crisis in which the blood supply to the gut is compromised. An absence of sound is also associated with alimentary paralysis as in postoperative ileus and grass sickness. A reduction in the intensity and frequency of ileocaecal sounds sometimes accompanies ileocaecal intussusception.

The presence of entrapped gas (tympany) is denoted by low-pitched tinkling sounds, which may be superimposed on other alimentary sounds – as, for example, in tympany associated with spasmodic colic. The localization of entrapped gas in a segment of the large bowel may be appreciated by simultaneous percussion and auscultation over the abdominal wall. A distinctive 'hollow' sound is audible where a volume of gas is trapped against the body wall. This area may be 'mapped out' on the side of the animal to determine its extent. The common site that can be readily localized is the base of the caecum when auscultating the right paralumbar fossa.

The sound of sand in the gastrointestinal tract may be detected, particularly when auscultating the lower regions of the abdomen. Sand tends to accumulate on the right side and sounds like sand in a paper bag that is being rotated slowly. Another way to think of this is the sound of water and sand rushing together at the seaside. These findings are only preliminary and should be confirmed with examination of the faeces for sand content (see later). Sand can also be seen on abdominal radiographs, but a powerful unit at a referral centre will be necessary. It is particularly useful to monitor gut sounds in assessing the progress of a colic or the postoperative recovery from a colic. The re-establishment of a normal frequency and intensity of sound is a good prognostic sign. We find it useful to record these sounds at each site using a simple scale of intensity: $0; \pm; +$ or ++ equivalent to absent, reduced, normal or increased sounds.

Comment

• In assessing the colic patient, the significance of gut sounds must be evaluated within the context of all other clinical signs. In the case of a return to normal sound the prognosis is probably favourable, whereas the outcome for a reduction or absence of sound is more difficult to predict.

Examination of the alimentary tract per rectum

Contributed by Professor G B Edwards

Rectal examination is the single most important part of the clinical work-up of a horse with colic. It should be carried out after reviewing the relevant aspects of the history and physical examination. In this way an attempt can be made to predict what should be felt and to compare the actual findings with the preconceived ideas. For the inexperienced, the pulse rate should be closely monitored to gauge the severity of a colic and the need for rapid referral (see later).

While rectal examination is of considerable value in the diagnosis of benign problems (such as primary impaction and tympany of the large intestine, and the intestinal hyperactivity of spasmodic colic), it is of greater importance in recognizing those cases that require surgical intervention. Many of these can be diagnosed by rectal palpation before the animal deteriorates or changes in the peritoneal fluid become apparent. *The early diagnosis and referral of these cases significantly improves the prognosis and reduces the occurrence of postoperative complications*. Rectal examination should therefore be performed in all colic cases whenever possible, but it must be approached with a respect for its value and the risks involved.

Restraint

Adequate restraint is essential to prevent damage to the horse or examiner. When rectal examination is carried out in a stable or barn, restraints may include the use of a twitch, sedation (e.g. xylazine) or raising a foreleg. For right-handed examination, the horse is positioned with its right flank against the wall and the handler restrains the animal to the left of the head, which should be drawn into the corner. In this way, forward and right-sided movement are restricted. If the clinician uses the left hand for rectal examination, the horse should be positioned with the left flank against the wall and the head should be restrained on the right side. Stocks provide a safeguard against being kicked but the examiner must always be wary of the horse going down suddenly.

Technique

The clinician should stand to the side of the tail base, close to the horse's quarters, with his/her back to the animal's head. This minimizes injury in the event of a sudden kick. The tail is raised with the free hand and a well-lubricated rectal sleeve is introduced into the rectum. In doing this the fingers and thumb should be brought together to form a cone for slow introduction through the anal sphincter. Any resistance on the patient's part usually relates to the width of the knuckles passing through the sphincter. Care should be taken to ensure that no tail hairs are carried into the rectum and all faeces within reach must be removed prior to any attempt to palpate structures. Thereafter, forward movement of the hand should always be performed slowly with the fingers and thumb brought together in a cone shape. Exploratory palpation should not be undertaken with a wide spread of fingers and thumb since this can predispose rectal trauma. If confronted with a strong peristaltic wave, the cone shape should be resumed and the examiner should be prepared to withdraw the hand.

Assuming patient compliance by the time the forearm has been introduced, the examiner can move to a position behind and in line with the horse in order to allow the full reach of his/her arm

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during examination. Once the arm has been introduced to its full length it is advisable to hold it still for 30 seconds, during which time the colon usually relaxes. In particularly difficult cases, injecting 60 ml of xylocaine into the rectum or applying xylocaine gel to the rectal sleeve may be helpful.

A systematic examination can now begin bearing in mind that it is limited to the caudal 40% of the abdomen even in small horses and ponies. The abdominal and pelvic contents should be palpated by running the hand along the surfaces rather than by grasping any structures through the gut wall. At the end of any examination the hand should be checked for any bloodstained faeces or frank blood. If there is concern that a rectal tear has occurred, greater sensitivity in exploring the lesion is achieved by wearing a plastic sleeve, with the fingers cut off, under a surgical glove. It is crucial to determine whether rectal trauma has occurred and to explore its position and extent by further rectal examination. If there is any concern that the rectal tear is deeper than the mucosa itself, rapid transport to a referral centre is warranted to confirm the extent of the trauma. This can be facilitated by an endoscope. If a horse has trauma that has extended beyond the mucosa, it will have typically penetrated at least the muscle layers and will require surgical attention to save the animal's life.

Normal structures

The normal structures palpable in the left dorsal quadrant include the spleen, the caudal pole of the left kidney and, linking the two, the nephrosplenic ligament (Fig. 2.7). Moving to the right and extending forwards in the midline below the spine, the root of the mesentery can be palpated – although in large horses the reach may be difficult. Specific arterial identification may be impossible and it is frequently easier to identify the caecocolic artery at the base of the caecum rather than the cranial mesenteric artery.

In the right dorsal quadrant the base of the caecum is identified. Normally, the caecum is not full and the caudal and medial teniae bands, running from dorsal to ventral, are fairly relaxed and allow

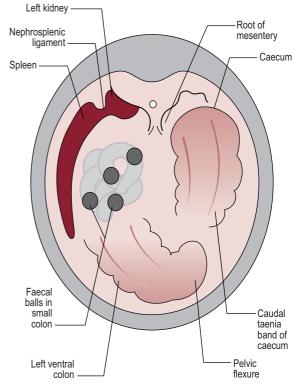


Figure 2.7 Normal disposition of structures that are palpable per rectum.

the fingers to be hooked around one or other of them so that painless traction can be applied to the caecum.

Moving ventrally to the pelvic brim and somewhat to the left, the pelvic flexure of the large colon containing soft ingesta can usually be detected. Extending cranially from the pelvic flexure are the left ventral colon, with its large diameter and clearly recognizable longitudinal bands, and the narrower, smooth left dorsal colon. However, attempting to define distinct components of the large colon will take a great deal of experience.

The space above this and to the left of the caecum is usually occupied by the small intestine and small colon. The normal small intestine is usually not palpable unless it happens to contract when touched, but the small colon is easily recognized by the formed faecal balls that it contains.

The inguinal canals can be felt in the stallion to either side of the pelvic opening at the pubic brim. The bladder, when distended, can limit palpation of organs cranial to it. This problem can be alleviated by catheterization, or stimulating the animal to urinate by putting it in a box with clean bedding.

In general, easy entry, the presence of normal faeces and a relaxed abdomen with ample room for movement of the arm, tend to rule out a serious lesion. However, tense painful loops of intestine distended with gas and fluid, which are displaced back towards the pelvic inlet and upwards towards the roof of the abdomen, indicate a severe obstruction.

Abnormal structures

Although specific diagnoses can be made on the basis of rectal findings, more often the examiner can only determine distension in a specific segment of bowel, or a particular position, which identifies an obstruction.

Abnormalities of the stomach and small intestine

Diseases of the stomach are rarely identifiable on rectal examination. The spleen may give the impression of being pushed caudally by gastric distension, but splenic enlargement is common and will mimic this finding.

Obstructions of the small intestine or adynamic ileus produce distension recognizable as one or more distended loops containing gas and fluid. Strangulating lesions usually lead to tighter distension. The number of loops palpable depends on the nature, duration and location of the lesion. In the early stages of obstruction, careful and patient palpation over a period of several minutes may be necessary before a distended loop is recognized. As the small intestine continues to distend it folds onto itself forming accordion-like loops (Fig. 2.8). These may be positioned vertically or horizontally and can occupy any quadrant of the abdomen, but eventually the tightly distended loops push back into the pelvic inlet making examination difficult. The presence of distended small intestine almost always

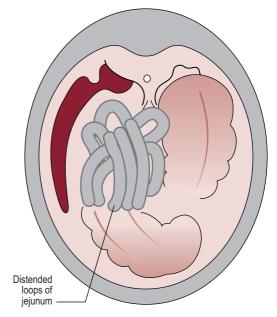


Figure 2.8 Well-established obstruction of the small intestine.

indicates a problem requiring surgical correction. Early identification greatly enhances the chances of recovery.

In *anterior enteritis* (proximal duodenojejunitis) the duodenum is distended and can readily be felt as a tubular structure over the base of the caecum in the right dorsal quadrant (Fig. 2.9). Some distension of the proximal jejunum may also be present, but the gut is not so tightly distended as in obstructions.

Impaction of the ileum can be identified in the early stages as a firm tubular structure 12–16 cm in diameter, medial to the base of the caecum (Fig. 2.10). However, the impaction cannot always be detected. A surrogate marker of ileal impaction is the presence of two or three loops of distended small intestine immediately adjacent to the caecum, although this would not be pathognomonic. In the later stages of ileal impaction, distension of much of the jejunum, if not all, will prevent palpation of the ileum.

Ileocaecal intussusception can be recognized as a firm, enlarged, tubular or coiled structure (depending on the length invaginated) within the base of the caecum in the right dorsal quadrant (Fig. 2.11).

Alimentary diseases

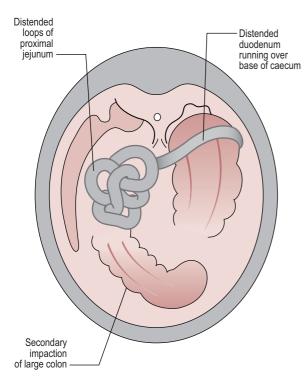
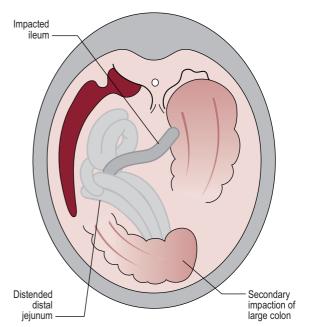


Figure 2.9 Palpable anomalies associated with anterior enteritis.

This can be corroborated by ultrasonic examination.

In stallions, palpation of the inguinal rings, either side of the midline at the public brim, should always be performed. In *strangulated inguinal hernia*, distended painful small intestine can be felt at the ring on the same side as the scrotal enlargement resulting from the engorged testis (Fig. 2.12).

Chronic obstruction causing intermittent bouts of colic, and often weight loss, may be due to a partial obstruction of the small intestinal lumen by intussusception, muscular hypertrophy or intramural neoplasia. As a result of the increased workload necessary to propel ingesta through the constricted segment, marked secondary muscular hypertrophy occurs in the intestine proximal to the obstruction. As a result, several metres of intestine may dilate to a diameter of 10 cm or more and develop a thickened wall (Fig. 2.13). A single loop can be mistaken for pelvic flexure but the presence of other identical



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Figure 2.10 Palpable anomalies associated with impaction of the ileum.

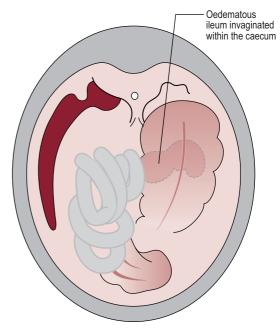


Figure 2.11 lleocaecal intussusception: high obstruction with a palpable caecal anomaly.

Diagnostic techniques in equine medicine

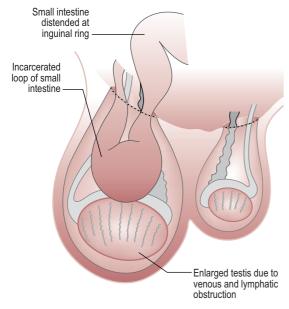


Figure 2.12 Strangulating inguinal hernia.

loops and the fact that they become 'solid' when they contract help to distinguish between them.

Although the precise cause of small intestine obstruction can be identified only infrequently, some indication of the level of obstruction is usually possible. The absence of palpable distension of the small intestine in the presence of gastric reflux indicates a gastric problem, a pyloric stricture or a high small-intestinal obstruction. Distended small intestine with no gastric reflux often indicates a distal small-intestinal obstruction, but this conclusion can only be reached with reference to the length of time that the obstruction has been in existence. Further evidence of a distal obstruction may be obtained by applying gentle traction to the medial caecal band. A pain response is often elicited in horses that have epiploic foramen incarceration or some other ileal obstruction.

Abnormalities of the caecum

A number of caecal obstructions can be identified per rectum. Located in the right caudal abdomen, the caudal aspect of the caecal base and the ventral aspect of the caecal body are within reach, even in large horses. In the normal horse the ventral taenia,

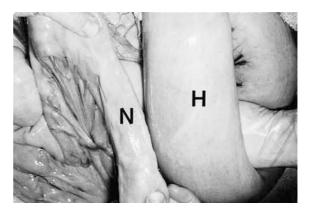
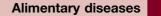


Figure 2.13 Chronic obstruction of small intestine: H = hypertrophied jejunum proximal to partial obstruction; N = normal jejunum.

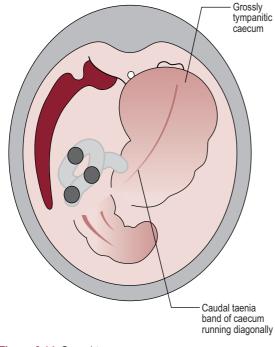
which is vertical in position, is readily identified as a flaccid narrow band that is without covering by mesocolon or vessels. In small horses the medial tenia is also palpable. The tension within the ventral band and its direction will vary with the contents and the degree of distension of the caecum. Because of the normal dorsal mesenteric attachment, the examiner's hand cannot pass dorsal to the caecum. This enables veterinarians to differentiate caecal impactions from large-colon impactions, which can shift to the right side of the abdomen. With largecolon impactions, the examiner's hand can pass dorsal to the colon.

Rectal examination allows differentiation of gaseous distension from accumulation of solid or fluid ingesta. Tympany pushes the caecum back to the pelvic inlet and the tense ventral taenia can be felt running diagonally from the right dorsal to the left ventral quadrant (Fig. 2.14).

Care must be taken to differentiate *caecal impaction* from impaction of the colon, right dorsal colonic displacement and caecal intussusception. Caecal impaction usually presents as a firm to hard digesta-filled viscus with a distinct ventral band. A hand can be passed to the right of, but not dorsal to, the viscus. Typically, the caecal base fills before the body and little or no gaseous distension is evident. Repeated rectal examinations over a period of 12 hours or more may be necessary before the







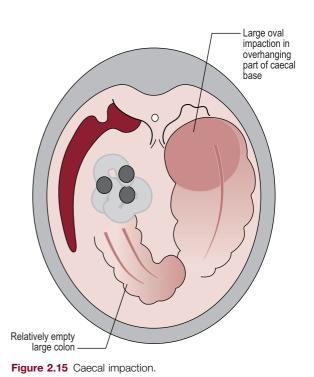


Figure 2.14 Caecal tympany.

impaction is palpable. Palpation of the overhanging part of the caecal base may be impossible in large horses. The mass of ingesta tends to be oval and can be moved from side to side like a pendulum (Fig. 2.15). Difficulty in recognizing the relatively empty large colon is another significant feature of caecal impaction.

Caecal intussusceptions take one of two forms. Following the initial invagination of the apex, intussusception of the body of the caecum into the base (caecocaecal intussusception) may occur or the invagination process may continue until most of the caecum has passed through the caecocolic opening into the right ventral colon (caecocolic intussusception). On rectal examination the firm, oedematous body of the invaginated caecum can be palpated in the right dorsal quadrant either within its base (Fig. 2.16) or within the right ventral colon.

Rectal examination is generally unhelpful in diagnosing non-strangulating infarction of the caecum. Infarctive changes commence at the apex, which is out of reach, but in some cases oedema of the caecal

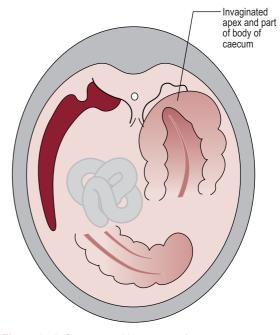


Figure 2.16 Caecocaecal intussusception.

body and pain on palpation are evident. Caecal torsion and typhlitis (due to enteric clostridiosis) also result in mural oedema, which is palpable on rectal examination. However, it is rare for either to be a primary disease process and usually there is concurrent involvement of the large colon.

Abnormalities of the large colon

Rectal examination is particularly helpful in diagnosing large-colon problems. Primary impaction of the pelvic flexure is characterized by an enlarged, firm, evenly filled viscus, which is often located on the pelvic floor or, alternatively, is palpated in the right ventral quadrant. In severe cases it is palpable within a few centimetres of the anal sphincter. Typically, as the hand and arm are moved cranially there is an obvious mass on the pelvic floor, which displaces the rectum in a dorsal direction. The doughy mass can be indented by manual pressure with the indentation remaining for 5-10 seconds. The gut wall around the impaction is smooth, taenia bands can be felt on the more cranial portions of the left colon and the impaction often extends beyond arm's reach. Occasionally there may be some gaseous caecal distension.

Evaluation of the firmness and extent of the impaction will give an indication of the severity of the problem and will allow the effectiveness of treatment to be assessed at subsequent examinations. The thickness of the colon wall should also be assessed. In the majority of cases it will feel normal, but oedema indicates a degree of vascular obstruction, usually due to torsion. This can occur (rarely) when the impaction is beginning to clear. In contrast to impaction, gaseous distension of the colon presents as a taut viscus that resists indentation.

A common error is to mistake a secondary impaction for a primary impaction. Secondary impactions are encountered in horses with grass sickness, anterior enteritis and ileal impaction, conditions in which gastric and small-intestinal fluid distension lead to hypovolaemia. As a result of the body's attempt to conserve as much fluid as possible, the contents of the colon become very dry and shrink. The large colon contracts on to the firm ingesta and the constrictions and sacculations give it a charac-

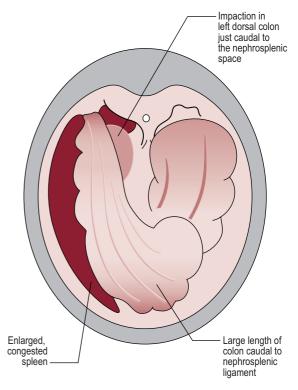


Figure 2.17 Nephrosplenic entrapment of the large colon.

teristic corrugated feel (Fig. 2.9), in contrast to the smoothly distended colon of the horse with a primary impaction. Recognition of a secondary impaction will avoid the mistake of giving large volumes of mineral oil by nasogastric tube with the risk of rupturing an already distended stomach.

In *left dorsal displacement* (nephrosplenic entrapment), varying lengths of left colon are draped over the nephrosplenic ligament. If the displaced portion is large the bands hang down in a diagonal direction and the pelvic flexure cannot be palpated (Fig. 2.17). Considerable tympany of the left ventral portion may obscure the spleen. In the absence of excessive tympany, the examiner may be able to palpate the colon with ease as it traverses the nephrosplenic ligament, which is pathognomonic for this type of obstruction. However, care has to be taken not to cause rectal trauma in attempting to palpate this region. An impaction is often palpable in the left dorsal colon just caudal to the nephrosplenic liga-

Alimentary diseases

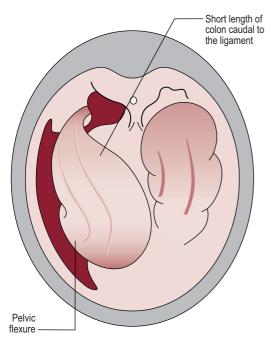
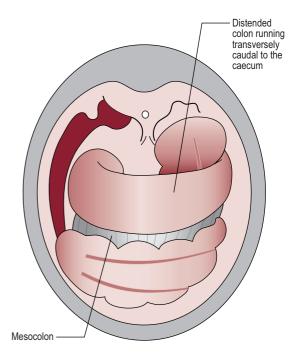


Figure 2.18 Nephrosplenic entrapment associated with a short length of palpable colon.



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Figure 2.19 Right dorsal displacement of the large colon.

ment. If only a short length of colon lies caudal to the spleen, the impacted pelvic flexure is easily recognized (Fig. 2.18). Deviation of the spleen away from the left abdominal wall suggests incomplete entrapment. Oedema of the colon wall indicates marked constriction in the nephrosplenic space or a degree of torsion.

In *right dorsal displacement* a slight to moderately distended large colon lies horizontally in front of the pelvic canal, caudal to a tympanitic caecum. The mesocolon containing fat and large vessels can be recognized and traced to the right, where it can be felt passing between the caecum and the abdominal wall (Fig. 2.19). Oedematous thickening of the mesocolon indicates a degree of torsion.

Severe *torsion of the large colon* produces such great distension that it is often impossible to explore beyond the pelvic inlet. The characteristic features are a horizontal colon with palpable thickening of its wall and mesocolon as a result of oedema (Fig. 2.20). Severe pain and a marked abdominal

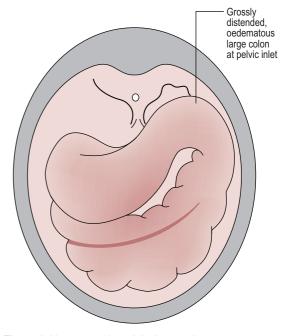


Figure 2.20 360° torsion of the large colon.

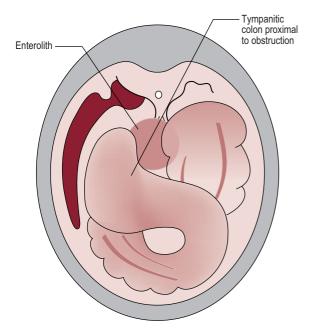


Figure 2.21 Obstruction of the large colon by an enterolith.

distension causing respiratory distress are other characteristic signs.

In obstruction of the large colon by an *enterolith*, the stone can occasionally be palpated, but in the majority of cases the only detectable abnormality is severe tympany because the stone is out of reach, typically in the right dorsal colon (Fig. 2.21).

Abnormalities of the small colon

Obstructions of the small colon are characterized by tympany proximal to the obstruction. Impactions present as a solid tube of ingesta without formed faecal balls. The antimesenteric band can be felt and helps to distinguish small colon from distended small intestine. Occlusion of the lumen by a submucosal haematoma may be identified if the lesion is located far enough distally. In cases where the overlying mucosa has split, frank blood will be present in the lumen. Abrupt deviation of the small colon to left or right may be felt in mares in which it has become hooked around an ovarian pedicle. Submucosal oedema, possibly associated with salmonella infection, can lead to small-colon impaction. Passage of a well-lubricated hand along the oedematous section is difficult because of partial occlusion of its lumen and roughening of the mucosa. Sand colic can be suspected on the basis of gritty rectal contents, and can be confirmed by floating faeces in water and allowing any sand to settle to the bottom.

Other abnormal masses

Occasionally, mesenteric abscesses or tumours are felt on rectal examination. Mesenteric abscesses, which usually develop secondary to upper respiratory infections several months previously, present as large firm masses in the midline. The closer their location to the root of the mesentery, the less mobile they are. Distended loops of adherent small intestine may also be palpable. Splenic abscesses or tumours cause splenic enlargement with medial and caudal deviation. The enlarged spleen feels irregular or nodular.

Gut rupture

Rupture of the stomach or intestine can be diagnosed on the basis of the roughened granular feel to the surface of the gut due to adherent particles of food. Emphysema of the bowel wall or gaseous distension of the abdominal wall may also be detected. Gas and fluid within the peritoneal cavity separate the loops of bowel, and movement of the arm is less restricted than would be expected when multiple loops of intestine are present.

Comments

- Rectal examination should be carried out routinely in all colic cases if possible – only rarely does the examination fail to provide significant information.
- Negative findings may indicate the absence of any serious problem, but on the other hand may simply mean that any affected bowel is out of reach.
- The examination should be repeated every 1–2 hours if the case is presented close to the onset of colic and/or other clinical findings indicate obstruction or strangulation of the bowel.
- Identification of the precise cause of the obstruction is more likely when it involves the

large intestine. Few causes of small intestinal obstruction can be recognized per rectum, but the presence of distended fluid and gas-filled loops is sufficient indication in the vast majority of cases for surgical intervention.

Abdominal paracentesis (peritoneal tap)

Changes in the composition of peritoneal fluid reflect changes occurring at the peritoneal surfaces of organs within the abdominal cavity. The analysis of peritoneal fluid is most useful in monitoring the progression of persistent, intractable colics and identifying peritonitis. It is also indicative of much rarer conditions such as pancreatitis, rupture of the bladder and chyloabdomen. Abdominal tumours may occasionally be revealed by paracentesis if they are sufficiently exfoliative. However, the commonest abdominal tumour of the horse, lymphosarcoma, is not usually exfoliative.

Technique

Peritoneal fluid may be collected using a sterile needle or, alternatively, using a sterile bovine teat cannula.

Needle technique

The horse is restrained with its head in a corner and the right side of its body against a wall. Additional physical restraints such as twitching are usually unnecessary. Sedation may be used in fractious animals providing the clinical circumstances permit.

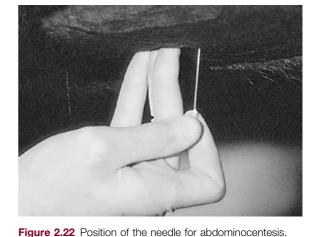
The clinician stands close to the left foreleg facing towards the animal's back. From this position of relative safety he/she can work in full view of the hindlegs and avoid injury if a foot strikes forward during the procedure.

The hair is clipped 5 cm either side of the linea alba from the xiphisternum to the umbilicus. The xiphisternum is recognized as the point at which the costal arches meet in a 'V' shape at the midline. The clipped area is then prepared as for surgical intervention.

Ideally, paracentesis is performed at the lowermost point of the belly since this forms a natural basin in which peritoneal fluid accumulates. In all cases the point of insertion should be approximately a handsbreadth behind the xiphisternum to avoid damage to its cartilage. It is also important to place the needle in the midline through the linea alba, since this is relatively avascular and free of sensory nerve endings. In most cases the linea alba is readily seen in the midline and is palpable at the fingertips as a shallow channel, roughly the width of a pencil.

A 1.5 inch 18G (40 1.2 mm) sterile needle is held by its hub between the forefinger and thumb of a surgically gloved hand. The site of insertion is visualized and the channel of the linea alba is located by the remaining fingers. If the needle is held at 90° to the linea alba and in line with the fingers as they are located in the channel (Fig. 2.22), then it is possible for the clinician to withdraw his/ her head to safety before placing the needle in the skin, knowing that it is positioned accurately. The needle is pushed through the skin and into the linea alba, gently but firmly, to a depth of no more than 5 mm. This should allow the needle to be selfretaining when released. As the point of the needle penetrates the peritoneum there may be a pain response, but this is not always apparent.

Having placed the needle, the clinician must exercise patience in manipulating it to obtain fluid. Unless some pathological process enhances the production of fluid, it is usual to obtain occasional drips at the needle hub. This dropwise collection occurs intermittently as the viscera move to and fro



with respiratory movements over the site of paracentesis. In trying to obtain a sample the clinician must therefore spend up to 10 seconds watching the hub, at a safe distance from the hindlegs, before attempting to manipulate the needle further. If no fluid is forthcoming, the hub should first be rotated between finger and thumb to free the point from any potential blockage. If there is still no fluid drip, the needle is advanced 2–3 mm only and the process is repeated. When the point of the needle comes into contact with the serosa of some part of the alimentary tract, the hub is seen to move or 'course' with respiratory excursions. At this stage the needle should be withdrawn slightly and redirected.

Once fluid appears it should be collected into EDTA and plain sample tubes for cytology and biochemistry/microbiology respectively (Fig. 2.23 (Plate 2)). One millilitre of fluid is sufficient for each purpose. NB: When attempting to obtain peritoneal fluid from the donkey it is essential to use a long needle (e.g. a disposable spinal needle, minimum 3.5 inch/90 mm), since there are usually deep deposits of retroperitoneal fat. This will be the case even when the animal is in poor condition.

Sampling failure

The accidental contamination of a sample with blood is recognized by a swirling of blood into the sample, possibly after a short period of clear fluid collection. Overt haemorrhage is recognized as fresh and accidental if it clots in a plain tube. On the other hand, blood which has collected in the abdomen as a result of some pathological process is defibrinated and will not clot.

Very dark blood that clots rapidly is likely to be the result of tapping an enlarged spleen. With splenic enlargement it may be impossible to obtain a fluid sample from the midline. In these cases ultrasound may be used to determine a 'window' to the side of the midline through which fluid may be reached.

A green-brown sample suggests accidental gut penetration (enterocentesis) or, alternatively, gut rupture. The fluid is likely to smell of ingesta and is obvious at microscopy. Fortunately, accidental gut taps are rarely attended by complications (see later).

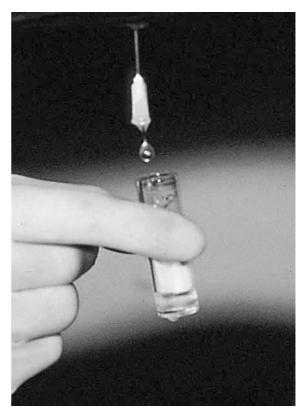


Figure 2.23 (Plate 2 in colour plate section) Dropwise collection of peritoneal fluid into EDTA for cytology.

Occasionally, no fluid is obtained; even after the needle is inserted to the hub. In such cases, if the hub shows no coursing movement the point may be resting in fat and a longer 2 inch 19G (50 1.0 mm) sterile needle should be used. In fat horses and ponies it is advisable to start with the longer needle.

In any of these instances the procedure may be repeated with a fresh needle 3–4 cm caudal to the original point of insertion. Up to three attempts to obtain a sample may be made at different sites along the midline.

Cannula technique

Although needle collection is quick and relatively simple, it suffers the disadvantage of occasional accidental contamination of the sample by blood or gut contents. An alternative technique is to collect fluid using a blunt bovine teat cannula. This technique is

2

preferable where intestinal distension against the belly wall is suspected, or where organs are repeatedly felt at a needle tip despite manipulation. The major disadvantages are its more invasive nature and the greater number of procedures to be undertaken under direct vision at a difficult location. In general, it requires greater patient cooperation.

The site is prepared as previously described and 1–2 ml of local anaesthetic is infiltrated below the skin at the site of proposed insertion using a 25G needle. A short stab incision is then made with a scalpel through the skin and a sterile 3.8 cm or 7.0 cm teat cannula is pushed through the linea alba using steady pressure. Once again, a pain response may be noticed as the blunt end of the cannula pierces the parietal peritoneum. A sterile swab wrapped around the hub should prevent blood contamination from the wound entering the sample. The cannula is manipulated, several millimetres at a time, until an adequate flow of fluid is obtained.

Gross appearance of peritoneal fluid

Useful empirical information may be obtained from the gross appearance of the sample as it is produced.

Volume

It is usual to obtain 5–10 ml of peritoneal fluid in dropwise fashion over 4–5 minutes. A copious flow of fluid under pressure is unusual and suggests that its production is enhanced by some pathological condition, but laboratory confirmation of pathology is required. An absence of fluid ('dry tap') may be experienced in dehydrated patients, but fluid is unobtainable from normal horses on occasion.

Colour and turbidity

Normal fluid is straw-coloured to deep yellow (depending on the bilirubin concentration) and is visually clear because of its low cell content. The yellow intensity increases with the bilirubin concentration during periods of reduced feed intake.

In colic patients, an amber colour and slight turbidity suggests vascular compromise of the gut (hypoxia), which is associated with diapedesis of red and white cells from serosal capillaries. Subsequent necrotic change causes a dark red-brown discoloration and an increase in fluid turbidity as the leukocyte count escalates. Visual assessment of sequential samples during the course of a colic is therefore valuable in confirming the need for laparotomy.

Dark sanguinous fluid should be run into a plain container to see if it clots. Clotting suggests accidental haemorrhage during the procedure. However, abdominal haemorrhage produces a reservoir of defibrinated blood that does not clot. In cases of colic this dark colour in a non-clotting sample could be indicative of gut necrosis; in which case the leukocyte count is also elevated.

A fawn colour in turbid solution is consistent with peritonitis and reflects a high leukocyte count. If the sample is left to settle for 10–20 minutes, the volume occupied by inflammatory cells is readily appreciated (Fig. 2.24 (Plate 3)). In healthy horses the cell deposit is scarcely visible.

A greenish brown colour in turbid solution is suggestive of gut contents. The sample usually has a distinctive pungent smell of ingesta and in the laboratory a stained smear will reveal food debris, protozoa and bacteria in the presence of few leukocytes. This sample may well be the result of enterocentesis. In a case of gut rupture the sample will have a similar appearance but in addition there will be a large leukocyte component and, more significantly, the clinical signs will be consistent with impending shock.

Complications of abdominal paracentesis

Potential complications of abdominal paracentesis are: gut perforation or laceration; the introduction of infection at the site (resulting in cellulitis or peritonitis); and damage/infection at the xiphoid cartilage by sampling too far forward.

Gut perforation is a relatively common accident that is rarely attended by complications. The small puncture hole quickly seals over but a local peritonitis is evoked which increases the nucleated cell count of the peritoneal fluid within a few hours. The count then remains raised for 4–5 days.

Laceration of the bowel by needle point is an extremely rare complication, but it is a potential

Diagnostic techniques in equine medicine



Figure 2.24 (Plate 3 in colour plate section) Huge volume of cellular deposit in the peritoneal fluid obtained from a patient with peritonitis.

catastrophe. It is more likely to occur if the bowel is abnormally distended, or suffering vascular compromise, and the procedure is interrupted by sudden violent movement on the patient's part.

Cellulitis of the ventral abdominal wall or iatrogenic peritonitis are also extremely rare but could follow the introduction of infection by careless technique. It is also possible that a needle contaminated by enterocentesis could infect the abdominal wall as it is withdrawn.

Comments

 When peritonitis is suspected the fluid in the plain sample container should be submitted for bacteriology. Ideally, fresh paracentesis samples should be drawn by syringe and transferred to aerobic and anaerobic blood culture bottles for immediate dispatch to a microbiology laboratory. However, it is not unusual to obtain a negative culture, even when the clinical signs and peritoneal fluid smears indicate sepsis.

- Turbid exudates may occasionally clot if the associated peritonitis is severe enough to allow fibrinogen to enter the peritoneal fluid.
- During the treatment of peritonitis, the composition of sequential peritoneal fluid samples may fluctuate considerably. Having established that a state of peritonitis exists, it is easier to assess progress by measuring plasma fibrinogen concentration. This is a sensitive and reliable monitor of septic inflammation, which simply requires a blood sample (in EDTA).
- Following castration or abdominal surgery, the cell counts and protein concentration of peritoneal fluid are usually raised, even in the absence of complications, and return to normal limits within 7–14 days. The use of abdominal paracentesis to distinguish between postoperative tissue reactions and postoperative infection is therefore limited. Equally, increases in the leukocyte count and protein concentration in peritoneal fluid after castration or abdominal surgery are not necessarily indicative of clinically significant peritonitis. Clear exceptions are where these parameters are greatly elevated in the presence of degenerate neutrophils and/or the presence of bacteria.

Interpretation of peritoneal fluid analysis

Cytology

Cytological analysis is highly specialized and requires the services of a veterinary pathologist.

Normal fluid has a packed cell volume (PCV) of less than 1% with a total leukocyte count below $10 \quad 10^9$ /l and frequently less than 5 $\quad 10^9$ /l. In the differential count, the predominant cell types are neutrophils, followed by mononuclear cells and a few lymphocytes.

Neutrophils. In cases of peritonitis, the cell count greatly exceeds $10 10^9/l$ and the neutrophil pre-

2

dominates. Neutrophils are often reported as either non-degenerate or degenerate. The presence of degenerate neutrophils in a sample indicates the activity of bacterial toxins – i.e. sepsis. These injured neutrophils may be seen in the absence of demonstrable bacteria but the supernatants of such samples should nevertheless be submitted for culture. Occasionally, degenerate cells are reported in association with intracellular or extracellular bacteria.

Mononuclear cells. Two types are recognized. Mesothelial cells form the peritoneal lining and are present in normal samples, where mitotic forms are occasionally seen. In acute inflammation they become pleomorphic and may be difficult to differentiate from neoplastic cells. In chronic inflammation they may be reported as phagocytic. Macrophages are present in low numbers during acute inflammation but their numbers increase as the condition resolves and they may be reported to contain degenerate neutrophils and aged erythrocytes.

Lymphocytes. Very low numbers are present in normal fluid. High numbers of lymphoblasts in a sample suggest abdominal lymphosarcoma, but exfoliation of abdominal tumours in the horse is rarely sufficient to enable diagnostic cytology in peritoneal fluid.

Other cells. Scant eosinophils are occasionally seen. Increased numbers within a slightly raised leukocyte count suggest strongyle parasite migration or hypersensitivity reactions. A few erythrocytes are usually present as a contaminant of collection or as a result of inflammation associated with abdominal pathology. Large numbers in a non-clotting sample indicate intra-abdominal haemorrhage. In these samples there is a lack of platelets and erythrophagocytosis may be reported. Although neoplastic cells are not commonly seen in cases of equine abdominal neoplasia, the total leukocyte count may increase according to the extent of any associated inflammation.

Biochemistry

The most useful biochemical parameter in peritoneal fluid is total protein, which is usually <20 g/l in health. Increases in the concentration of protein reflect the severity of inflammatory effusion. Peritoneal alkaline phosphatase activity (ALP) is increased in conditions of bowel ischaemia owing to the release of the intestinal isoenzyme with which the intestinal mucosa is richly endowed. An elevation in conditions of colic is therefore consistent with the need for laparotomy. However, the colour of the fluid sample and the associated clinical signs would be of more immediate value in electing laparotomy.

Peritoneal amylase activity is elevated in acute necrotizing pancreatitis. However, acute pancreatitis is extremely rare in horses although the presenting signs, acute intractable colic, are relatively common. Because the indications for choosing to estimate amylase are not specific, the condition is usually diagnosed as a post-mortem finding.

When rupture of the bladder is suspected, the urea concentration of peritoneal fluid may be very similar to that of the blood, because it dialyses freely. However, creatinine is less freely diffusible and its peritoneal concentration will usually be more than double that of blood, despite the presence of azotaemia.

Significance of effusions

The leukocyte count and total protein concentration of peritoneal fluid can be used to identify effusions as transudates, modified transudates or exudates. The type of effusion may reflect the mechanism of its formation. However, it must be remembered that the nature of the effusion may change swiftly with the dynamics of the disease process.

Transudates are clear, colourless fluids of low cell count, normal cell differential and low protein concentration (<20 g/l). These fluid characteristics may be present in healthy horses, but a large volume of sample, pouring out under pressure, is abnormal and may be associated with hypoalbuminaemia or venous congestion.

Modified transudates are transudates featuring a modest increase in cell count and/or total protein (20–30 g/l). They therefore appear slightly turbid and are amber to red in colour. They reflect early or low-grade abdominal disease or, alternatively, they accompany a systemic disease. *Exudates* are turbid, amber to red fluids of high cell count $(10 10^9/l)$. The predominant cells are neutrophils, and the protein concentrations are high (>30 g/l). These fluids usually reflect inflammation of the peritoneal surface. Much more rarely, the predominant cell type may indicate a chylous effusion (small lymphocytes amongst numerous fat globules) or neoplasia (exfoliative cells).

Nasogastric intubation

Apart from therapeutic applications, a nasogastric tube may be used to deliver glucose or xylose solutions for the purpose of performing absorption tests, to assess fluid reflux and permit decompression in cases of high alimentary obstruction, or (with great care) to indicate the site of oesophageal obstruction.

Proprietary tubes are manufactured in foal, pony or horse sizes. Soft tubes that are easily folded or misdirected in the warmth of the oropharynx should be avoided. Tubes that are too narrow for the patient may also be folded or misdirected during attempted intubation. However, this is preferable to attempting to pass a tube that is too large, since the inevitable result is a traumatic nosebleed. Tubes with an additional hole in the side of the leading end are recommended and transparent tubes are preferable because they allow the clinician to see the passage of fluid.

As tubes are rarely graduated along their length, it is extremely useful to make an indelible mark around their circumference at a point which indicates that the leading end is approaching the entrance to the larynx or oesophagus. This distance is approximately 30 cm for pony tubes and 35 cm for horse tubes. It is also useful to make an orientation mark indicating the 'top' of the tube – i.e. on its outer curvature.

In cold weather a rigid tube may be softened by passing warm tap water through it. This also reduces objection by the patient as it traverses the sensitive mucosa of the nasal cavity.

Restraint

The horse is stood diagonally in a corner with its quarters against the wall to restrict backward and

lateral movements. The handler should stand to the left of the horse's head with his/her back to the horse to minimize injury if rearing occurs. A sound headcollar is essential but additional restraints will depend upon the horse's temperament. A horse that struggles during intubation is more likely to suffer a nosebleed and such patients are best twitched. Where clinical circumstances permit, sedation is possible – but this will diminish the swallow reflex as the tube is passed and could affect the results of an absorption test if intubation is used for this purpose. In extreme circumstances the handler may apply an ear hold, but this must only be undertaken by a competent, experienced handler.

Passing the tube

A coiled tube is cumbersome to handle and an uncoiled tube will trail on the floor. The uncoiled tube is therefore draped around the clinician's neck, leaving the hands free to control its passage.

The first 10–12 cm of the leading end is liberally coated with a water-soluble lubricant and the tube is grasped just behind this point for controlled insertion. The clinician must avoid getting lubricant on the hands, otherwise the tube will constantly slip beneath the grasp.

The right-handed clinician will be most comfortable if standing to the right of the horse's head with his/her back to the horse. The handler should attempt to keep the head in a flexed position and the clinician rests his/her left hand on the bridge of the nose above the muzzle. Care should be taken not to occlude the opposite nostril inadvertently. The thumb is then used to elevate the alar cartilage of the right nostril, thus opening wide the entrance to the nasal cavity. The lubricated end of the tube is then placed on the floor of the open nostril, slightly inclined towards the nasal septum, with its curvature directed downwards (Fig. 2.25). It is then pushed gently forwards so that it follows the floor of the ventral meatus and the alar cartilage is released. Failure to place the tube on the floor of the nasal cavity may result in its passage along the middle meatus with consequent trauma to the ethmoid turbinates. High placement may direct the tube into the nasal diverticulum ('false nostril').



Figure 2.25 Placement of the stomach tube prior to passage along the ventral meatus.

Passage of the leading end of the tube through the nasal cavity is usually the part of the procedure which is most resented by the patient. The tube's advance is stopped once its preset mark arrives at the nostril, indicating that the leading end is approaching the larynx or oesophagus. In most cases, onward passage will result in entry into the larynx and trachea. To avoid this, the tube should be turned through 90° before being advanced further. This has the effect of raising the level of the leading end with respect to the larynx, thereby bringing it closer to the opening of the oesophagus, which is above the larynx. If successful, gentle pressure by advancing the leading end against the oesophageal opening will cause the tube to be admitted by a swallow. Without the horse swallowing, it is virtually impossible to pass the tube. As the tube induces a swallowing reflex, it will be pushed slightly towards the clinician. This is a sign that pressure needs to be maintained, so ensuring that the tube passes down the oesophagus.

If the tube is accidentally passed into the larynx, it should be withdrawn to the nostril mark, given an additional 90° turn to raise the leading end higher, and advanced again.

Alternatively, if gentle pressure meets total resistance the tube is withdrawn 2–3 cm and gently readvanced in the hope of provoking a swallow. If this fails on three or four occasions, the operator should suspect that the end is pushing against the pharyngeal recess above both the larynx and the oesophagus. In this instance, the leading end is lowered by turning the tube back through approximately 90° before advancing it again. This trial and error manipulation of the tube to bring it adjacent to the oesophagus and provoke a swallow is the most difficult part of the procedure to master.

Checking the tube position

The commonest error is to pass the tube into the larynx. Telltale signs are as follows:

- Air can be blown or sucked through the tube without resistance
- Shaking the larynx produces a palpable 'rattle' because of the tube within
- The tube cannot be seen in the oesophagus (typically on the left side of the neck).

If the tube is clean, untoward effects are unlikely – it is simply withdrawn and repositioned as described above. NB: If the tube does enter the larynx, there may be no associated coughing. Equally, when coughing does occur it may coincide with swallowing of the tube and is not necessarily indicative of misplacement.

When the tube enters the oesophagus, there is often an accompanying swallow, which may be repeated on the downward passage of the tube. Signs of successful intubation are as follows:

2

- There is some resistance to passage (oesophageal tone)
- A swelling may appear in the upper third of the left jugular groove and move down the neck as the leading end follows the line of the oesophagus along the left side of the trachea
- There is resistance to air being sucked through the tube due to oesophageal collapse at the leading end
- When the leading end is in the neck region, a short, sharp blow of air down the tube produces a momentary inflation of the oesophagus, which is seen in the jugular groove. This is a useful test if a distinct swelling has not been seen to travel down the jugular groove. Blowing should be repeated until the clinician is satisfied that the effect is truly inflation and not incidental swallowing
- The fail-safe method of ensuring that the tube is in the correct place is to feel the tube in the oesophagus. This is best accomplished shortly after the tube is swallowed. The most common mistake when attempting this is to pass the tube too far at first, in which case it may be in either the trachea or the lower oesophagus. If the tube can be seen and felt in the upper third of the oesophagus, it is in the right place.

Once the tube is correctly placed, it is advanced to the stomach. On entry, there is usually an audible release of gas and listening at the open end reveals gaseous 'popping' sounds.

Aspiration of reflux

High intestinal obstruction causes fluid accumulation in the small intestine and stomach. The release of fluid and gas from the stomach at intubation is therefore indicative of high obstruction (Fig. 2.26). However, it is not always the case that fluid is released spontaneously and it is often necessary to create a siphon in the tube's dead space. This can be achieved by filling the tube with water, but the most consistent success is achieved by sucking on the open end of the tube – providing the clinician ensures that the tube is dropped from his/her mouth as soon as fluid is seen to reflux!



Figure 2.26 Reflux of fluid from the distended stomach of a pony with high intestinal obstruction.

It should always be borne in mind that the tube's leading end may not be immersed in gastric fluid and attempts to create a siphon should occupy at least 2 minutes of aspiration, moving the tube to and fro over 15–30 cm, before abandoning the procedure. In the absence of any fluid accumulation, gastric mucus is often seen in the leading end of the tube after withdrawal.

Tube withdrawal

Any fluid medication that has been given by tube and is occupying its dead space should be blown through to the stomach before removal. Failure to do so may result in inhalation of spilt fluid as the tube is withdrawn over the larynx.

The tube should be withdrawn slowly and carefully. Particular care should be taken not to rush out the last 50 cm, otherwise trauma to the highly vascular turbinate mucosa will result in a nosebleed.

Potential problems

Nosebleeds look dramatic but are seldom a clinical problem. Raising the head may help to slow the bleed and promote clotting. Packing the nostril may reduce the external signs of haemorrhage but rarely hastens clotting time.

An unsuitably small or soft tube that folds over in the oropharynx may emerge from the opposite nostril, or may emerge from the mouth as it wraps around the soft palate (in which case excessive chewing may be noted). The tube may also be swallowed with the leading end doubled on itself. In this instance, a swelling will probably be seen in the jugular groove, but inflation will not be possible because of a complete seal at the tube's end. If this is suspected, the tube should be passed into the stomach to release the kink rather than raking it back through the oesophagus.

There are rare but harrowing reports of excess pressure being exerted to pass the tube when it is not engaged with the oesophageal opening. In these cases the tube has been pushed through the pharyngeal recess and onward progression has caused it to dissect down the neck towards the thoracic inlet. The result is invariably sepsis. Attention to the technique outlined above should avoid this disaster.

Tubes should be kept in good condition and replaced as necessary. Frayed or chewed ends will traumatize mucosal surfaces. There are also infrequent reports of tube severance with retention of the leading end in the oesophagus, necessitating endoscopic or surgical removal.

Comment

• It is almost impossible to pass a stomach tube in an agitated patient without practice. While acquiring the technique it is advisable to twitch the horse, irrespective of temperament. In addition, despite the effects of sedatives on swallowing and oesophageal motility, heavy sedation (such as xylazine or detomidine) should be strongly considered, in appropriate cases, to ensure safety of all concerned, including the patient.

Rectal biopsy in the horse

Lesions within the mucosa/submucosa of the hindgut are usually associated with chronic diarrhoea and can be characterized with surprising frequency in the histopathology of the rectal mucosa. Since rectal biopsy is easily undertaken in the standing horse it offers a clear advantage over intestinal biopsies, which must be obtained under general anaesthesia. Bacterial colitis may be demonstrated

by inflammatory changes in the specimen, while verminous colitis may be revealed by the presence of cyathostomin larvae and/or eosinophil infiltration. Rarer causes of chronic diarrhoea that may be identified by biopsy are the malabsorption syndromes associated with cellular infiltrations such as lymphosarcoma, granulomatous enteritis and avian tuberculosis. Additionally, it may be possible to isolate salmonellas from homogenized biopsy samples when faecal isolation has proved unsuccessful.

A variety of human rectal and cervical biopsy instruments are suitable for this purpose. The most suitable have a folding upper jaw that cuts the specimen against a rigid lower jaw (Fig. 2.27).

Technique

The horse is restrained as for rectal palpation. Apart from passing the hand into the rectum, the procedure is usually without discomfort to the patient and the necessary restraints are minimal. A lightly lubricated gloved hand is introduced through the anal sphincter to wrist depth and the closed end of the sterilized instrument is passed into the cupped palm using the other hand (Fig. 2.28).

A mucosal fold in the roof of the rectum is palpated and held between finger and thumb and the instrument is advanced with the jaws open to 'snag' the fold in an adjacent dorsolateral position. Taking biopsies from a dorsolateral position (at '1 or 11 o'clock') avoids damage to the dorsal vasculature. The jaws are closed and the sample is removed and transferred to fixative.

Figure 2.27 Rectal biopsy instrument.





Figure 2.28 Passing the rectal biopsy instrument along the wrist and into the cupped palm.

A second biopsy for microbiology may be attempted in the opposite dorsolateral position. This specimen should be transferred to sterile saline.

Comments

- It is essential that the instrument is well maintained and cuts efficiently, otherwise the surrounding mucosa tears as the closed jaws are withdrawn.
- While rectal biopsies can reflect pathology in the more cranial large bowel, normal (negative) specimens do not rule out the presence of colonic lesions.

Ultrasonography of the alimentary tract

Abdominal ultrasonography may occasionally be used to complement other investigations of the alimentary tract. However, a prerequisite for successful examination is a thorough knowledge of the normal topographical anatomy of the abdomen and the ultrasonographic appearance of organs. Both percutaneous and rectal approaches are possible, depending upon the area of interest.

Equipment

Adequate sound penetration is vital for a satisfactory examination. Low-frequency transducers, in the

range of 2–3.5 MHz, are preferred for the general examination. Linear array transducers allow a more rapid evaluation of a large area but sector scanners, with a smaller transducer–patient contact area, may be more useful where access is restricted, between ribs. A higher-frequency transducer, in the range 5–8 MHz, is an advantage when a greater degree of resolution is required after initial scanning reveals a superficial area of interest. For percutaneous ultrasonography the hair must be clipped and the skin cleansed with povidone-iodine, before finally degreasing with spirit.

A rectal transducer is particularly useful for evaluation of the mid-caudal abdomen, especially where an abnormal structure can be palpated per rectum. In addition, a higher-frequency transducer can be used per rectum, as the depth of penetration required is not so great, and a corresponding increase in image quality may be achieved.

Appearance of organs

In the normal horse the greater curvature of the stomach can be imaged on the left side, in close apposition to the spleen. At this site the echogenic reflection of gas in the stomach allows the normally thin hypoechoic gastric wall to be identified. Thickening and abnormal echogenicity of the stomach wall suggests the development of a tumour. Although rare, the commonest gastric tumour in horses is the squamous cell carcinoma. Ascites is commonly found with these tumours and secondary spread may be recognized ultrasonographically as nodules associated with the liver, spleen, omentum, intestines or diaphragm.

Normal intestine is less easily identified and examined. Large intestine is usually seen as a thinwalled hypoechogenic structure around the echogenic reflection of gas in the lumen. Small intestine can be recognized as a tubular structure with a thin hypoechoic wall. Both are recognized by their heterogeneous contents and the movement associated with peristalsis. The most recent advance is the use of abdominal ultrasound to detect thickened right dorsal colon, which is most commonly associated with excessive or prolonged use of phenylbutazone. This segment of the colon can be detected behind the liver on the right side of the abdomen between the rib spaces.

Recognizable abnormalities in cases of obstruction include distended lengths of intestine containing fluid with little or no peristalsis. The appearance of a double-walled area of intestine (with a 'doughnut' appearance in transverse section) is suggestive of an intussusception.

Ultrasound is now commonly used to assess the presence of nephrosplenic entrapment, particularly where rectal palpation is inconclusive because of the difficulty of detecting the nephrosplenic ligament. Ultrasound is used in these cases to rule out entrapment by detecting the left kidney immediately behind the last rib in the upper quadrant of the abdomen. However, if the kidney cannot be found, it could mean that the ultrasonographer is simply unable to locate this organ, or that a gas-filled large colon is entrapped in the nephrosplenic space and is therefore obscuring the kidney. Even the most experienced ultrasonographer has to admit that there is a chance that he/she simply cannot locate the kidney.

In cases of intestinal neoplasia only the secondary effects may be detected and these can include changes to the volume or echogenicity of peritoneal fluid. In these cases ultrasound will determine an accurate location for abdominocentesis. The commonest intestinal tumour of horses is lymphosarcoma, which may or may not be associated with recognizable thickening of the bowel wall; this is also true of gross inspection at post-mortem examination.

When an abdominal mass is identified, the commonest differentials for neoplasia are abscessation or haematoma formation. The fluid-filled nature of an abscess or haematoma may be appreciated by the swirling motion of its contents as seen by real-time ultrasonography. This may be enhanced by external ballottement or moving the patient. However, it may be impossible to differentiate a primary abscess from an area of tumour necrosis and/or infection using ultrasonographic criteria alone.

II. CLINICAL EVALUATION OF THE COLIC PATIENT

This section deals with the clinical assessment of colic and the indications for exploratory surgery (laparotomy), and summarizes a strategy for dealing with the colic patient.

Several of the practical techniques described in this chapter are of particular relevance to clinical evaluation of the colic patient. The vast majority of colics seen in practice are benign in nature; i.e. they are resolved by medical treatment alone and sometimes in the absence of any treatment at all. Most of these are either 'spasmodic' colics or of unknown cause.

In clinical examination it is rare to establish a precise diagnosis of the lesion causing colic but it is essential to determine whether the situation is life-threatening or not. In the case of spasmodic colic this conclusion can be achieved fairly quickly. However, in the case of persistent colic continued monitoring of the clinical parameters is essential. In simple terms, the clinician must decide on the basis of these parameters whether the colic can be managed and resolved by medical treatment alone or whether referral for surgical intervention is necessary. The success of surgery is directly proportional to the speed at which a decision is made following the onset of colic. The veterinarian in the field does not need to be certain that a horse requires surgery, he/she just has to have a high enough index of suspicion to indicate that referral is best for the horse and its owner.

Clinical parameters of colic

Once a history is obtained, the following observations should be made before handling the animal.

Behavioural signs

A state of colic is characterized by an abrupt change in normal behaviour in which various degrees of restlessness are seen. Mild colic produces behaviour such as: stretching of the abdomen; looking at the flanks; repeated yawning and/or teeth grinding. Geldings will occasionally prolapse the penis for protracted periods and even achieve erection. Signs of moderate colic include: persistent pacing of the box; pawing at the ground; kicking at the belly; adopting a crouching stance; occasional grunting; getting up and down frequently. Prolonged periods of lateral recumbency are possible. Severe colic signs include the above, but with profuse sweating, rolling and self-inflicted trauma.

Exceptions to these behaviour patterns occur in donkeys and heavy draught horses. In the USA, exceptions also occur in certain breeds, particularly the Standardbred and Tennessee Walking Horse. These animals are often more stoical and less demonstrative of pain, even in a state of severe colic. Another consideration at this stage of observation is that the patient's behavioural signs may not reflect gut-associated pain, or even abdominal pain. Table 2.1 indicates some differential diagnoses of colic behaviour.

Abdominal enlargement

Distension of the abdomen is often indicated by a convexity of the paralumbar fossae and suggests tympany or, in cases of severe pain and rapid deterioration, torsion of the large bowel. Rupture of the gut will also cause gaseous distension of the abdomen, accompanied by signs of toxic shock.

Respiration

Rapid, shallow respiration can be a feature of pain and/or metabolic acidosis. Dyspnoea can accompany pressure exerted upon the diaphragm by severe gastric distension or hindgut tympany. Rupture of the diaphragm (rare) with prolapse of the gut into the thorax can also cause dyspnoea; particularly if the hindgut is prolapsed.

Muscle tremors

Occasionally, muscle fasciculation is seen over the flank and shoulders in moderate-severe colics. This is probably an autonomic response. Together with patchy sweating it is one of the characteristic features of grass sickness.

owing to discomfort that is not of gut origin						
Straining to	urinate, e.g. urolithiasis and/or cystitis					
	titis, cholangitis or cholelithiasis – pain ± pyrexia					
Peritonitis that is unassociated with a gut lesion						
Rhabdomyolysis ('azoturia') – associated with exertion						
lliac thromb	osis - usually associated with exercise					
Laminitis – o recumbency	can be associated with prolonged					
Acute pleuritis – anxiety; pain on movement + pyrexia						
Complicatio	Complications following castration					
Pregnancy	Hypermotility of the fetus in late gestation					
	Abortion					
	Parturition or dystocia					
	Postparturient haemorrhage from the uterine artery					
	Retained placenta					
	Uterine contractions at involution					
Pancreatitis	- extremely rare in horses					
Hypocalcae diaphragma	mia – muscle stiffness ± synchronous tic flutter					
Hepatic end	cephalopathy – behavioural disturbances					
Pendulous (ovarian tumours or haematomas					
	aly (uncommon), e.g. abscessation; nune-mediated haemolysis					

 Table 2.1
 Conditions presenting colic behaviour

Cessation of defaecation

Defaecation ceases for the duration of any gut obstruction, although the faeces behind an obstruction may be passed initially. Small quantities passed irregularly suggest a partial obstruction. However, if faeces are passed regularly over 24 hours in a patient showing colic behaviour, the diagnosis of alimentary colic should be reviewed (see Table 2.1).

Having checked these observations, a 'hands on' clinical evaluation must include the following.

Heart and pulse rate

The heart and/or pulse rates are important indicators of the severity of a colic. They are influenced to some extent by pain, but most particularly by haemoconcentration (dehydration), decreased venous return and toxaemia (as in gut devitalization). A heart/pulse rate increasing beyond 60 beats per minute in a patient with moderate-severe colic behaviour indicates a deterioration of the circulation and a need to scrutinize other parameters with a view to laparotomy. A persistent high pulse rate, the quality of which gradually becomes weaker, suggests impending shock. On occasion, a paradoxically low heart rate will be detected in horses with large-colon torsion, even though immediate surgery may be needed. The cause is unknown, but it may be related to increased tension on the vagal nerve by the colon, which in turn reduces the heart rate. Nevertheless, heart/pulse rate is the most important clinical sign in assessing a colic and should be obtained prior to administering analgesics if possible, because drugs such as xylazine will lower the heart rate.

Rectal temperature

Slight increases can be associated with pain. However, temperatures in excess of 38.6 °C (101 °F) suggest a differential diagnosis of a systemic disease for which colic is an early incidental sign. The major differentials are *salmonellosis* and *acute peritonitis*. *Anterior enteritis*, an uncommon form of colic featuring ileus with thickening and haemorrhage of the anterior small intestine, is also associated with pyrexia.

A decreasing temperature, coupled with a rapid weak pulse, indicates the development of shock and carries a grave prognosis.

Mucous membrane colour and capillary refill time

The membrane colour and capillary refill time (CRT) reflect the circulatory status of the animal. The normal membrane appearance is moist and pink. Dry, congested membranes suggest dehydration and circulatory disturbance. The CRT, observed by blanching out the gum adjacent to an incisor tooth and judging the time to colour restoration, indicates whether perfusion, hydration and vascular tone are

impaired. In health, the normal CRT occupies less than 2 seconds. Increasing refill times indicate progressively inadequate perfusion and are usually accompanied by dryness and discoloration of the membranes.

Gut sounds

Gut sounds reflect gut motility (see above under: 'Abdominal auscultation'). In a healthy individual there should be sounds of movement at all sites. An absence of sound is abnormal and suggests gut stasis (ileus). An excess of sound suggests hyperperistalsis and is often a feature of spasmodic colics. Lowpitched tinkling suggests associated tympany.

Rectal examination

All cases of colic should be examined per rectum (see above under: 'Examination of the alimentary tract per rectum'). Essentially, the examination is a systematic search to reveal one or more of the following abnormalities:

- Distended loops of small intestine, indicating a high obstruction
- Impaction of the large bowel. This may variously be associated with nutritional impaction, large bowel displacement and/or entrapment, or reduced gut motility – as in peritonitis or grass sickness
- Taut distension of the large bowel, indicating tympany
- Taut bands of mesentery that are painful on manipulation, indicating a dependent lesion such as volvulus
- Solid masses. Possibilities include enlarged lymph nodes, tumours, enteroliths or adhesions.

Stomach intubation

The release of fluid and/or gas following stomach intubation is consistent with obstruction or stasis of the stomach and/or small intestine (see above under: 'Nasogastric intubation'). The normal amount of fluid that can be retrieved from the equine stomach is less than 2 litres.

Abdominal paracentesis

Pathological change, in particular vascular compromise of the gut, is reflected in the colour changes seen in peritoneal fluid. This technique is particularly useful for monitoring persistent colics (see above under: 'Abdominal paracentesis').

Laboratory aids

In general, the most useful laboratory aids assess the extent of the physiological problems associated with a developing 'crisis', i.e. fluid and electrolyte losses and the development of metabolic acidosis. Of these, the most convenient in the field are assessments of dehydration by PCV and/or total plasma protein estimation. In most cases PCVs above 45% indicate haemoconcentration.

In summary, clinical evaluation of the colic patient should encompass all the above parameters. A trend to improvement or deterioration in the patient's condition is readily appreciated by monitoring these parameters over a period of time. The usual experience in practice is that spasmodic colic resolves fairly quickly; however, it is important that the clinician identifies as soon as possible the 'acute abdomen' that requires exploratory surgery. One way to improve the survival of horses with colic is to focus primarily on whether a horse should be referred or not, instead of whether surgery is needed. For the best prognosis, surgery must be carried out within a few hours of an obstruction. Under 6 hours the prognosis is good; at 8-12 hours it becomes doubtful, and after 12 hours the prognosis for a successful recovery is progressively poorer.

Indications for surgical exploration (laparotomy)

Only on rare occasions is the precise cause of a 'surgical colic' diagnosed prior to laparotomy. One such example is the umbilical hernia in which the intestine is incarcerated and strangulated. Most usually, the collective evaluation of clinical parameters indicates a deterioration in the patient's condition that requires surgical intervention. The collective indications for urgent laparotomy are as follows:

- Relentless pain despite analgesia*
- Pulse rate rising (>60 bpm) and deteriorating in quality
- Congested mucous membranes and extended CRT
- Distension of the abdominal wall
- Gut sounds much reduced
- Fluid reflux on nasogastric intubation
- Positive findings on rectal examination*
- Abdominocentesis indicating gut devitalization.

In most instances the clinician will seek to refer the horse to a specialist centre. It must be emphasized that in these cases time is of the essence for a favourable prognosis. The delay in arranging transport and the distance to be travelled should therefore be borne in mind and the present condition of the patient must be carefully assessed. *It is inhumane to subject a mortally sick animal to a protracted and stressful journey*. In the client's interests the potential costs should be discussed with the centre when referral is requested.

A final indication for laparotomy is the undiagnosed chronic or recurrent colic that persists for days or weeks. In these cases laparotomy may provide the only remaining diagnostic step. However, the clinician should ensure that exhaustive clinical examinations have been undertaken and that both larvicidal and cesticidal anthelmintics have been administered before electing for exploratory surgery in these cases (Table 2.2).

A strategic approach to the colic case

If the behaviour suggests mild-moderate pain and there are no systemic complications (i.e. no evidence of high obstruction or circulatory collapse), and a positive response follows the administration of a spasmolytic/analgesic drug, the prognosis is fair-good. A state of affairs is established; the colic can be treated medically for the present but should continue to be monitored. NB: The use of non-

^{*}NB: Both intractable pain and positive rectal findings are justifications alone for surgical exploration.

Table 2.2 Some causes of chronic or recurrent colic

Possible cause	Aids to diagnosis		
Gastric ulceration	Gastroscopy at a specialist centre		
Gastric squamous cell carcinoma (rare)	Gastroendoscopy and biopsy (specialist centre); ultrasonography; check abdominal paracentesis for exfoliatio		
Ileal obstruction:	Colic often follows soon after feeding		
- intussusception	See below		
– hypertrophy (uncommon)	Rectal examination		
- tapeworm	Response to treatment		
Intussusception (fairly common):			
- ileocaecal/caecocaecal/caecocolic	Rectal examination; ultrasonography		
Hindgut impaction (common)	Rectal examination; response to intravenous fluids and liquid paraffin		
- usually pelvic flexure			
- occasionally descending colon			
- rarely caecum			
Non-strangulating displacement of the large colon (fairly common)	Rectal examination		
Sand colic (uncommon)	Relates to sandy topsoil or muddy streams; rectal examination may indicate impaction; sand in diarrhoeic faeces		
Enteroliths; faecaliths; foreign bodies in the colon (all rare)	Rectal examination		
Recurrent ischaemia due to redworm migration	Recurrent 'spasmodic type' colics; check parasite parameters; response to larvicidal anthelmintics		
Chronic grass sickness (fairly common in the UK)	Subtle signs of dysphagia; patchy sweating and muscle tremors; radiography to check mega-oesophagus and oesophageal transit time; ileal biopsy (specialist centres); histopathology of the coeliacomesenteric ganglion at post-mortem		
Peritonitis (fairly common cause of chronic colic)	Abdominal paracentesis		
Adhesions:	Rectal examination; ultrasonography		
- post-operative complications	History or evidence of previous surgery		
- chronic peritonitis	Abdominal paracentesis		
- transabdominal parasite migration	Check beta globulin; worming history		
Progressive obstruction – tumour or abscess interfering with gut patency or peristalsis (uncommon)	Rectal examination; abdominal paracentesis		
Infiltrative bowel diseases (uncommon)	Not usually associated with appreciable colic; investigate chronic wasting/malabsorption		
Right dorsal colitis (uncommon)	History of phenylbutazone administration, ultrasound		
Differentials of colic pain	See Table 2.1		

Туре	Aids to diagnosis		
Spasmodic colic (very common)	Response to spasmolytic treatment; good prognosis		
Tympanitic colic (fairly common) – often accompanies other types	Gut auscultation; rectal examination; response to treatment of underlying colic; good prognosis. NB: Extreme distension is a surgical emergency		
Hindgut impaction (common)	Rectal examination; responsive to treatment with intravenous fluid and liquid paraffin; good prognosis		
Gastric colic – grain overload or impaction (both uncommon)	Response to decompression by stomach intubation; prognosis guarded		
Acute peritonitis (uncommon)	Abdominal paracentesis; response to antibiotics; guarded prognosis since aetiology unknown		
Heat exhaustion/exercise dehydration	Immediate history; PCV and electrolyte estimations; blood gas estimations; usually fair-good prognosis		

Table 2.3 Acute colics that are usually responsive to medical treatment

PCV, packed cell volume.

steroidal anti-inflammatory drugs with antiendotoxic effects (e.g. flunixin, ketoprofen, phenylbutazone) should be avoided in the first instance since these analgesics can mask the clinical signs of a developing crisis, and valuable time can be lost in diagnosing an acute abdomen. Table 2.3 lists acute colics that are usually responsive to medical treatment.

If the pain is severe and difficult to control using various analgesics, the prognosis is much poorer. If the collective clinical parameters indicate deterioration, there are only two choices: surgery or euthanasia. In all cases where surgery is elected there will be one of three conclusions, which the owner should understand in advance:

- The lesion is operable, but the procedure constitutes major surgery, which is necessarily expensive and carries a guarded prognosis in the first instance
- The lesion is inoperable and the situation demands euthanasia while the horse is under general anaesthesia
- Nothing of significance is found because the lesion is functional or inaccessible. This is rarely the case in the acute abdomen but can arise at surgical exploration of chronic or recurrent colic. The inevitable dilemma is then between

reviving the animal or destroying it under general anaesthesia. To avoid this predicament, the course of action to be taken must be agreed by prior consultation with the owners before surgery. It is for this reason that elective laparotomy for chronic or recurrent colic must only follow exhaustive clinical examinations (see Table 2.2).

III. CLINICAL PATHOLOGY

Serum biochemistry

Total protein

Sequential total protein estimations may be used to monitor dehydration in cases of colic. However, in the severely compromised gut there may be a concurrent and progressive loss of protein into the peritoneal cavity or bowel lumen, thus rendering the technique inferior to sequential determinations of PCV in whole blood. Similarly, mucosal lesions associated with enteropathies such as malabsorption, parasitism or diarrhoea are usually accompanied by protein loss (hypoalbuminaemia) and in these cases progressive dehydration must also be judged by changes in the PCV.

Albumin	Alpha 2	Beta-1	Beta-2	Gamma	
Normal	++ (APPs)	Normal	Normal	Normal	
Normal	+ (APPs)	+ (IgG(T))	+ (lgs)	++ (lgs)	
Normal	Normal	+ (IgG _(T))	+ (lgs)	++ (lgs)	
Low (PLE)	++ (APPs)	++ (IgG _(T))	Normal	Normal	
Low	Normal	Normal	+++ (lgs)	+++ (lgs)	
	Normal Normal Normal Low (PLE)	Normal++ (APPs)Normal+ (APPs)NormalNormalLow (PLE)++ (APPs)	Normal++ (APPs)NormalNormal+ (APPs)+ (IgG_{(T)})NormalNormal+ (IgG_{(T)})Low (PLE)++ (APPs)++ (IgG_{(T)})	Normal++ (APPs)NormalNormalNormal+ (APPs)+ (IgG_{(T)})+ (Igs)NormalNormal+ (IgG_{(T)})+ (Igs)Low (PLE)++ (APPs)++ (IgG_{(T)})Normal	

 Table 2.4 Empiric interpretation of serum protein shifts as revealed by electrophoresis

PLE: protein losing enteropathy; APPs: acute phase proteins; Igs: Immunoglobulins; IgG₍₇₎: Immunoglobulin G (subclass T).

Albumin

In horses hypoalbuminaemia is almost invariably associated with a *protein-losing enteropathy* as a result of some lesion of the intestinal mucosa. Much rarer causes are glomerulonephropathy, liver failure or massive exudative effusion. In all these lesions albumin is lost preferentially from the circulation because it has the smallest molecular weight of the plasma proteins. The exception is end-stage liver failure, in which case the synthesis of albumin declines.

Globulins

Apart from dehydration, total globulin concentrations may also be increased by acute and chronic inflammatory processes (caused by increases in acute phase protein and immunoglobulin concentrations respectively), strongyle parasitism (caused by increases in beta-1 globulin) or liver failure (possibly related to systemic immune stimulation from intestinally derived antigen following the loss of protective Kupffer cells). Some commercial laboratories offer beta-1 globulin estimation by electrophoresis; if raised above the normal range, it is good evidence of active strongyle migration.

Albumin: globulin (A/G) ratios

In health, the A/G ratio approximates to 1.0. Shifts in the ratio may occur in a number of pathological states. However, the information is seldom useful since it lacks specificity. It follows from the preceding paragraphs that a fall in this ratio, due to a decrease in albumin and/or an increase in globulin, may be a feature of either inflammatory intestinal disease, strongyle parasitism or liver failure.

Serum protein electrophoresis

Routine serum protein electrophoresis may help the clinician to identify certain categories of disease, some of which will be alimentary in nature. Agarose gel electrophoresis separates equine serum proteins into four fundamental bands, characterized in order of their electrophoretic mobility. These bands are stained and identified as albumin with subdivisions of alpha, beta and gamma globulins. Once the total protein concentration is known, the individual protein concentration within each band may be determined in the laboratory by densitometer. However, the results of electrophoretic analysis of horse serum are not always comparable between laboratories because of differences in the separative technique. As a result there are conflicting data regarding the 'normal' concentrations and ranges of the various protein fractions. It is therefore advised that clinicians interpret protein shifts as empirical increases or decreases rather than absolute values. Table 2.4 shows an empirical interpretation of protein shifts.

Comment

 In diarrhoeic horses, the identification of high beta globulin levels is suggestive of cyathostominosis. However, the presence of normal beta globulin levels cannot be regarded as a reliable indicator of the absence of significant parasitism.

Serum alkaline phosphatase (SAP or ALP)

The brush border of the intestinal epithelium is richly endowed with alkaline phosphatase and cellular damage increases the circulating SAP concentration. However, alkaline phosphatase is not organ-specific and damage to bone or the biliary tract of the liver will also cause an increase in the circulating SAP concentration. Many laboratories will assay the isoenzyme intestinal alkaline phosphatase (IAP). However, in our experience the accurate quantification of this enzyme on a reproducible basis is technically difficult and it is arguably preferable to consider the total SAP. Thus an increased SAP concentration, in the absence of either bone disease or clinicopathological evidence of liver disease (see Ch. 4: 'Liver diseases'), is indicative of gut pathology.

Pancreatic enzymes

Pancreatitis is extremely rare in horses and the indication for pursuing its clinicopathological diagnosis only follows the exclusion of other causes of moderate to severe abdominal pain.

Albeit on the basis of limited experience, useful indicators of acute necrosing pancreatitis seem to be estimations of amylase and lipase activity in both serum and peritoneal fluid. In health, amylase and lipase estimations are usually very low in horses, but normal ranges vary between laboratories, reflecting different assay techniques. In horses that have had demonstrable pancreatitis, the concentration of these enzymes has been greatly elevated. However, these enzymes are not organ-specific and relatively modest amounts may be released following injury to the intestinal mucosa or renal tubules. Modest increases could also occur following ischaemic change to the pancreas (secondary pancreatitis), as it may be associated with an intercurrent disease such as distension of the adjacent bowel.

Fluid, electrolyte and acid–base balance

Fluid, electrolyte and acid-base disturbances are associated with those acute colics in which fluid is

sequestered in the gut lumen and/or there is associated strangulation. Examples include all forms of high obstruction, and displacement with torsion of the large intestine. In diarrhoea, the extent of fluid and electrolyte losses and the development of acidosis depends upon the severity of the enteric lesion and whether or not the patient continues to drink during the illness. The diagnostic assessment of fluid, electrolyte and acid–base balance in various disease states is considered in depth in Chapter 11: 'Fluid, electrolyte and acid–base balance'. Brief details of clinical pathology are given below.

Fluid balance

Simple blood parameters such as PCV and total plasma protein can be used to indicate the severity of dehydration. However, where facilities are available they are best used in a serial manner to follow the course of dehydration over a critical period.

Packed cell volume

In general terms, a PCV of more than 45% indicates a reduction in extracellular fluid volume and a loss of sodium. Colic patients with a PCV of more than 60% usually have a poor prognosis, but this is not invariably so.

Total plasma protein (TPP)

TPP estimation can be undertaken in the field using a refractometer. However, a patient suffering a concurrent protein loss (e.g. protein-losing enteropathy) as well as dehydration may show a total plasma protein that is within the normal range.

Urea and creatinine concentrations

Most serum or plasma biochemistry parameters, including urea, are raised by acute dehydration. However, increases in both urea and creatinine beyond their normal ranges could reflect prerenal failure associated with hypovolaemia (i.e. renal hypofusion).

Electrolyte balance

The interpretation of serum or plasma electrolytes in alimentary disease should be undertaken with caution. Increases in sodium, potassium and chloride concentrations are consistent with dehydration but there may be a concurrent loss of electrolytes to the gastrointestinal tract. High obstructive colic is associated with a loss of water, sodium and chloride from the plasma. In cases of lower-bowel pathology, relatively more potassium and bicarbonate ions are lost. A meaningful interpretation of electrolyte shifts can only be undertaken with a knowledge of the concurrent acid–base status.

Acid-base balance

Metabolic acidosis is the most common acid-base disorder in horses. It occurs most frequently in association with obstructive gastrointestinal disease and diarrhoea. The underlying causes of acidosis in these situations are increased base loss and/or reduced peripheral perfusion causing a switch from aerobic to predominantly anaerobic metabolism in tissues, with a consequent build-up of lactate.

Although blood gas and pH measurements provide the only accurate guide to acid–base status, plasma bicarbonate estimations are acceptable for most clinical situations. Even so, this requires venous blood samples to be collected anaerobically into syringes treated with lithium heparin and processed as soon as possible using sophisticated equipment that is not usually available in practice. In practical terms, however, the need to correct a metabolic acidosis by specific bicarbonate therapy is rare if fluid and electrolyte requirements are met (see Ch. 11: 'Fluid, electrolyte and acid–base balance').

Haematology

Useful aspects of haematology in the evaluation of alimentary disease are the PCV, indicators of anaemia and the white cell count. In chronic conditions the plasma fibrinogen concentration should also be requested. Although strictly a biochemical parameter, fibrinogen is often estimated by the haematologist and must be submitted in anticoagulant (either EDTA or sodium citrate depending upon laboratory requirements).

Erythrocyte parameters

As indicated above, the PCV is a useful monitor of dehydration and hypovolaemia if used on a sequential basis.

Chronic anaemia in the horse is often nonregenerative and associated with chronic inflammatory processes, but a chronic regenerative anaemia could reflect chronic haemorrhage into the gut or abdomen. Techniques for investigating anaemia are detailed in Chapter 8: 'Blood disorders'.

Acute haemorrhage is only reflected in the haematology profile after 12–24 hours, by which time there is a compensatory influx of tissue fluid to expand the plasma volume. The effect is to reduce the PCV, red blood cell and haemoglobin concentration, and dilute plasma protein concentrations.

Leukocyte parameters

Leukopenia

Leukopenia (white cell count <6.0 $10^9/l$) is a feature of peracute/acute diseases of the gastrointestinal tract, e.g. gut ischaemia (as in surgical colics), gut perforation or salmonellosis. In these situations the count may fall to 2–3 $10^9/l$. It is attributed to localization of cells at the site of injury and is most pronounced in the presence of endotoxin. A number of morphological changes to the border and cytoplasm of neutrophils may be reported as toxic changes. These reflect the production of chemicals by neutrophils that are toxic to bacteria. The magnitude of these changes is proportional to the severity of sepsis, and persistence over several days in sequential samples is consistent with a poor prognosis.

Leukocytosis

Leukocytosis may accompany acute, progressive or more chronic inflammation of the gastrointestinal tract. This 'reactive leukocytosis' usually features neutrophilia and may be accompanied by immature band forms in acute conditions (left shift) and a monocytosis in chronic conditions.

Eosinophilia

Eosinophilia is popularly associated with parasitism, but high burdens of mature worms do not seem

to affect the eosinophil count. In many instances eosinophilia probably reflects some form of hypersensitivity response.

Plasma fibrinogen concentration

The fibrinogen concentration is raised by inflammation, most particularly septic inflammation, and its level indicates the severity of disease. Concentrations increase within 1–2 days of an infection but peaks are not attained until 3–4 days. A modest increase may therefore reflect early disease or, alternatively, a chronic low-grade inflammation. High concentrations indicate advanced and serious disease with a poorer prognosis.

Comments

- In horses, plasma fibrinogen concentration is usually a more sensitive and reliable monitor of recovery from inflammation, or efficacy of treatment, than the peripheral white cell count.
- The normal range of fibrinogen concentration varies markedly between laboratories depending upon the technique used for its quantification. *The clinician should always refer to the reference range given by the laboratory.*

Tests of intestinal malabsorption

These tests are indicated where weight loss is occurring in the absence of an obvious cause, despite an adequate food intake. The tests assess the functional integrity of the small intestine by measuring the efficiency of sugar absorption from the intestinal lumen. Pathological changes that interfere with cellular transport mechanisms reduce uptake into the bloodstream.

The oral glucose absorption test (OGAT)

This test is inexpensive, simple to perform using readily available reagents and offers good empiric information on the efficiency of small-intestinal absorption.

Technique

• The horse's weight is estimated as accurately as possible (e.g. girth weigh band) and it is fasted

overnight on an inedible bedding. Access to water can be allowed until 2 hours before the test begins.

- 1 g/kg bodyweight of anhydrous or monohydrate D-glucose is weighed out and a fresh solution is prepared as 20% w/v in warm water. The quantity and concentration of glucose are important since stomach emptying is delayed by excessive concentrations of glucose and the test depends upon the rapid entry of the administered solution into the lumen of the small intestine.
- A 'fasting' sample of blood is taken immediately before the test and designated 'time zero'. All samples must be collected into potassium oxalate-sodium fluoride anticoagulant.
- A nasogastric tube is passed and the entire solution is delivered as a bolus into the stomach.
- Further blood samples are taken at 30, 60, 90, 120, 180 and 240 minutes and submitted to the laboratory for glucose estimation. These samples will be sufficiently stable in oxalate–fluoride to send in the post.

NB: If the patient is insufficiently fasted, residual food in the stomach will mix with the incoming glucose solution and reduce its rate of delivery to the small intestine, thus producing a spurious result.

Interpretation

The absorption curve is plotted arithmetically and in conditions of normal absorption has two phases (Fig. 2.29A). In the first 2 hours glucose is continuously absorbed from the small intestine and the plasma glucose concentration doubles. Quite apart from mucosal cell integrity, this absorption phase is influenced by the rate of gastric emptying, intestinal transit time and previous dietary history. A recent dietary history of a high-energy intake will be associated with the production of reduced peaks. The second phase is insulin-dependent and shows a progressive fall to a resting level, which is achieved by 6 hours. The sampling times suggested above should reveal these features in cases where absorption is not compromised.

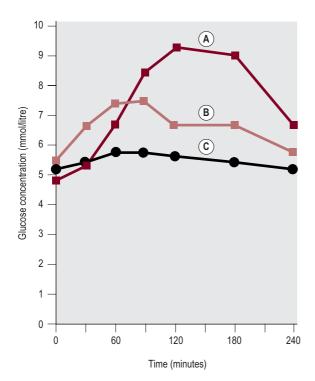


Figure 2.29 Oral glucose absorption curves for three horses: (A) normal absorption; (B) partial malabsorption; (C) total malabsorption.

A flat line indicates a state of total malabsorption (Fig. 2.29C) and usually constitutes a grave prognosis because the principal causes are progressive inflammatory cellular infiltrations of the gut wall. These include lymphosarcoma, granulomatous enteritis, eosinophilic gastroenteritis and avian tuberculosis. Diagnosis is defined by histopathology of the small intestine; gross lesions are usually not visible or palpable at laparotomy or post-mortem examination.

An intermediate curve between normal absorption and total malabsorption suggests a state of partial malabsorption (Fig. 2.29B), which is more difficult to interpret. Causes are likely to be variable and could include, for example, circulatory disturbances, villous atrophy or reversible inflammatory changes associated with parasitism. In some cases the associated histology may be normal, suggesting other causal factors such as protracted stomach emptying, rapid intestinal transit time, inherent anomalies of cellular uptake and metabolism of glucose, or an overgrowth of intestinal bacteria that metabolize the test sugar. Without knowing the precise nature of the lesion or functional disturbance, it is not possible to be certain that such cases will not revert to normal given time and supportive treatment. However, the test can easily be repeated at a later date to monitor the patient's progress. Repeated 'partial malabsorption' results require bowel wall biopsy for further diagnosis. Alternatively, subsequent deterioration to a state of 'total malabsorption' suggests the end stage of a severe infiltrative lesion in the small intestinal wall.

Comment

• Lesions causing malabsorption in the small intestine may also infiltrate the hindgut, where malabsorption causes chronic diarrhoea. Lesions causing malabsorption may therefore affect the small intestine alone, the large intestine alone or the whole intestinal tract. In patients with chronic diarrhoea of unknown cause, an OGAT will indicate whether or not there is associated small intestinal malabsorption.

The D-xylose absorption test

The principle of this test is essentially the same as the OGAT, but the shape of the xylose absorption curve is unaffected by the endogenous metabolic events that can influence the blood glucose concentration. In addition, xylose is not a normal constituent of the plasma. Because of this, it is said to provide a more accurate assessment of absorption. It is also believed to be a more sensitive indicator of malabsorption, registering decreases in absorptive function before the glucose uptake curve. This may be because xylose is passively absorbed from the intestine whereas glucose absorption is active. However, the shape of the curve is influenced by a number of factors, which can also cause anomalies in the glucose absorption curve, i.e. the rate of gastric emptying, the intestinal transit time, intraluminal bacterial overgrowth and the immediate dietary

history. In addition, the costs of xylose and its assay are considerably more than those of glucose and at present commercial laboratories do not process the samples routinely. On balance, the practitioner is advised to use the OGAT.

Technique

- The horse is weighed and prepared as for the OGAT (above).
- The xylose solution is prepared as 0.5 g/kg bodyweight in 10% solution.
- A 'fasting' sample of blood ('time zero') is taken into potassium oxalate-sodium fluoride anticoagulant immediately before the test.
- A nasogastric tube is passed and the solution is delivered as a bolus to the stomach.
- Further blood samples are taken at 30-minute intervals for 2 hours.

Interpretation

In conditions of normal absorption, blood xylose concentrations rise from zero to a peak concentration of 1.33–1.67 mmol/l within 60–90 minutes of administration.

As with interpretation of the oral glucose absorption curve (above), normal absorption and total malabsorption are easily appreciated. A flattened, intermediate curve suggests partial malabsorption and requires re-evaluation.

Faecal analysis

Intestinal parasites

The faeces should be examined grossly for large parasites and tapeworm proglottids.

Faecal egg count (FEC)

Parasite eggs are separated from the faecal mass by a floatation technique using solutions of high specific gravity. The results are calculated as eggs per gram (epg) of faeces. Faecal samples should be fresh and taken from the rectum if possible. A half universal volume (approximately 10 ml) is sufficient. Samples may be stored in a refrigerator for a short time before submission if necessary. Strongyle eggs are readily identified in the laboratory but it is difficult to distinguish between large and small species. However, small strongyle (cyathostomin) eggs usually comprise the vast majority of the count (>90%).

Interpretation

It is impossible to determine the number of parasites present in the gut on the basis of a faecal egg count. Egg production varies greatly between, and possibly within, species of worms and also varies with individual host factors such as age and immune status. Most importantly, intermediate larval stages do not produce eggs – thus parasitic infection may be a significant problem without a significant faecal egg count. However, some positive counts do reflect the severity of strongyle burdens: 500 epg suggests a mild burden; 800–1000 moderate and more than 1500 severe. In general, counts greater than 500 epg indicate the need for control measures to be implemented.

Faecal egg reduction and anthelmintic resistance

Resistance amongst cyathostomin species to several benzimidazole anthelmintics is well known and reports of resistance are accumulating for other classes of anthelmintic. When evaluating the efficacy of a parasite control programme, particularly where drug resistance is suspected, it is useful to monitor the faecal egg count prior to worming and again 10–14 days after the routine anthelmintic treatment.

Following effective anthelmintic treatment, the faecal egg count reduction (FECR) at 10–14 days should be at least 90% and preferably close to 100%. If not, resistance should be suspected and the test should be repeated following a change of anthelmintic from one chemical group to another. The FECR is calculated as follows:

$$FECR\% = 1 - \left(\frac{epg after 10-14 days}{epg at worming}\right) \times 100\%$$

Within a group of horses, the FECR will vary between individuals (because of shifts in the individual host– parasite relationship) and as many horses as possible should be monitored to give an overview of the efficacy of control. In the case of a large group it is ideal to divide the horses in order to compare a subgroup treated with the usual anthelmintic with a positive control group that has been treated with an anthelmintic against which resistance is less likely.

Presence of faecal larvae

Unlike parasite eggs, faecal larvae are separated from a sample by sedimentation using the Baermann apparatus. Alternatively, a wet faecal smear may be examined under the microscope. Samples should be taken freshly for rapid analysis and not subject to refrigeration.

Presence of tapeworms

The laboratory test for diagnosing an *Anoplocephala* burden is unsatisfactory. Eggs are rarely floated out of faecal solution and are found primarily in gravid proglottids from which they are released after the segments have been passed in the faeces. Gravid segments or even whole tapeworms may be seen intermittently in the faeces, but usually there is no conclusive evidence of infection.

A serological test is available for detecting tapeworm infections in horses and correlates well with the intestinal burden. However, when investigating the possibility of tapeworm infection it may be more cost-effective to treat prophylactically with pyrantel embonate or praziquantel than to attempt laboratory diagnosis. In positive cases, tapeworms may appear in the faeces 24 hours after treatment.

Presence of Oxyuris equi

Oxyuris (pinworm) eggs may be identified on the anal sphincter by pressing a strip of transparent adhesive tape on to the mucosal folds of the external sphincter and attaching it, adhesive side down, over a water droplet on the surface of a clean microscope slide. The operculated eggs show at 100 magnification.

Bacterial culture of faeces

Faecal samples inevitably contain a great many organisms with differing requirements for culture *in*

vitro. When submitting samples it is therefore necessary to define the organism(s) of interest to enable selective culture.

Salmonella

In patients suffering salmonellosis the numbers of salmonellae shed may be very low, even during the acute stage of disease. In consequence, a minimum of three and preferably five faecal samples should be collected from the rectum at 24-hour intervals to increase the possibility of detection. An adequate sample should occupy half a universal tube (approximately 10 ml) – swabs are unsatisfactory.

At the laboratory the sample is inoculated into direct medium and an enrichment broth. A positive culture in the direct medium can be reported within 24 hours but this does not always grow sufficiently well. The enriched culture needs to be subcultured to a selective medium for a further 12–24 hours. Suspect cultures are then subcultured again for biochemical test. The turn around time for samples is thus a minimum of 48 hours and may be as long as 72 hours.

Comments

- There appear to be no host-adapted salmonellae that affect horses, but horses are known to excrete salmonellae asymptomatically and they may act as carriers or reservoirs of infection. This occasionally calls into question the significance of some salmonella isolates in horses with chronic diarrhoea. Whereas a positive isolate always carries the suspicion of being the primary aetiological agent in patients with colitis, excretion may accompany concurrent bowel diseases such as verminous colitis or lymphosarcoma.
- Despite the fact that salmonella may not be isolated in the faeces of a patient during life, it may be detected at fresh post-mortem in homogenates of the large-intestinal mucosa and/or mesenteric lymph nodes.
- The concurrent submission of rectal mucosal biopsy specimens for culture improves the likelihood of detecting invasive salmonellae.

Clostridia

Clostridiosis (usually *Clostridium perfringens*) is uncommon in horses but is certainly a differential diagnosis to salmonellosis in cases of peracute/acute toxaemic colitis. Whilst faecal samples should always be cultured for salmonellae, clinical circumstances may also dictate examination for clostridia. In some cases this may be an investigation of intestinal contents post-mortem. A half universal of faeces taken from the rectum is submitted for anaerobic culture as soon after collection as possible – swabs are unsatisfactory. A positive result is indicated by a high faecal count (>100 colony-forming units (cfu) per gram of faeces).

In the acute case it is possible to submit blood in anaerobic blood culture bottles (see 'Blood culture' in Ch. 8: 'Blood disorders'). Up to three samples should be taken during a 24-hour period. The isolation of clostridia from the blood is obviously significant.

Faecal leukocytes

The presence of leukocytes and occasionally epithelial cells in faecal samples suggests inflammatory injury to the distal intestinal mucosa. Consequently, they are a feature of severe diarrhoea (fluid faeces), particularly in the acute stage. High numbers suggest the presence of an intestinal pathogen such as *Salmonella* or *Clostridium*.

Technique

- Fresh faeces should be taken from the rectum and strained through a thin medical gauze to remove fibrous matter.
- If necessary, the sample is diluted to a watery consistency with saline (0.9%). A drop is smeared on a 1 3 inch microscope slide.
- The smear is air dried, stained with modified Wright's stain and mounted with a cover slip.
- The feathered (trailing) edge of the sample is viewed under low power (10) to assess the presence or otherwise of leukocytes.

Interpretation

Large numbers, often in the presence of epithelial cells, are significant and suggest salmonellosis, but their presence is not pathognomonic for salmonellosis. Equally, the absence of faecal leukocytes does not rule out salmonellosis.

Faecal blood

When blood is clearly visible in the faeces, a red discoloration suggests a recent, distal source such as the small colon or rectum. A dark to black discoloration (melaena) suggests a source in the proximal gastrointestinal tract or large colon. Chronic gastrointestinal loss is usually occult and may be associated with a state of chronic regenerative anaemia.

Occult blood may be detected qualitatively using a commercial kit. A small amount of specimen from deep within a faecal mass is smeared on a reagentimpregnated paper slide. Two smears are made from different parts of the mass to increase the chances of detection. In the laboratory, the presence of haemoglobin is detected in the smear by reagents that produce a dye. Specimen preparations are stable if kept dry and may be sent through the post for development.

Comment

 Occult bleeding may be intermittent and ideally three faecal samples should be checked on separate occasions. Chemical tests for the determination of blood in faeces are highly sensitive but not specific. Potential sources are neoplastic infiltration of the bowel, parasitism or mucosal ulceration. A positive finding must be interpreted with great care, taking into consideration all the presenting clinical signs and the associated clinical pathology.

Faecal sand

Sand ingestion from topsoil or water courses may be associated with colonic impaction and, following abrasion of the intestinal mucosa, severe diarrhoea. If this is suspected, faeces should be tested for the presence of sand.

One volume of faeces is mixed vigorously with two volumes of water in a clear container and allowed to settle. Sand sediments to the base of the mixture.

Comment

• Minimal amounts of sand are often present in the faeces of grazing horses but amounts vary with the regional differences in soil type. A clearly defined layer of sand in a small faecal sample is abnormal, but if there is doubt as to its significance, the faeces of a healthy individual from the same region should be tested for comparison.

Chapter appendices

The appendices suggest applications of some of the diagnostic techniques covered in this chapter for the

investigation of two common problems of the equine alimentary tract: dysphagia (Appendix 2.1) and diarrhoea (Appendix 2.2).

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APPENDIX 2.1 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF DYSPHAGIA

Possible cause	Aids to diagnosis
Choke/oesophageal foreign body	Stomach intubation to determine the level of obstruction (care!); endoscopy of the oesophagus; radiography of the oesophagus
Foreign body lodged in the mouth/oropharynx	Examination of the mouth; endoscopy of the upper alimentary tract
Oesophageal stricture	Endoscopy; contrast radiography
Oesophageal ulceration	Endoscopy
Squamous cell carcinoma involving the oesophagus	Endoscopy and biopsy (specialist centre); thoracic radiography
Teeth problems	Mouth examination; radiography
Pharyngitis, e.g. acute 'strangles' or viral infection	Endoscopy; nasopharyngeal swab (see Ch. 12)
Obstruction of the oropharynx or oesophagus, e.g. 'strangles' abscessation	Radiography of the oropharynx/oesophagus
Pharyngeal paralysis:	Endoscopy of the nasopharynx
- guttural pouch infection	Endoscopy of the guttural pouches
- trauma of the head or neck	Neurological examination (see Ch. 14)
- lead poisoning	Lead estimation in: blood (check lab's preferred anticoagulant); liver or kidney, and topsoil
– botulism	Check clinical signs and feedstuff (see Ch. 14)
Hyoid abnormalities	Radiography of the oropharynx
Hepatic encephalopathy	Check blood ammonia (specialist centre); serum enzymes; liver function (see Ch. 4)
Tetanus	Check clinical signs
Grass sickness	Check clinical signs; radiography of the oesophagus (mega-oesophagus and pooling of contrast medium); endoscopy of the oesophagus ('reflux oesophagitis'); ileal biopsy (specialist centres); post-mortem histopathology of the coeliacomesenteric ganglion
Myopathies	Estimation of serum muscle enzymes (see Ch. 13)
Hypocalcaemia	Estimation of serum calcium, magnesium and phosphorus; response to treatment (see Ch. 5)

APPENDIX 2.2 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF DIARRHOEA

Possible cause	Aids to diagnosis
Acute diarrhoea	
Dietary changes	Dietary history
Salmonellosis (fairly common)*	Check clinical signs (colic, pyrexia, toxaemia and leukopenia suspicious); sequential culture of faeces; faecal leukocytes; rectal biopsy for histopathology and culture of homogenized specimen; post-mortem examination of fresh carcase (haemorrhagic inflammation of the caecum and colon/culture of tissues)
Intestinal clostridiosis (uncommon)	Clinical signs and investigation as above for acute salmonellosis; the major differential between all three is the ability to isolate a causative organism; Clostridiosis: high faecal bacterial counts on anaerobic culture (>100 cfu/g faeces); colitis X: no organisms isolated
Potomac fever (Neorickettsia risticii)	Increase in antibody titre between paired serum samples; antigen detection in white blood cells (WBC)
Colitis X (rare)	
latrogenic causes, e.g. antibiotics	History of drug treatment
Poisons, e.g. mycotoxins in feed, acorns in grazing	Check spoilage/adulteration of feedstuffs; grazing history
Endotoxaemia, e.g. acute peritonitis	Clinical signs; check WBC and plasma fibrinogen concentration
Sand colitis	Sandy grazing or muddy streams; sand in faeces
Hyperlipaemia (ponies and donkeys)	Milky opacity to plasma; a metabolic consequence of some other problem (see Ch. 16)
Chronic diarrhoea	
Salmonellosis (fairly common)*	Repeated faecal culture (at least five occasions)
Cyathostominosis (common)	Faecal redworm egg and larvae counts; serum albumin, globulin and beta-globulin estimations; rectal biopsy for histopathology
Malabsorption syndromes associated with various cellular infiltrations (uncommon)	Check serum albumin and ALP (or IAP); oral glucose absorption test (to assess small intestinal involvement); rectal biopsy; full-thickness colonic biopsies under general anaesthesia

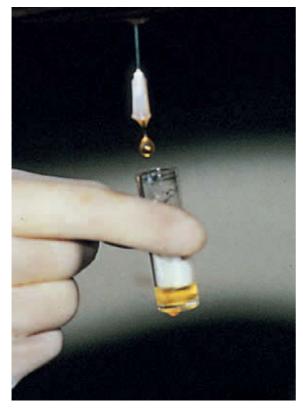
ALP, alkaline phosphatase; IAP, intestinal alkaline phosphatase.

*The clinician should always investigate the possibility of salmonellosis in a diarrhoeic horse.

2



Plate 1 (Fig. 1.1) Various tubes suitable for collecting specific blood samples from horses (see Table 1.1). (Left) Becton Dickinson's Vacutainers. (Right) Sarstedt's Monovettes.



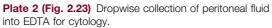




Plate 3 (Fig. 2.24) Huge volume of cellular deposit in the peritoneal fluid obtained from a patient with peritonitis.

CHAPTER

Chronic wasting

Chapter contents

I. Inadequate intake or utilization of feed 73

Inadequate intake in an otherwise healthy horse 73

Inadequate intake due to intrinsic disease in the absence of obvious clinical signs 74

Dental abnormalities 75

Chronic low-grade pain 75

Inadequate utilization of ingested feed 77

II. Increased metabolic rate or requirement for energy 78

III. Organ dysfunction 79

IV. Clinical pathology and chronic wasting 79 Haematology 79

Blood biochemistry 79 Additional tests 80

Further reading 80

The purpose of this chapter is to consider an approach to the investigation of the horse that is losing weight without a readily identifiable predisposing cause. The number of conditions that can result in wasting is extensive, but a methodical approach is likely to produce an appropriate list of differential diagnoses and/or a definitive diagnosis. The basis of this approach is allocating causes of weight loss to one of three categories:

- Inadequate intake or utilization of feed
- Increased metabolic rate or requirement for energy
- Organ dysfunction.

These are the fundamental pathophysiological mechanisms underlying weight loss (Fig. 3.1). However, they are not mutually exclusive and some diseases associated with wasting may embrace all three.

I. INADEQUATE INTAKE OR UTILIZATION OF FEED

This category can be further divided into:

- Weight loss due to inadequate intake *in an* otherwise healthy horse
- Inadequate intake due to intrinsic disease in the absence of obvious clinical signs
- Inadequate utilization of ingested feed.

Inadequate intake in an otherwise healthy horse

Several management factors can result in an inadequate intake by healthy horses and may be revealed during a careful scrutiny of the history and the environment in which the animal is kept. The clinician should consider the following possibilities.

Diagnostic techniques in equine medicine

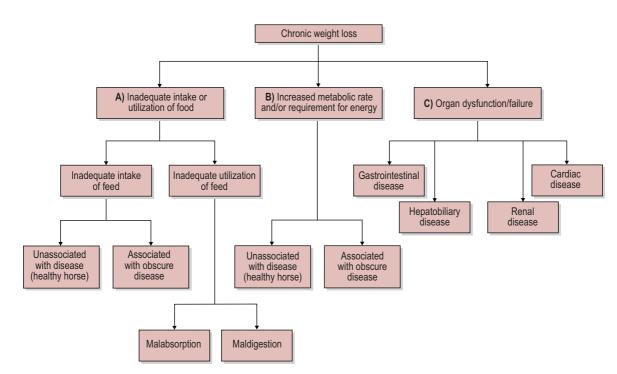


Figure 3.1 Basic algorithm of chronic weight loss in the horse.

- Provision of insufficient food; especially when supplementation is required because of poor grazing or a high stocking density. In addition, increasing workload, cold weather, pregnancy and lactation will increase nutrient demands (see below: 'Increased metabolic rate or requirement for energy'). Infrequent feeding of stabled animals may be insufficient
- Poor quality/unpalatable feed
- Social hierarchy; competition between horses at feeding time, reducing access for the individual.

Inadequate intake due to intrinsic disease in the absence of obvious clinical signs

Disease may prevent the adequate intake of feed by reducing appetite, reducing mobility or causing dysphagia. Such diseases are often obscure and careful observation and investigation of the patient may be required over a protracted period of time. Horses are often distracted and exhibit aroused mentation during visits by a veterinarian, such that subtle signs of disease are obscured. In such circumstances it may be preferable to hospitalize the horse for several days to assess its behaviour and ability to eat. Investigation requires careful observation of behaviour, locomotion, interest in feed, ability to ingest both water and a variety of feedstuffs, and a *thorough clinical examination*. Depending upon the findings, further investigation may require the use of ancillary tests. Potential causes of obscure chronic wasting include:

- Dental abnormalities
- Chronic low-grade pain
- Chronic hepatic disease
- Chronic renal disease
- Chronic cardiac disease
- Chronic low-grade infectious disease
- Neoplasia
- Dysphagia.

These conditions will now be considered in more detail, together with pointers to diagnosis. At appro-

priate points the reader is referred elsewhere in the text for details of the relevant practical techniques.

Dental abnormalities

See 'Examination of the mouth' in Chapter 2: 'Alimentary diseases'.

Chronic low-grade pain

Chronic low-grade pain can cause considerable loss of weight due to a reduction in appetite or an unwillingness to move, or as a result of the neuroendocrine responses to stress. Causes of chronic pain include the following.

Chronic musculoskeletal disease of the axial skeleton or bilateral limb pain

Examples include laminitis (most commonly), osteoarthritis, navicular disease and occasionally myopathies.

Diagnostic aids. If the length of stride is shortened or pain is suspected in both fore- or hindlimbs, a unilateral nerve block will reveal obvious lameness in the contralateral limb (see Ch. 13: 'Musculoskeletal diseases'). Evidence of musculoskeletal disease may warrant the use of nerve/joint anaesthesia, radiography, ultrasonography and more specialized modalities such as scintigraphy, muscle biopsy, computed tomography (CT) or magnetic resonance imaging (MRI), offered by specialist centres.

Chronic abdominal disease

Examples include gastric ulceration, mesenteric abscessation, enterolithiasis, sand colic, neoplasia, chronic low-grade peritonitis, inflammatory bowel disease, chronic grass sickness and endoparasitism. Several of these diseases also interfere with utilization of ingested feed (see below under 'Inadequate utilization of ingested feed'). Clinical signs may be subtle: persistent yawning; stretching of the abdomen; teeth grinding; poor performance; protracted penile prolapse in geldings; increased periods of recumbency; flank watching and occasional pawing. Chronic grass sickness is suspected if patchy sweating, dry rhinitis, intermittent muscle fascicula-

tions, the development of a 'tucked up' abdomen and/or signs of dysphagia are detected.

Diagnostic aids. A thorough colic assessment is essential (see 'Clinical evaluation of the colic patient' in Ch. 2: 'Alimentary diseases'). Abdominocentesis will demonstrate peritonitis but rarely discloses alimentary neoplasia. A serum biochemistry profile may reveal hypoalbuminaemia consistent with a protein-losing enteropathy. If so, an oral glucose absorption test is indicated to determine whether intestinal absorption is compromised. If warranted, intestinal biopsies may be obtained by laparotomy, laparoscopy or transendoscopic duodenal biopsy at specialist centres.

Chronic hepatic disease

Hepatic dysfunction is often associated with weight loss and obvious clinical signs may not be present. In the UK, significant chronic hepatic disease is often the result of ragwort poisoning (pyrrolizidine alkaloid toxicity). Other causes include neoplasia, chronic active hepatitis, cholangiohepatitis and cholelithiasis.

Diagnostic aids. Estimation of serum liver enzyme concentrations and liver function tests (see Ch. 4: 'Liver diseases').

Chronic renal disease

The functional reserve of renal tissue is extensive (75% of renal function is lost before azotaemia occurs) and chronic renal failure (CRF) is rare in horses. CRF may occur subsequent to congenital disease, nephrotoxicosis, pyelonephritis or neoplasia, and is invariably associated with weight loss. Other signs that are often present in horses with CRF are polyuria/polydipsia, dental calculus, inappetence, exercise intolerance and lethargy.

Diagnostic aids. Estimation of blood urea and creatinine concentrations. A state of azotaemia indicates the need for further investigation of renal function (see Ch. 6: 'Urinary diseases').

Chronic cardiac disease

Weight loss in horses with cardiac disease is probably due to a reduced cardiac output associated with an inadequate delivery of nutrients to tissues. Weight loss is usually associated with advanced disease such as decompensated congestive heart failure, endocarditis and pericarditis.

Diagnostic aids. Cardiac auscultation, which may reveal dysrhythmias or murmurs, electrocardiography, echocardiography, estimation of serum cardiac troponin I concentration (see Ch. 9: 'Cardiovascular diseases').

Chronic infectious disease

Horses with chronic infectious diseases frequently lose weight because of increased catabolism and the inhibitory effects of bacterial toxins and inflammatory mediators on appetite. Additionally, chronic pain may be a contributing factor. Associated clinical signs are often absent. Chronic infectious diseases include bacterial diseases (e.g. peritonitis, internal abscessation, lymphadenitis, pyelonephritis, endocarditis and pericarditis), equine infectious anaemia (EIA) and, rarely, systemic fungal diseases (e.g. histoplasmosis, coccidiomycosis, blastomycosis and cryptococcosis). Bacterial infectious disease occurs worldwide but systemic fungal diseases are geographically restricted, predominantly to warm and humid environments. Rarely, infection with Mycobacterium spp., Brucella spp. and Leptospira interrogans serovars can cause weight loss without localizing signs. These pathogens are zoonotic and have implications for human health.

Diagnostic aids. Frequent monitoring of the rectal temperature by competent owners may reveal intermittent pyrexia (>38.2°C), often in association with malaise. Evidence of a chronic and active inflammatory process may be obtained from haematology and raised blood concentrations of fibrinogen or serum amyloid A. Evidence of inflammation on abdominocentesis may localize pathology to the abdomen and ultrasonography of the abdomen may then be helpful. Demonstration of tubercular mycobacteria requires acid-fast staining of suitable biopsy specimens. Unfortunately, tissue lesions only become obvious at an advanced state of disease and intradermal tuberculin testing is unreliable in horses. Specific serology is required to implicate leptospirosis or brucellosis.

Neoplasia

Chronic wasting is a common feature of neoplastic disease, often in the absence of localizing signs. However, wasting may not be obvious if ascites is present because of the expansion of the abdominal contour. Neoplasia can produce a variety of interrelated effects, which result in wasting:

- Reduced/variable appetite
- Dysphagia (see below)
- Malabsorption (see below)
- Chronic/recurrent pain
- Increased metabolic demand and competition for nutrients resulting in net catabolism
- Chronic bleeding/protein loss
- Secondary anaemia.

The most common internal tumour of clinical significance in the horse is lymphosarcoma, which usually takes an abdominal form; either as a diffuse cellular infiltration of the intestine associated with malabsorption (see below under 'Inadequate utilization of ingested feed'), or as a discrete mass acting as a space-occupying lesion – both forms may occur together. Less common manifestations are mediastinal, multifocal, generalized or cutaneous forms. Although rare, a number of other neoplastic diseases can involve body cavities or tissues, resulting in weight loss. These include melanoma, haemangiosarcoma, adenocarcinoma, squamous cell carcinoma, plasma cell myeloma and mesothelioma.

Diagnostic aids. Haematological and serum biochemical examination often reveal non-specific findings of inflammation and occasionally evidence of specific organ involvement. Leukaemias associated with lymphosarcoma are extremely rare in horses, such that haematology is often unhelpful as a diagnosis (see also 'Lymphosarcoma in horses' in Ch. 10: 'Lymphatic diseases').

Dysphagia

Dysphagia is defined broadly as difficulty in prehension, mastication or deglutition. Owners may notice that horses have difficulty in eating or drinking. This may include: prolonged feeding time; dipping and splashing the muzzle in water; dropping feed/water

3

while eating/drinking; nasal discharge of feed/water, and signs of pain associated with these actions. Direct consequences of dysphagia are inadequate food and water intake, resulting in wasting and dehydration. Dysphagia associated with chronic wasting may encompass a diverse number of diseases, which are conveniently categorized as follows.

- *Painful conditions* dental disease, persistent oral/pharyngeal foreign body, inflammation or neoplasia, oesophageal ulceration
- *Obstructive disorders* retropharyngeal lymphadenopathy, neoplasia of the upper alimentary tract, oesophageal disease
- Neuromuscular dysfunction guttural pouch mycosis, temporohyoid osteoarthropathy, botulism, nutritional myodegeneration, equine protozoal myeloencephalitis, rabies, lead toxicity, organophosphate/carbamate toxicity, chronic grass sickness, hypocalcaemia, leukoencephalomalacia, nigropallidal encephalomalacia, brainstem neoplasia, mega-oesophagus.

Diagnostic aids. Examination of the mouth using a gag and light source allows visualization of clinical dental crowns, oral mucosa and the tongue. Radiography may reveal involvement of tooth roots, surrounding bone and the hyoid apparatus. Neurological examination may reveal evidence of dysfunction of the cranial nerves involved in eating (V, VII, IX, X, XII) and other neurological deficits in cases of brainstem or basal nuclei lesions. Endoscopy of the pharynx, larynx and guttural pouches is used to assess the swallowing reflex and structural or functional disease, while examination of the oesophagus allows detection of structural disease (see also 'Endoscopy of the upper alimentary tract' in Ch. 2: 'Alimentary diseases'). Contrast radiography/fluoroscopy is useful in detection of functional and structural disorders of the oesophagus. Serum biochemistry may reveal evidence of rare causes of dysphagia, including hypocalcaemia and myopathy (increased concentrations of aspartate aminotransferase (AST) and creatine phosphokinase (CK)). At specialist centres more advanced modalities such as CT and MRI may contribute to the diagnosis of neurological, osseous and dental diseases.

Inadequate utilization of ingested feed

Malassimilation, collectively referring to *malabsorption* and *maldigestion*, results in impaired utilization of ingested feedstuffs despite a normal or increased appetite. In adult horses, malabsorption is most common, while in foals malabsorption and maldigestion may coexist with mucosal damage and loss of brush-border disaccharidases (especially lactase). Maldigestion is ill defined in adult horses and, in the absence of available laboratory tests, is either rare or underdiagnosed. However, it remains possible that conditions that alter the intestinal mucosa result in impaired nutrient absorption.

Malassimilation may occur with diseases involving the small intestine, large intestine or both. In adult horses, an important distinction is that diseases of the large intestine frequently result in diarrhoea while diseases confined to the small intestine do not. Diarrhoea is an obvious clinical sign and a diagnosis is therefore pursued by investigating the cause of diarrhoea (see Ch. 2: 'Alimentary diseases'). Malassimilation may result from one of more of the following pathophysiological mechanisms.

Diffuse cellular infiltration of the intestinal wall by inflammatory or neoplastic cells (usually lymphosarcoma)

Horses often present with a normal to increased appetite and may demonstrate capricious eating habits. These infiltrative bowel diseases include:

- Alimentary lymphosarcoma
- Inflammatory bowel disease, eosinophilic enterocolitis, lymphocytic-plasmacytic enteritis, granulomatous enteritis, multisystemic eosinophilic epitheliotropic disease, equine granulomatous disease (equine sarcoidosis)
- Avian tuberculosis
- Proliferative enteropathy (Lawsonia intracellularis)
- Alimentary histoplasmosis.

It is important to realize that these diseases are defined by histopathology; in such cases no visible or palpable lesion is likely to appear at laparoscopy, laparotomy or gross post-mortem examination.

Diagnostic aids. Hypoalbuminaemia in the wasting horse, in the absence of renal disease or severe hepatopathy, is suggestive of a protein-losing enteropathy and the absorptive capacity of the small intestine should be tested using an oral glucose absorption test (see 'Tests of intestinal malabsorption' in Ch. 2: 'Alimentary diseases'). Evidence of malabsorption may justify collection of fullthickness intestinal biopsies via laparotomy or laparoscopy. Transendoscopic duodenal mucosal biopsies may be diagnostic in cases where this bowel segment is affected. Some inflammatory bowel diseases (e.g. multisystemic eosinophilic epitheliotropic disease, equine granulomatous disease) often have involvement of other organs (e.g. skin, lung, liver, kidneys, lymph nodes), biopsy and histopathological examination of which may be rewarding.

Deficiency/dysfunction of bile salts

This may impair absorption of fats, but this remains unexplored in horses. However, involvement is plausible with hepatobiliary disease, infiltrative disorders of the ileum (the site of bile salt absorption) and bacterial overgrowth in the small intestine after resection or surgical bypass of the ileum.

Diagnostic aids. Direct determination of fat absorption is not possible. However, hepatobiliary function can be investigated (see Ch. 2: 'Liver diseases').

Endoparasitism

Large burdens of larval and adult strongyles may cause a protein-losing enteropathy and compete for nutrients. Infestation with large strongyles is uncommon since the advent and widespread use of modern anthelmintics. However, infestation with small strongyles (larval cyathastominosis) is a common cause of weight loss and hypoalbuminaemia (and frequently diarrhoea), particularly in young horses.

Diagnostic aids. Detection of larvae in faeces or on a rectal glove after rectal examination is supportive of larval cyathastominosis. However, definitive evidence of larvae in faeces may only be obtained some 48 hours following the onset of larvicidal anthelmintic treatment. Unlike the faecal egg count, larvae are identified by sedimentation of the sample. By itself, the faecal egg count is only useful to detect patent infections and cannot be used to accurately determine the size of the parasite burden. Hypoalbuminaemia is usual in endoparasitism and an inflammatory leukogram may also be present. Serum protein electrophoresis may detect a beta-1 globulin peak in the presence of strongyle infections, but this assay is of low sensitivity.

Immunological causes

Immunological causes of malassimilation in the horse are poorly defined. However, on rare occasion food allergies have been reported in horses and are likely to operate in a similar manner to infiltrative diseases.

Diagnostic aids. Similar to infiltrative bowel diseases. Selected dietary trials can be used in an effort to identify (and avoid) causative feedstuffs.

II. INCREASED METABOLIC RATE OR REQUIREMENT FOR ENERGY

A variety of exogenous and endogenous factors can increase a horse's metabolic rate or energy requirement. If the nutritional intake is not increased accordingly, then wasting results.

Exogenous influences include the nutritional demands of exercise or reproduction (breeding stallions) and the effects of cold ambient temperatures.

Endogenous factors may or may not be associated with disease. Those unassociated with disease are pregnancy and lactation. Examples of diseases that may increase metabolic rate/energy requirements include chronic infectious conditions, neoplasia and those causing chronic pain. Others include endocrinopathies (e.g. pituitary pars intermedia dysfunction (equine Cushing's disease); see Ch. 5: 'Endocrine diseases'), organ dysfunction (see below), and equine motor neuron disease.

Equine motor neuron disease is most prevalent in north-eastern USA and Canada but cases are increasingly being recognized in Europe. Weight loss occurs despite a normal appetite. There is progressive weakness of postural muscles with tremors and fascicula-

3

tion (reminiscent of grass sickness), particularly at rest. Serum muscle enzyme concentrations (CK and AST) may be elevated but definitive diagnosis requires histopathological examination of a biopsy of the medial tail head muscle (sacrocaudalis dorsalis medialis) or the ventral branch of the spinal accessory nerve. In addition, a mosaic pattern of pigment deposition involving the retina may be present; however, this is a non-specific finding. Chronic vitamin E deficiency is a predisposing factor.

III. ORGAN DYSFUNCTION

Dysfunction or failure of organs that are associated with nutrient metabolism or transport can result in weight loss, often with few localizing signs. Examples include hepatic, renal, cardiac and chronic gastrointestinal disease. As indicated earlier in this chapter, these diseases may also be associated with reduced feed intake.

IV. CLINICAL PATHOLOGY AND CHRONIC WASTING

Heavy reliance on the use of laboratory tests is often tempting when faced with chronic weight loss featuring vague and non-specific signs. However, such an approach can be expensive and unrewarding. Initial laboratory investigations are usually haematology and blood biochemistry. Further clinicopathological tests are then selected according to results of these tests, combined with information from the clinical findings and history.

Haematology

Anaemia is common in horses with chronic wasting and may be associated with:

- Chronic infection a mild to moderate anaemia caused by reduced erythrocyte life span and reduced erythropoiesis (dyserythropoiesis)
- Neoplasia associated with infiltrative disease of the bone marrow and non-regenerative anaemia

- Blood loss associated with neoplasia or inflammatory lesions
- Immune-mediated haemolysis associated with neoplasia or infection
- Inadequate erythropoietin production associated with chronic renal failure (rare).

Leukocytosis featuring a mature *neutrophilia* and *monocytosis* is typical of chronic infectious and noninfectious conditions (e.g. neoplasia, trauma and immune-mediated diseases). Further evidence of a chronic and active inflammatory process is provided by increased concentrations of the acute phase proteins fibrinogen and serum amyloid A (see Ch. 1: 'Submission of samples and interpretation of results').

Blood biochemistry

Globulin concentrations are frequently increased in a variety of septic and non-septic conditions:

- Chronic infection
- Neoplasia
- Immune-mediated disease
- Hepatopathy
- Endoparasitism.

Globulin fractions can be further differentiated using *serum protein electrophoresis*: an increase in the beta-1 globulin concentration is suggestive of larval cyathostominosis; polyclonal increases in gamma globulins (polyclonal gammopathy) may be found in chronic inflammatory conditions or hepatopathies, while a large monoclonal gammopathy is suggestive of a plasma cell myeloma (rare).

Hypoalbuminaemia usually reflects a proteinlosing enteropathy due to parasitism, alimentary lymphosarcoma or idiopathic infiltration of the gut wall with inflammatory cells. Occasionally, hypoalbuminaemia may result from effusive disorders (e.g. pleuritis, peritonitis) and on rare occasions it is associated with a protein-losing nephropathy. In cases of hypoalbuminaemia where parasites have been ruled out (faecal analysis, serum protein electrophoresis and aggressive larvicidal anthelmintic treatment) and there is no evidence of liver or kidney disease, then an oral glucose absorption test

is indicated to assess absorption in the small intestine (for technique and interpretation see Ch. 2: 'Alimentary diseases').

Mild increases in urea concentration may reflect tissue catabolism, exercise, a high protein diet, dehydration or early renal dysfunction. More substantial increases reflect renal failure. *Creatinine* is a more specific indicator of azotaemia and should be estimated with urea. Further investigation of renal disease includes urinalysis, renal ultrasonography and biopsy (see Ch. 6: 'Urinary diseases').

Indicators of hepatobiliary disease include increases in *hepatocellular enzymes* (glutamate dehydrogenase (GLDH) and AST) and *biliary enzymes* (gamma glutamyltransferase (GGT), alkaline phosphatase (ALP)). In such cases the *serum bile acid* concentration should be requested in order to assess liver function (see also Ch. 4: 'Liver diseases').

Muscle enzyme concentrations (*CK* and *AST*) are raised in all forms of myopathy.

Additional tests

Abdominocentesis may provide evidence of an abdominal inflammatory condition and on rare occasions neoplasia (although most intraabdominal neoplasms are non-exfoliative).

The *oral glucose absorption test* is a simple test of the absorptive capacity of the small intestine. Reduced absorption is expected in diffuse infiltrative disorders, including chronic idiopathic inflammatory bowel conditions and lymphosarcoma (see above under 'Hypoalbuminaemia').

A *faecal egg count* may provide supportive evidence of patent endoparasitism but it will not reflect the burden of encysted parasitic larvae (particularly larval cyathostomins). Cyathostomin larvae may be detected in the faeces during rectal examination or subsequent to larvicidal anthelmintic treatments.

Serology can be used to support a diagnosis of equine infectious anaemia, *Lawsonia intracellularis* proliferative enteropathy and, rarely, brucellosis and leptospirosis.

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CHAPTER

Liver diseases

Chapter contents

I. Clinical pathology 81 Blood biochemistry 82 Liver enzymes 82 Indicators of liver function 83 Total serum bile acids 83 Serum bilirubin 84 Serum proteins 84 Blood glucose 84 Blood ammonia 84 Hyperlipaemia 85 Haematology 85 Liver fascioliasis 85

II. Practical techniques 85

Ultrasonography of the liver Liver biopsy Instruments Site of biopsy Procedure Complications of liver biopsy

Further reading 88

I. CLINICAL PATHOLOGY

Liver disease is relatively common in the horse, but often occurs in the absence of specific clinical signs and sometimes in the absence of any clinical signs. It is usually diagnosed on the basis of a serum biochemistry profile and for this reason clinicopathological techniques are considered first in this chapter. Any horse with an obscure, non-specific history of malaise, lethargy, inappetence and/or weight loss should be screened for the possibility of liver disease.

The liver has great powers of regeneration and the more overt clinical signs associated with its failure do not appear until some 70–80% of the functional capacity is lost. *Obscure signs of liver disease are there-fore much more common than overt signs of liver failure.* Signs of failure include central disturbances and are

usually acute, even if the underlying liver disease has developed over a protracted period. This 'hepatic encephalopathy' is associated with toxic levels of ammonia and intestinally derived neurotoxic metabolites that would normally be detoxified by the liver.

Acute liver disease can present as a mild, reversible condition of non-specific malaise with depression and occasionally colic. In acute disease with liver failure a severe encephalopathy develops rapidly. There may be defective vision, head pressing, ataxia, compulsive walking and, at the extreme, hyperexcitability with mania.

Chronic liver disease often features an obscure subclinical malaise with mild anaemia and weight loss over many months. This may be punctuated by acute episodes of more obvious depression and mild encephalopathy. With progressive deterioration, the condition can terminate in a severe depressive encephalopathy, without the excitability of acute failure.

In all forms, there is occasionally an associated photosensitization of the white or flesh-coloured areas. This is caused by the increased circulating concentration of phylloerythrin, a photodynamic derivative of chlorophyll, which is normally excreted by the liver. Any horse showing signs of photosensitization should be screened for the possibility of liver disease.

As will be seen, it is relatively easy to define a state of liver disease (hepatopathy) but it is difficult and often impossible to identify the precise causative agent. Although the pathology of the condition can be defined in histopathological terms, it is rarely indicative of the exact cause. In consequence, the associated history is of particular diagnostic importance in that it may implicate potential agents. Causes of liver disease in horses include the following.

- *Plant hepatotoxins* are probably the commonest recognized cause. These plants are rarely eaten at pasture but the toxic principles survive the haymaking process and they are consumed with the hay. The best known is ragwort, but many other pasture plants contain hepatotoxic principles.
- Other food toxins. Mouldy feeds containing aflatoxins are potential causes.
- *Infections* are an occasional cause. Chronic active bacterial hepatitis can result in chronic neutrophilic cholangitis, multiple liver abscessation or even (rarely) cholelithiasis. Viral infections are often implicated in outbreaks of lymphoplasmacytic hepatitis, but rarely proven.
- *Parasitism* is rarely a problem in the adult horse liver. Occasionally, liver fluke may be acquired from infected grazing.
- *Haemosiderin deposition* is often found in liver biopsy samples and may contribute to oxidative damage in hepatocytes. Excessive dietary iron supplementation may be a potential concern in such cases.

- Fatty infiltration can occur secondary to the development of hyperlipaemia (see below).
- Serum hepatitis (Theiler's disease) is rarely reported as an acute liver failure associated with the previous administration of an equinederived biological, usually an antiserum.
- *Tumours of the liver* such as biliary carcinoma or lymphosarcoma are rare.

Blood biochemistry

Liver enzymes

Laboratory evidence of liver disease is substantially based on enzyme release from damaged hepatocytes. These enter the circulation and are measured in the serum or plasma. Enzymes may be subdivided according to their primary source: biliary or hepatocellular. Comparison of the magnitude of the increases seen in these two subclasses of enzymes may suggest the site of primary pathology. However, cases are rarely seen where the increased serum enzyme concentrations are absolutely restricted to one of these two groups.

Biliary enzymes

Increased circulating concentrations of the so-called 'biliary enzymes' suggest biliary tract disease and/or biliary hyperplasia as a secondary response to hepatocellular disease. The most dramatic increases in these enzymes are usually found in association with true primary biliary tract disease.

Gamma glutamyltransferase (GGT or γ GT). This enzyme is the most sensitive indicator of liver disease. It is persistently elevated in both acute and chronic liver disease and reflects damage to the biliary tract. However, mild to moderate increases may sometimes be seen in horses in which a diagnosis of liver disease is not confirmed by subsequent biopsy. The reason for this lack of specificity is not clear; it may reflect a non-biopsied focal liver injury or the release of the enzyme from another source, e.g. pancreas or renal tubules.

Serum alkaline phosphatase (SAP or ALP). This enzyme is predominantly associated with the biliary system and is therefore released in conditions of hepatobiliary insult. Unfortunately, it is not liver-

4

specific and is also released during bone and gut (brush border) damage. It is often high in normal, young, growing horses.

Hepatocellular enzymes

Several enzymes are primarily associated with hepatocytes rather than biliary epithelial cells and include arginase, aspartate aminotransferase (AST or AAT), glutamate dehydrogenase (GLDH), sorbitol (iditol) dehydrogenase (SDH/IDH) and lactate dehydrogenase (LDH). Relatively higher increases in these enzymes, compared to biliary enzymes, suggest primary hepatocellular injury.

Aspartate aminotransferase (formerly GOT – glutamine oxaloacetate transaminase) is released early in cell disruption and clears slowly from the circulation. Increased AST is very sensitive for liver disease, but it is not liver-specific because it is also released on disruption of other soft tissues such as skeletal and cardiac muscle. In cases of uncertainty, a cross-check on the serum concentration of a muscle-specific enzyme, most conveniently *creatine phosphokinase* (CPK or CK), will indicate whether or not the likely origin is muscle.

Sorbitol dehydrogenase is liver-specific and is released early after hepatocyte insult. It has a short half-life and therefore declines once the damage ceases to be progressive. Unfortunately, it is not stable in blood and the assay must be undertaken as soon as possible after sampling; certainly within 24 hours. Its serum activity is halved in 2–3 days at ambient temperature.

Glutamate dehydrogenase is another liver-specific enzyme with a short serum half-life whose presence indicates current damage. It is more popular than SDH because of its greater stability ex vivo. Significant increases may be seen with relatively minor liver pathology.

Comments

• In screening for evidence of liver insult using a serum biochemistry profile, it is not necessary to request analysis of all the enzymes listed above. The authors recommend the use of one hepatocellular and one biliary enzyme to signal disease – e.g. AST and GGT. Very few horses

with significant liver disease have both AST and GGT within reference ranges.

- Lactate dehydrogenase (LDH) is usually raised in liver disease. However, it is widely distributed in all tissues and specific evidence of a liver origin requires further estimation of its liverderived isoenzymes LDH₄ and LDH₅ (see Ch. 1, under: 'Serum enzymes').
- Although analysis of liver-derived enzymes is an important part of the diagnostic evaluation of suspected liver disease, results are never absolutely sensitive, nor specific, and should always be interpreted with a degree of caution.

Indicators of liver function

Substances whose serum concentrations depend upon normal hepatic function offer additional information when investigating hepatopathies, because abnormal results imply compromised liver function or at worst failure.

Total serum bile acids (TSBAs)

Bile acids are synthesized and conjugated with amino acids in the liver and excreted in the bile. They are important for the digestion of dietary fats and the absorption of fat-soluble vitamins. Following excretion they are largely reabsorbed from the gut, taken up by the liver and re-excreted in the bile, thus undergoing an 'enterohepatic cycle' (Fig. 4.1). A small portion enters the peripheral circulation after reabsorption and can be measured as the total serum bile acid concentration.

Most cases of liver disease will have normal serum bile acids. In cases of liver failure however, the take up and re-excretion of bile acids is reduced, resulting in higher circulating levels of TSBAs. The estimation of TSBAs from a single blood sample therefore provides a liver function test. It also allows liver function to be monitored routinely during disease and treatment.

Comments

- TSBAs will be raised (but not grossly elevated) by fasting or inappetence.
- Poor liver circulation will increase TSBAs.

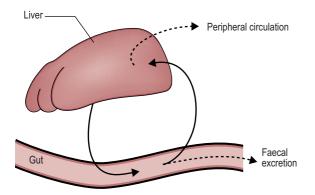


Figure 4.1 The enterohepatic cycle of bile acids. In liver malfunction the circulating concentration of bile acids is increased.

Serum bilirubin

Total serum bilirubin is not usually elevated in equine hepatopathies. An increase may be diagnostically useful, but normal values do not rule out liver disease.

On those occasions where jaundice does occur in association with equine hepatopathy, the greater part of serum bilirubin is always unconjugated, irrespective of the underlying pathology. Comparisons of the relative concentrations of direct (conjugated) and indirect (unconjugated) bilirubin, using the classic Van den Bergh reaction, are less useful in classifying jaundice in horses than in other species.

Increases in total bilirubin are also seen in a variety of equine diseases that are unrelated to primary liver disease, such as haemolysis, impaction colic and any condition associated with reduced food intake. In fasting (or inappetence) there is a physiological decrease in the removal of bilirubin by hepatocellular transport. *Anorexia, for whatever reason, is the commonest cause of jaundiced membranes in the horse.*

Serum proteins

Serum protein analysis is often very useful in the assessment of liver disease. Serum albumin is derived from hepatic synthesis and may be found to be low in some cases of chronic liver failure. However, hypoalbuminaemia is rare in equine liver disease and, when present, tends to be only mild to moderate (rarely <20 g/l). In contrast, hyperglobulinaemia is seen more frequently, so that reduced total protein (hypoproteinaemia) is very rare in equine liver failure. The exact cause of hyperglobulinaemia is unclear, but may relate to systemic immune stimulation from intestinally derived antigen following the loss of protective Kupffer cells. Significant hyperglobulinaemia is a negative prognostic indicator in cases of liver failure.

Blood glucose

Hypoglycaemia occasionally develops in conditions of liver failure and requires therapeutic support. The situation is aggravated by inappetence and a loss of efficient gluconeogenesis by the liver. Hypoglycaemia contributes to hepatic encephalopathy, the central disturbance associated with liver failure. However, many cases of equine liver failure (with or without encephalopathy), are actually found to be hyperglycaemic as a result of insulin resistance and stress.

Blood ammonia

Because detoxification (conversion to urea) is impaired as a consequence of liver failure, there may be an increase in the circulating concentrations of intestinal amines and ammonia (the products of protein degradation in the gut). These are associated with the development of encephalopathy. However, hyperammonaemia is poorly correlated with clinical signs of encephalopathy. This is probably explained by variable blood–brain barrier permeability to ammonia in hepatic encephalopathy cases.

Blood ammonia is the most convenient for routine laboratory estimation, but unfortunately it is labile and requires collection into anticoagulant (EDTA) for rapid plasma separation, followed by prompt laboratory analysis (within 2 hours). Where facilities are available, serial estimations of blood ammonia can indicate an impending encephalopathy and also provide a sensitive monitor of the response to treatment.

Hyperlipaemia

Hyperlipaemia is a metabolic disease in which there is increased mobilization of fat deposits in response to some form of stress or nutritional deprivation. It is most often seen in female ponies and donkey mares and is frequently associated with non-specific illnesses or inadequate nutrition during late pregnancy. An increase in serum liver enzyme concentration may reflect either a cause or effect of hyperlipaemia. Thus, hyperlipaemia may be triggered by a primary hepatopathy. Alternatively, fatty liver disease is often a secondary effect of hyperlipaemia.

Evidence of hepatopathy associated with any disease process, or with advanced pregnancy in inappetant ponies and donkeys, should raise the suspicion of intercurrent hyperlipaemia. The condition is diagnosed by an abnormal increase in serum triglyceride concentration (>5 mmol/l), which produces a milky discoloration of the plasma. The latter is readily apparent to the naked eye if a blood sample is allowed to stand and settle (see 'Hyperlipaemia' in Ch. 16: 'Fat diseases').

Haematology

Haematology provides no specific information in cases of liver disease. Anaemia may accompany chronic disease and patients suffering with neoplasia or bacterial infection, as in cholangitis or abscessation, will show leukocyte shifts and an increase in plasma fibrinogen and serum amyloid A concentrations.

In advanced liver failure there is a decrease in the production of coagulation factors and a consequent increase in clotting time. In practical terms, the clinician is more likely to appreciate significant clotting defects as petechiation of the mucosae, or haematoma formation following venepuncture, than by pursuing elaborate coagulation tests.

Liver fascioliasis

In very rare instances horses are known to be parasitized by the sheep/cattle fluke *Fasciola hepatica*. The longevity of the adult fluke could mean that infected horses suffer low-grade hepatitis and associated poor performance for several months after removal from the source of infected grazing. However, in an abnormal host the parasite does not develop to full patency and few (if any) eggs are produced in the faeces.

As proof of infection, the conventional faecal analysis requires adaptation to detect a low number of eggs in a large amount of faeces. In most instances this is not practical. Where there is circumstantial evidence in the history of access to fluke-infested pastures, it is advisable to treat the horse for fascioliasis (e.g. triclabendazole 15 mg/kg per os) and assess the clinical/clinicopathological response.

II. PRACTICAL TECHNIQUES

Clinical examination and, in particular, clinical pathology can establish whether or not a state of hepatopathy is likely to exist, but in most instances the cause, severity and choice of appropriate therapy are unclear. Ultrasonography and liver biopsy may help to provide aetiological information - but not invariably. However, they will provide information concerning the severity and prognosis of the lesion and may help to guide the choice of specific therapy. They also establish a comparison for future examinations. It is not unusual to find reassuring biopsy results in cases with marked increases in serum liver enzyme concentrations. Equally, biopsy results of concern may be found in cases with only mild to moderate increases in liver enzyme concentrations. Biopsy is therefore indicated early in the course of almost any case in which increased liver enzymes are found. Delaying biopsy carries the risk of potentially treatable early disease progressing to liver failure with an inevitably poorer prognosis.

Ultrasonography of the liver

In normal adult horses the bulk of the liver is situated on the right side of the abdomen but it may be 'scanned' via the intercostal spaces of both sides by percutaneous ultrasonography. A variable frequency transducer, in the range 2–6.0 MHz, is suitable for liver ultrasonography and a sector scanner, with a small transducer/patient contact area, is preferred because of the restricted access between ribs.

On the left side, the liver is found ventral to the lung margin extending from the diaphragm in a caudal direction over several rib spaces to where it lies adjacent to the spleen (somewhere between the 7th and 12th intercostal spaces). On the right side, the liver is found ventral to the lung margin extending from the diaphragm to the level of the right kidney (somewhere between the 7th and 15th intercostal spaces). The exact size and position is variable and alters with age, bodily condition and breed. Careful skin preparation is essential for high-quality percutaneous ultrasonographic images and in most cases this involves clipping the hair, cleansing the skin with povidone-iodine and then degreasing with surgical spirit before applying coupling gel. Satisfactory diagnostic images can usually be obtained in thin-skinned horses by simply soaking the haircoat with surgical spirit.

Ultrasonography of the liver should include an assessment of the following:

- *The volume and nature of the surrounding peritoneal fluid*. Increases in peritoneal fluid cause displacement of the liver away from its normal close contact with the body wall and deeper viscera. The echogenicity of the fluid increases with its turbidity and cellularity.
- *The overall size of the liver*. Liver size is very variable in horses. However, a subjective assessment of overall size can be made by considering the area of surface contact between the liver and body wall relative to the animal's size, and the depth of tissue that is present over this area. Typically, approximately 10 cm of liver will project ventrally to the expiratory lung border in the right 13th intercostal space.
- The capsular surface of the liver and the angle of its ventral margin. The healthy liver has a capsule, which provides a smooth and sharply defined border, with an acute (rather than rounded) angle at its ventral limit.
- *The texture of the hepatic parenchyma*. Healthy hepatic parenchyma has a uniform echogenicity

interspersed with blood vessels. Variations in overall echogenicity may result from changes in transducer/patient contact, transducer frequency and control settings, or hepatic disease. Experience with a standard examination protocol will allow a subjective assessment of hepatic echogenicity to be made. Diffuse increases in echogenicity may result from fibrosis, lipidosis, haemosiderosis or cellular infiltration. Focal changes in echogenicity are more readily appreciated and may result from liver abscessation, hydatid cysts, cholelithiasis or neoplasia. The appearance of these abnormal areas may suggest an aetiology and usually enable a 'targeted' biopsy to be obtained.

• The appearance of the hepatic vasculature. Images of hepatic veins and hepatic portal veins can be identified. The latter tend to have more echogenic borders. Alterations in the size or shape of these vessels can be appreciated readily and usually reflect other (e.g. cardiovascular) disorders. In the peripheral liver, blood vessels should rarely be greater than 8 mm diameter.

Liver biopsy

Most lesions that afflict the horse liver are diffuse, so that a biopsy usually provides a representative sample for histopathology. The severity of the lesion is usually apparent, which may help in terms of prognosis, but the precise cause is not always revealed. Exceptions are cases where the pathology is characteristic, e.g. the hepatomegalocytosis of ragwort poisoning. Possible contraindications for biopsy are evidence of concurrent coagulopathy, or suspicion of liver abscessation, but these risks are largely overcome by ultrasound guidance, making it a very safe procedure.

Instruments

Several medical biopsy instruments are suitable for the purpose. Automatic, spring-loaded 14 gauge disposable biopsy needles (e.g. Cook, Ranfac) retrieve good specimens and are easy to use. Most biopsies are taken at a 5–10 cm depth from the skin surface and so a 15–20 cm needle length is suitable for most horses. Biopsy guides can be purchased for most transducers, which maintain the biopsy needle in the plane of the ultrasonographic image and facilitate correct needle direction. However, they are not essential for satisfactory biopsy collection.

Site of biopsy

Liver biopsies should ideally be taken under ultrasonographic guidance to limit the possibility of adverse effects and maximize the likelihood of collecting a diagnostic biopsy. If ultrasound is available, the optimal site for biopsy will be shown by the liver image and may be taken from the right and/or left sides. In the absence of ultrasound the approach is the same for all instruments. A site is selected in the 13th intercostal space on the right hand side, just in front of the 14th rib, midway within a wedge whose upper and lower limits are delineated respectively by imaginary lines drawn from the point of the hip to the point of the shoulder and from the point of the hip to the point of the elbow (Fig. 4.2). It should be emphasized that in some cases a biopsy will be unsuccessful because of absence of liver tissue at the expected anatomical location.

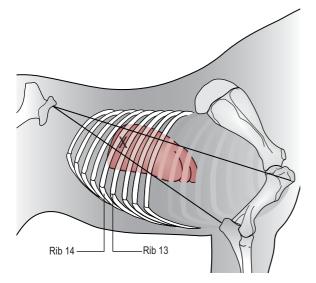


Figure 4.2 Landmarks indicating a suitable site for liver biopsy in the absence of ultrasound guidance.

Procedure

- An area 10 × 10 cm is clipped and surgically prepared at the chosen site.
- Depending upon temperament, the horse will usually require to be sedated.
- Using sterile precautions the skin and intercostal muscle beneath are infiltrated down to the parietal pleura with 4–5 ml of local anaesthetic using a 1.5 inch × 21G (39 × 0.8 mm) needle.
- A 5 mm stab incision is made in the skin just in front of the 14th rib. The biopsy needle is introduced through the incision, into intercostal muscle and then directed some 10° backwards to pass through the lung and diaphragm. If it is inserted at the point of full expiration, the amount of lung involved is minimized. It is possible to feel the diaphragm 'pick up' on the instrument as it passes through. If relaxed from the operator's grip, the instrument will now be seen to move with the respiratory excursions of the diaphragm.
- The biopsy needle is advanced 5 cm or so through the diaphragm and into the liver, which has a 'solid' feel, and at this point the instrument is operated. At withdrawal the core of tissue should be dark in colour and sink in fixative. If the first attempt yields nothing (or a pale tissue that does not readily sink), two further attempts may be made through the same incision, redirecting the needle slightly and maintaining sterile precautions. If there is clinical/ clinicopathological evidence of liver infection, a sample (or part of a sample) should be submitted for culture in a sterile container.
- A single interrupted suture is placed in the wound, although a dab of wound powder/spray is often sufficient for such a small incision. The horse is rested for at least 1 hour to permit clotting within the biopsy tract.
- If unsuccessful, it is possible to repeat the procedure at a different site, preferably after 24 hours. Using a 'blind' procedure it is advisable to try one intercostal space further back, but in older horses atrophy may cause the liver to be

drawn forward. The advantages of progressing to an ultrasound-guided biopsy are obvious: passage through the diaphragm and lung tissue can be avoided and the biopsy needle can be visualized throughout its manipulation into the site selected for biopsy.

- The patient's tetanus status should be assessed and the appropriate action taken.
- A single dose of phenylbutazone (or other non-steroidal anti-inflammatory drug) may be given as a routine analgesic, although clinical evidence of discomfort following liver biopsy is very rare.

Complications of liver biopsy

These are very rare. Tissues other than liver (e.g. diaphragm, lung, colon) may be inadvertently sampled without undue effect. However, if the core of tissue obtained does not have the 'feel', colour or texture of liver, it is advisable to give a short course of antibiotics in case of bowel penetration.

Haemorrhage may occur into the abdomen or thorax. Serious haemorrhage is a very rare complication of liver biopsy, even in advanced disease. Where there is evidence of extended clotting times, such as haematoma formation following venepuncture, an estimation of bleeding time may be helpful (see 'Coagulation tests' in Ch. 8: 'Blood disorders'). The use of ultrasound guidance avoids large intrahepatic vessels.

Comments

• On occasion, normal tissue will be obtained at biopsy. Depending upon the clinical and

clinicopathological circumstances, this may offer reassurance regarding the horse's condition or might possibly suggest a discrete liver lesion such as abscessation, fascioliasis or (rarely) neoplasia. In such circumstances repeat biopsy is indicated in 1–3 months' time if serum liver enzymes are found to be persistently elevated. In these cases biopsy is preferably ultrasoundguided and should be undertaken at a different site from the original biopsy.

• The ultrasound-guided technique offers the opportunity to biopsy the liver from the left side of the horse. Blind biopsy of the left side is not recommended because of the close proximity of the left ventricle.

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CHAPTER

Endocrine diseases

Chapter contents

Equine Cushing's syndrome: pituitary pars intermedia dysfunction (PPID) 89 Clinical recognition of PPID 89 Diagnosis of PPID 89 Equine metabolic syndrome/insulin resistance 91 Clinical recognition of EMS 92 Diagnosis of EMS 92 Adrenocortical insufficiency 93 Clinical signs 93 Diagnosis of adrenocortical insufficiency 93 Phaeochromocytoma 94 Clinical signs 94 Diagnosis of phaeochromocytoma 94 Polyuria/polydipsia in the horse 94 Psychogenic polydipsia and PU/PD 94 Renal disease and PU/PD 95 Diabetes insipidus and PU/PD 95 Diabetes mellitus and PU/PD 96

Equine Cushing's syndrome: pituitary pars intermedia dysfunction (PPID)

Excessive salt consumption and PU/PD 96

Equine Cushing's syndrome is probably the most commonly recognized equine endocrinopathy and results from age-associated oxidative damage to dopaminergic neurons in the periventricular aspects of the hypothalamus. These neurons normally serve to inhibit the secretion of pro-opiomelanocortin (POMC)-derived peptide hormones by melanotropes in the pars intermedia of the pituitary gland. These hormones include adrenocorticotrophic

Thyroid disorders 96

Thyroid gland enlargement Hypothyroidism Congenital hypothyroidism Hyperthyroidism Diagnostic evidence of thyroid dysfunction in horses **97**

Disorders of calcium metabolism 97

Hypocalcaemia 98 Hypocalcaemic tetany 98

Hypoparathyroidism and hyperparathyroidism in the horse 98

Hypoparathyroidism 99 Hyperparathyroidism 99

Endocrinopathies of the reproductive tract 99

Granulosa cell tumour 99 Cryptorchidism 99

Further reading 100

hormone (ACTH, corticotropin), beta-endorphin, alpha-melanocyte stimulating hormone and corticotropin-like intermediary peptide. The loss of normal inhibitory dopaminergic influence on melanotropes leads to both POMC-peptide hypersecretion and clonal expansion of melanotropes. The clinical abnormalities by which equine Cushing's syndrome is recognized may be attributable to both the effects of excessive melanotrope-derived POMC peptides and physical encroachment of the enlarging pars intermedia on adjacent structures.

It is important to recognize that the resultant endocrinopathy follows the combined effects of all the POMC-derived peptides and not just ACTH. This situation is different from that in other species, in which pituitary-dependent Cushing's disease results simply from ACTH hypersecretion by corticotropinomas in the *pars ventralis*. Therefore, the term 'pituitary pars intermedia dysfunction' (PPID) more accurately describes the equine condition than the traditional reference to 'Cushing's disease'.

Clinical recognition of PPID

Horses affected with PPID tend to develop a characteristic physical appearance. PPID should be suspected in older horses and ponies that are presented for ill thrift, laminitis, polyuria/polydipsia (PU/PD) and a failure to shed their winter hair coat in the spring and/or inappropriate hirsutism (Fig. 5.1). Indeed, inappropriate hirsutism is widely regarded as pathognomonic for PPID. The underlying mechanism is unknown; excessive circulating cortisol or melatonin and pressure on the hypothalamic thermoregulatory centre by an enlarged pituitary have been implicated. In early cases, long hairs under the mandible and at the palmar and plantar aspects of the lower limbs may be suggestive of PPID, even if the remainder of the hair coat has been shed appropriately.

Affected animals tend to lose skeletal muscle mass and acquire a characteristic regional distribution of body fat similar to that described below for equine metabolic syndrome. Chronic laminitis is readily apparent in many affected horses and ponies and has been reported to be the most common clini-

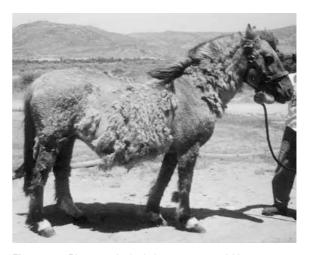


Figure 5.1 Photograph depicting a 22-year-old horse affected with pituitary pars intermedia dysfunction. Notice the unusual appearance of the hair coat and that it had failed to shed out completely and appropriately during the spring.

cal sign in these animals. Other clinical signs that may be presented include inappropriate sweating (hyperhidrosis), lethargy, infertility, inappropriate lactation in females, blindness, seizures and miscellaneous chronic infections resulting from immune compromise. Although most commonly diagnosed in horses and ponies older than 18 years of age, PPID has been reported in younger horses, but rarely under 7 years old.

Diagnosis of PPID

Routine clinicopathological findings in horses and ponies affected with PPID are non-specific. A stress leukogram (mature neutrophilia with mild-tomoderate lymphopenia) may be present. Hyperfibrinogenaemia and mild anaemia may also be evident in those patients affected by a chronic inflammatory disease. Plasma biochemical abnormalities may include mild-to-moderate hyperglycaemia, hyperlipidaemia and mildly elevated plasma hepatic enzyme activities.

Endocrine tests

Although pharmacological management of PPID is readily available in the form of orally administered

dopaminergic agonists (such as pergolide and cabergoline), it is expensive and consequently specific endocrine testing may help to justify the cost of treatment for the remaining life of the horse. However, although various endocrine tests have been proposed for the diagnosis of PPID there is no single unequivocal test at present.

The 'overnight dexamethasone suppression test' is easily accomplished in clinical practice and is often regarded as the 'gold standard' for making a clinical diagnosis of PPID. Following the collection of a plasma sample for baseline cortisol estimation, a low dose of dexamethasone (40 g/kg by intramuscular injection) is administered at 5:00 pm and the plasma cortisol concentration is determined at approximately 10:00 am the next morning. In healthy horses, plasma cortisol concentration is suppressed to less than 1 g/dl at 17-19 hours following dexamethasone treatment. Failure to demonstrate a plasma cortisol concentration of less than 1 g/dl 17–19 hours following dexamethasone treatment supports a clinical diagnosis of PPID.

An elevated resting plasma ACTH concentration also supports a diagnosis of PPID. However, plasma ACTH concentration alone can yield false-negative results. There are also seasonal effects: false-positive results are believed to be more likely during the fall/ autumn in North America. It is important to note that ACTH determination requires careful sample handling: the plasma must be quickly separated from the blood and the sample must be submitted on ice. It is always advisable to check in advance the shipping and handling requirements for a particular laboratory.

There is increasing interest in North America in the domperidone challenge test for diagnosis of PPID in horses. Domperidone is an orally administered dopamine (D_2) receptor antagonist that has no effect upon the plasma ACTH concentration of healthy horses. In PPID-affected horses, treatment with domperidone causes a significant elevation of the plasma ACTH concentration. This effect has been attributed to the possibility that domperidone neutralizes residual dopaminergic inhibition in hypertrophied melanotropes, leading to an elevation in the plasma ACTH concentration. The plasma cortisol concentration is measured before and 4 hours after a single oral dose of domperidone (2.5 mg/kg). Diagnosis of PPID is supported by a significant increase in plasma cortisol concentration (two- to threefold) at 4 hours. Such increases are not observed in unaffected horses. Preliminary results indicate that the domperidone challenge test is practical and safe. This test promises high diagnostic accuracy but further work on a larger number of normal and PPID-affected horses is required to determine its sensitivity and specificity.

The demonstration of insulin resistance in horses affected by PPID may provide specific guidance to veterinarians regarding the type of treatment (e.g. dietary adjustments) and the subsequent prognosis for their patients. Long-term survival is poor in patients where hyperinsulinaemia is also identified at the outset of treatment. Moreover, it is likely that the presence of insulin resistance further predisposes these patients to laminitis.

Equine metabolic syndrome/insulin resistance

Equine metabolic syndrome (EMS) is characterized by an increased risk of laminitis resulting from the development of obesity. Insulin resistance appears to be central to the risk of laminitis. The term 'equine metabolic syndrome' is controversial because there are no agreed diagnostic criteria and further work is needed to better define the syndrome. However, the name is pragmatic in order to distinguish it from both PPID and suspected hypothyroidism, conditions with which it is often confused.

Currently EMS describes horses and ponies with evidence of obesity, insulin resistance and laminitis (clinical evidence of laminitis, physical appearance of the hoof and/or radiographic evidence of laminitis). Despite there being physical evidence of laminitis in some EMS-affected horses, the owner may report that lameness has not been apparent. Structural changes in the hoof-lamellar interface may therefore occur in the absence of laminitic pain.



Figure 5.2 Obesity typical of the equine metabolic syndrome.

Clinical recognition of EMS

Equine metabolic syndrome should be considered in horses and (especially) ponies that are presented for laminitis or found to be obese (Fig. 5.2). Some breeds appear to be predisposed: ponies, miniature horses and the Morgan, Peruvian Paso and Paso Fino breeds. Thoroughbreds and Standardbreds appear to be at less risk. Although generalized obesity suggests a state of insulin resistance, some insulin-resistant horses develop regional thickening in the crest ('cresty neck') and expanded subcutaneous adipose tissue at the base of the tail, in the prepuce, in the supraorbital fossae and near the shoulders (Fig. 5.3). Horse owners commonly refer to affected horses as 'easy keepers' or 'good doers', because their perception is that these horses tend to maintain their obese body condition when being fed minimal rations.

Clinically affected animals tend to be recognized in their 'teenage' years when they develop laminitis while grazing good-quality pastures ('pastureassociated laminitis'). However, it is likely that in these individuals insulin resistance and obesity has been developing for several years prior to the clinical expression of overt laminitis (Fig. 5.4). Careful examination of the feeding strategy often suggests that the EMS patient has been provided with dietary carbohydrates far in excess of the requirements.

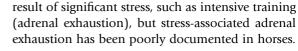
Diagnosis of EMS

Clinical suspicion of EMS is based on results of the physical examination of the patient. Physical abnor-



Figure 5.3 Regional thickening of the crest ('cresty neck') associated with the equine metabolic syndrome.

malities that are commonly identified in EMSaffected patients include: generalized or 'regional' obesity, a 'cresty' neck and evidence of subclinical or overt laminitis (based on either physical or radiographic abnormalities). It is important to eliminate PPID as an underlying cause of regional obesity and laminitis. It has become clear that PPID sometimes arises in teenage horses without the 'classic' physical appearance (inappropriate hirsutism, loss of musculature, PU/PD, etc.). In these cases the clinical presentation of PPID in young horses may be very similar to that of EMS and the extent to which these conditions may or may not be related is the subject of some controversy. Nevertheless, before a diagnosis of EMS can be established, diagnostic tests should be undertaken to demonstrate that the patient's clinical signs are not related to PPID (see above under: 'Diagnosis of PPID').



Clinical signs

Unfortunately, clinical signs of adrenocortical insufficiency are non-specific, vague and mimic other more common conditions. Veterinarians should consider a diagnosis of adrenocortical insufficiency when presented with the following clinical signs: lethargy, anorexia/inappetence, exercise intolerance, loss of body condition, poor hair coat (including inappropriate hirsutism), lameness, recurrent mild colic and seizures. Plasma biochemical profiling may yield unremarkable results or the following suggestive abnormalities: hyponatraemia, hypochloridaemia, hyperkalaemia, low sodium-to-potassium ratio and hypoglycaemia. Azotaemia, when present, may be attributable to prerenal factors such as hypovolaemia. Although a diagnosis of adrenocortical insufficiency may be suspected on careful consideration of the medical history, it should be noted that trainers and owners are not necessarily forthcoming regarding the drugs that have been administered to the patient.

Diagnosis of adrenocortical insufficiency

Primary adrenocortical insufficiency is characterized by an elevated plasma ACTH concentration (loss of cortisol-driven negative feedback) and must be carefully differentiated from PPID. Diagnosis may be supported by an ACTH stimulation test result in which the plasma cortisol concentration does not increase appropriately following administration of ACTH. A blood sample to determine cortisol concentration is taken between 8:00 and 10:00 am and ACTH is administered (ACTH gel: 1 IU/kg by intramuscular injection). In healthy horses, administration of ACTH leads to a two- to threefold rise in plasma cortisol when retested at +2 and +4 hours. Alternatively, Cosyntropin (synthetic ACTH subunit) may be used: plasma is collected between 8:00-10:00 am and the patient receives 100 IU (1 mg) of Cosyntropin intravenously. The plasma cortisol

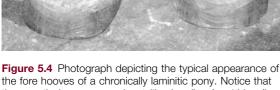


Figure 5.4 Photograph depicting the typical appearance of the fore hooves of a chronically laminitic pony. Notice that the growth rings are prominent ('laminar lines' or 'ridges') and that there is divergence of the growth rings toward the palmar aspect of the hoof.

The simplest diagnostic test for insulin resistance is estimation of the plasma insulin concentration. Compensatory hyperinsulinaemia is a common finding in horses affected by insulin resistance. However, serum insulin concentration can be influenced by factors other than insulin sensitivity; e.g. time since the animal was last fed; circulating cortisol concentration (diurnal variance, excitement, pain and stress, PPID); the type of food on which the ration is based; reproductive status; and physiological status (fitness/illness). Nevertheless, fasting concentrations of both insulin and glucose tend to be relatively constant and may be used to provide insight into the patient's insulin sensitivity. Insulinresistant animals tend to be characterized by a high normal or slightly elevated plasma glucose concentration and hyperinsulinaemia.

Adrenocortical insufficiency

Adrenocortical insufficiency is rare in horses. It is likely that most cases represent a secondary insufficiency, consequent to the discontinuation of long-term ACTH, glucocorticoid, or anabolic steroid treatment ('steroid letdown' phenomenon). However, the adrenal glands are shock organs in the equine species and primary adrenocortical insufficiency may develop in horses that have been recently affected by endotoxaemia, or in foals affected with sepsis. It has also been suggested that some horses might develop adrenocortical insufficiency as a concentration should double after 2 hours in a healthy horse.

Phaeochromocytoma

This is a rare tumour of the adrenal medulla that may be associated with excessive secretion of adrenaline (epinephrine) and/or noradrenaline (norepinephrine), which antagonize insulin and promote glycogenolysis. Although both difficult and rare to diagnose in living horses, veterinary pathologists report that non-functional tumours are a common incidental finding at necropsy. While the clinical signs are mostly attributable to the manifestations of excessive catecholamines, phaeochromocytomas tend to bleed and should always be considered when faced with perirenal haemorrhage and haemoperitoneum.

Clinical signs

Commonly reported clinical signs of equine phaeochromocytoma include excessive sweating (hyperhidrosis), anxiety, abdominal pain (ileus), generalized muscle fasciculations, muscle tremors, ataxia, mydriasis, polydipsia/polyuria, azotaemia, bladder paralysis, hyperthermia, tachypnoea, tachycardia and cardiac arrhythmia. These signs may be episodic. Phaeochromocytoma has been mostly reported in mature to older horses, in which it is usually benign and unilateral.

Diagnosis of phaeochromocytoma

Diagnostic corroboration of phaeochromocytoma is difficult. Although not pathognomonic, the combination of the following plasma biochemical abnormalities has been reported for some cases (in the absence of azotaemia): hyponatraemia, hyperkalaemia, hypocalcaemia and hyperphosphataemia. In humans, the diagnosis of phaeochromocytomas depends primarily upon the demonstration of catecholamine excess in urine and plasma. Subsequently, tumour location is based on either computed tomography or magnetic resonance imaging of the adrenal glands and abdomen. Unfortunately, aside from scintigraphy, none of these advanced imaging techniques is readily available or practical for large animal patients. Definitive diagnosis is usually obtained at post-mortem examination.

Polyuria/polydipsia in the horse

In a temperate climate, healthy adult horses that are housed and fed a standard ration generally consume 40-60 ml of water per kilogram of body weight every 24 hours (approximately 25 litres for a typical Quarter Horse). Voluntary water consumption declines substantially (50%) in horses that are not being fed or have developed inappetence or anorexia. Similarly, horses at pasture consume relatively less water because of the high water content of the grass. Although rarely quantified, adult horses normally produce 5-15 litres of urine per day. Since the volume capacity of the bladder is approximately 4 litres, healthy horses typically urinate three or four times per day. Normal urine is usually 3-4 times more concentrated than plasma and is characterized by a specific gravity (USG) between 1.030 and 1.045 (equivalent urine osmolality: 900-1400 mosmol/ kg). At pasture, adult horses tend to produce relatively dilute urine.

Owners may seek veterinary attention for horses that appear to have PU/PD in the absence of an obvious explanation. An accurate measurement of voluntary water consumption should be made at the outset (usually by providing known quantities of water by bucket and disabling automatic water suppliers).

PU/PD in older horses can be caused by PPID. Although PU/PD is not an inconsistent finding in horses affected by PPID, it should be considered in older horses that develop muscle wasting, regional obesity ('cresty neck'), laminitis and inappropriate hirsutism; the diagnostic tests for PPID are described above. Other causes of PU/PD include psychogenic polydipsia, renal disease, diabetes insipidus, diabetes mellitus and excessive salt consumption.

Psychogenic polydipsia and PU/PD

Psychogenic polydipsia resulting from the boredom associated with prolonged stall confinement is one

of the most common causes of PU/PD. In these cases, voluntary water consumption often exceeds 100 ml/kg/day and results in the passage of large quantities of dilute urine. Diagnosis of psychogenic polydipsia can usually be based on inspection of the patient's circumstances, ruling out other causes of PU/PD and, when necessary, performance of a water deprivation test.

Water deprivation test for psychogenic polydipsia

In psychogenic polydipsia, renal tubular function and antidiuretic hormone (ADH) activity are not impaired, so that water deprivation should induce urinary concentration. The deprivation test should begin in the evening to ensure that regular hydration checks can be made during the daylight hours of the following day. The procedure is as follows:

- All feed and water is removed. The bladder is emptied by catheter and the urinary specific gravity (USG) is determined
- The USG, blood urea and PCV or total protein are checked every 4–8 hours for a maximum of 20 hours. The test should be stopped when the USG indicates an adequate urinary concentration or, alternatively, if azotaemia or dehydration develop
- If the USG reaches no more than 1.020 by 20 hours, then deprivation to 24 hours may be considered if it is safe to do so.

NB: Water deprivation tests should not be undertaken in dehydrated horses or those demonstrating azotaemia.

An increase in USG above 1.020 following water deprivation suggests psychogenic polydipsia. A low or suboptimal USG suggests either diabetes insipidus or 'medullary washout'. Any longstanding case of PU/PD, of whatever cause, may be associated with the 'washout' of sodium and chloride from the medullary interstitium of the kidney. This reduces the osmolarity of the renal medulla, causing an inability to concentrate urine. An otherwise healthy kidney may therefore show poor concentrating ability following a water deprivation test. If urine does not concentrate to more than 1.020 at 24 hours, then an extended *modified* water deprivation test should be considered to overcome the confusion in diagnosis caused by medullary washout. In the modified test the daily water intake is restricted to 40 ml/kg for several days, after which the USG is reassessed. An increase in USG above 1.020 indicates psychogenic polydipsia. A lack of concentration over a 4-day period suggests diabetes insipidus, for which an exogenous ADH test is then indicated (see below).

Renal disease and PU/PD

Kidney disease should be considered when routine laboratory tests are suggestive of renal dysfunction (see Ch. 6: 'Urinary diseases').

Diabetes insipidus and PU/PD

Diabetes insipidus is a rare cause of PU/PD that results either from insufficient release of arginine vasopressin ('antidiuretic hormone') from the pars nervosa of the pituitary gland, or from unresponsiveness of the renal tubules to vasopressin, which may be inherited or acquired. Central 'neurogenic' diabetes insipidus is most commonly attributed to compression of the pars nervosa by an expanding pars intermedia adenoma in PPID-affected horses. Nephrogenic diabetes insipidus may result from genetically determined tubule defects or be acquired as a consequence of certain renal diseases. Horses affected with both forms of diabetes insipidus produce a copious volume of dilute urine (with secondary polydipsia) and are unable to produce concentrated urine during a water deprivation test.

In suspected cases of central diabetes insipidus, the diagnosis may be confirmed by demonstrating that PU/PD resolves following administration of a synthetic vasopressin analogue, desmopressin acetate. Water intake is monitored per 6 hours for 24 hours following the intravenous administration of 20 g of desmopressin acetate. In central diabetes insipidus the consumption of water (and output of urine) should decrease, while the USG increases. On the other hand, treatment with desmopressin will not alleviate polyuria in horses affected with nephrogenic diabetes insipidus.

Diabetes mellitus and PU/PD

Diabetes mellitus is a rare disease in horses. As with other species, PU/PD associated with diabetes mellitus is attributable to marked hyperglycaemia (typically >280 mg/dl) and glycosuria.

Excessive salt consumption and PU/PD

Water consumption may be increased (with a concomitant increase in urine volume) in horses that ingest too much salt (sodium chloride). Most commonly, excessive salt ingestion arises in some horses that develop a 'craving' for salt blocks (possibly another manifestation of boredom). Alternatively, the ration may contain too much added salt. Diagnostic support for PU/PD associated with excessive salt ingestion may be obtained by demonstrating that the fractional urinary excretion of sodium is increased (see Ch. 6: 'Urinary diseases').

Thyroid disorders

The thyroid hormones, thyroxin (T_4) and triiodothyronine (T_3) , affect almost every organ system by assisting the regulation of growth, cell differentiation and metabolism. However, despite the potential for causing an enormous diversity of clinical signs, conditions directly attributable to thyroid dysfunction are evidently rare in adult horses.

Making a diagnosis of hypothyroidism in an adult horse based upon low circulating plasma concentrations of (total) T₃ and T₄ is inaccurate as low plasma T₃ and T₄ concentrations are more commonly attributable to non-thyroidal factors. Nonthyroidal factors that may suppress the plasma concentration of thyroid hormones in horses with normal thyroid function include treatment with phenylbutazone, time of day, composition of the ration (energy, protein and micronutrients), conditions associated with glucocorticoid excess, equine metabolic syndrome (see above), food deprivation, level of training and stage of pregnancy. The terms 'non-thyroidal illness' and 'sick euthyroid syndrome' have been used to describe situations in which plasma T₄ concentrations are depressed by drugs and illness in the face of normal thyroid gland func-



Figure 5.5 Swelling associated with a thyroid adenoma to the left of the larynx.

tion. Simply demonstrating low plasma T_3 and T_4 concentrations is of little diagnostic value and the use of either thyroid stimulating hormone (TSH) or thyrotropin releasing factor (TRH) stimulation tests should be employed to determine whether the secretory function of the thyroid gland is normal.

Thyroid gland enlargement

The most common thyroid disorder of the horse is the so-called *thyroid adenoma*. It is frequently seen in older horses as an enlarged, palpable, unilateral swelling on one or other side of the larynx (Fig. 5.5). *They are usually non-functional, unassociated with endocrinopathy and rarely merit any interference*. The enlarged lobe of the thyroid gland can be quite mobile and may shift, quite normally, between a superficial and a deeper location, giving the impression that there is intermittent 'swelling'. If necessary, confirmation of the true (thyroid) identity of the evident mass can be accomplished by a combination of palpation, ultrasonography and biopsy.

In rare cases, enlargement of the thyroid gland in old horses is associated with neoplastic transformation and *hyperthyroidism*. Surgical removal of an enlarged thyroid gland should only be considered if hyperthyroidism is demonstrated or the size of the gland leads to coughing or dysphagia.

Excessive iodine in the ration, as seen sometimes in horses fed kelp (seaweed)-based supplements, may cause thyroid gland enlargement ('goitre'). Paradoxically, the gland may also enlarge as a result of iodine deficiency, although dietary deficiency of iodine is very unlikely. Secondary iodine deficiency may result from ingestion of excessive calcium, *Brassica* plants (such as broccoli, cabbage, cauliflower and mustard), white clover, rapeseed, linseed, or feed contaminated by sewage. However, if a dietary factor is responsible for goitre, the thyroid gland should be enlarged on *both* the left and the right sides of the neck. Moreover, other signs of iodine imbalance may be evident, such as an abnormal hair coat.

Hypothyroidism

Hypothyroidism may be classified as primary, secondary or tertiary depending on the location of the primary defect. Intrinsic thyroid gland disease constitutes primary hypothyroidism. Secondary hypothyroidism results from inadequate production and/or release of TSH by the pituitary gland. Tertiary hypothyroidism is caused by inadequate production and/or release of TRH from the hypothalamus. The true prevalence of bona fide hypothyroidism (of any type) is currently unknown but it is regarded as rare. Until a validated assay for the measurement of equine-specific TSH becomes available it will remain difficult to differentiate between the categories of hypothyroidism in horses. Assays based on canine TSH are not valid for horses.

Congenital hypothyroidism

True primary hypothyroidism has been recognized in neonatal foals in western Canada and the northwestern USA and is believed to be associated with exposure to a combination of high environmental nitrate levels and low levels of iodine in the feed of pregnant mares. Affected foals have enlarged thyroid glands and exhibit skeletal abnormalities (including mandibular prognathism, flexural deformities of the thoracic limbs, ruptured digital extensor tendons and incomplete ossification of cuboidal bones in the carpi and tarsi). Many affected foals die and those that survive exhibit persistent musculoskeletal disease that is unresponsive to thyroid hormone supplementation. The diagnosis is corroborated by 5

demonstrating low T_3 and T_4 levels and a reduction in the response to either TRH or TSH.

Hyperthyroidism

Hyperthyroidism is extremely rare in adult horses. Affected horses exhibit clinical signs similar to those described for other animal species affected with hyperthyroidism, including enlargement of the thyroid gland, weight loss, heat intolerance, hyperexcitability, tachycardia, tachypnoea, PU/PD and polyphagia. Affected horses are usually older than 20 years. Cases of hyperthyroidism have been associated with hyperfunctioning thyroid gland neoplasia (adenocarcinoma or adenoma) and are characterized by elevated plasma levels of thyroid hormones.

Diagnostic evidence of thyroid dysfunction in horses

The majority of diagnoses of equine hypothyroidism are inappropriately based on clinical signs, spot measurements of serum levels of total T_3 and T_4 and/or response to therapy using thyroid hormone supplementation. Plasma thyroid hormone concentrations, when used alone, are insensitive and misleading.

The TRH stimulation test is the only practical method for evaluation of the hypothalamic-pituitary-thyroid axis. A serum sample is obtained immediately prior to intravenous administration of 1 mg of TRH. Serum samples are obtained at 2 and 4 hours following administration. In normal horses, serum concentrations of total T_3 and T_4 at least twice those of the baseline should be achieved after 2 and 4 hours respectively. However, this test does not serve to distinguish primary hypothyroidism from other forms. Until validated assays for equine TSH become available for routine testing, bona fide hypothyroidism in adult horses remains difficult to corroborate.

Disorders of calcium metabolism

Calcium is important for diverse physiological processes such as blood coagulation, muscle contraction, hormone release, bone formation, maintenance

of the heartbeat and diverse metabolic activities. Plasma calcium concentration is usually estimated as the 'total calcium' concentration in the sample. Wherever possible, the physiologically important plasma *ionized calcium concentration* should be measured.

Hypocalcaemia

Hypocalcaemia develops when the circulating concentration of ionized calcium falls below the homeostatic requirement. Although it may be possible to demonstrate a low serum or plasma calcium concentration, this often represents total calcium and is not a measure of the biologically available (ionized) calcium. Consequently, successful diagnosis and treatment rely heavily on recognition of the circumstances and clinical signs associated with hypocalcaemia. Clinical hypocalcaemia is uncommon but tends to occur in particular circumstances that help its recognition (see below). Although the mechanism(s) associated with development of hypocalcaemia are often incompletely understood, a positive response to treatment supports the diagnosis. The delivery of 20% calcium, magnesium, phosphorus solution, diluted 1:4 in saline, by slow intravenous infusion, is often rapid and diagnostic. However, the amount required for effect varies enormously between cases.

Hypocalcaemic tetany

Low ionized calcium causes increased neuromuscular irritability and decreased smooth muscle contractility. The net result includes spontaneous skeletal muscle fasciculations, anxiety, lethargy, musculoskeletal stiffness, tremors and tetany. It also causes inhibition of normal gastrointestinal motility. Less commonly, ionized hypocalcaemia may cause increased neuroexcitability within the central nervous system, leading to seizures. Other clinical manifestations of hypocalcaemic tetany include: tachypnoea, dyspnoea, dysphagia, hypersalivation and sweating.

Synchronous diaphragmatic flutter (SDF, 'thumps') is a well-recognized consequence of ionized hypocalcaemia in which the phrenic nerve is stimulated by atrial contractions, such that the diaphragm contracts synchronously with each heartbeat. This abnormal activity is visibly evident as a twitch or contraction in one or both flanks; thoracic auscultation reveals that it is synchronized with each heartbeat. In some cases, violent diaphragmatic contractions occur and there develops a characteristic 'thumping' sound that is audible at some distance from the animal. SDF is usually seen in fatigued horses and commonly includes other fluid, electrolyte and acid–base disturbances (especially metabolic alkalosis).

Ionized hypocalcaemia has been reported in 'lactation tetany'; 'transit tetany'; gastrointestinal diseases (typhlocolitis, colic, systemic inflammatory response syndrome); blister beetle toxicosis; rapid intravenous administration of tetracycline; use of sodium bicarbonate by racehorse trainers ('milkshake'); exhaustive exercise; heat stroke; administration of furosemide; acute renal failure; dystocia/ retained placenta and exertional rhabdomyolysis. Lactation tetany (also known as eclampsia) is typically seen in mares several weeks after parturition or, less commonly, just after weaning. Clinical signs include an apprehensive appearance about the eye, sweating, muscle tremors, tachycardia and tachypnoea. In particular, there is stiffness in the limbs ('tetany'), producing an abnormal gait in which the animal appears to 'walk on tip-toe'. Some cases may also exhibit SDF. Untreated cases can proceed to collapse and develop tetanic convulsions. Transit tetany is associated with transportation fatigue and has most commonly been reported in ponies (of either sex). The clinical signs are similar, but with a marked tetany of all superficial muscles.

Hypoparathyroidism and hyperparathyroidism in the horse

Clinical disease associated with the parathyroid glands is rare in the horse. Although parathyroid conditions are commonly considered in the context of abnormalities of plasma calcium concentration, it may be necessary to determine the plasma concentrations of phosphate, magnesium, intact parathyroid hormone (PTH), vitamin D metabolites and parathyroid hormone-related protein (PTHrP) in order to more completely characterize the nature of the underlying aetiology.

Hypoparathyroidism

Hypoparathyroidism should be considered when faced with clinical or plasma biochemical evidence of hypocalcaemia. Although primary hypoparathyroidism is rare in horses, it has been reported to cause the clinical signs of hypocalcaemia given above. Diagnosis of primary hypoparathyroidism is supported by the following clinicopathological abnormalities: hypocalcaemia, hyperphosphataemia, hypomagnesaemia and a low plasma (intact) PTH concentration. It is important to consider the plasma magnesium concentration in the diagnosis of hypoparathyroidism for two reasons: 1) PTH stimulates renal magnesium retention and 2) hypomagnesaemia is a cause of secondary hypoparathyroidism.

Primary hypoparathyroidism may be differentiated from magnesium-dependent secondary hypoparathyroidism by demonstrating that the plasma PTH concentration is elevated following treatment with magnesium. Although there are very few published descriptions of secondary hypoparathyroidism in horses, it should be considered in the context of hypomagnesaemia and the sepsis/ systemic inflammatory response syndrome (PTH secretion is inhibited by pro-inflammatory mediators).

Secondary hypoparathyroidism should be considered in cases of hypocalcaemia when the plasma PTH concentration is not appropriately increased.

Hyperparathyroidism

Hyperparathyroidism should be considered when faced with clinical or plasma biochemical evidence of *hypercalcaemia*. Primary hyperparathyroidism is rare in horses and has been reported as a result of parathyroid adenoma or hyperplasia. In those cases, the secretion of PTH is excessive and is not negatively inhibited by the resulting hypercalcaemia. Secondary hyperparathyroidism arises when stimulated PTH secretion is driven by persistent hypocalcaemia, hyperphosphataemia or hypovitaminosis D. Noteworthy conditions associated with secondary hyperparathyroidism include renal dysfunction and nutritional secondary hyperparathyroidism (such as 'bran disease').

Clinical signs of hyperparathyroidism are characterized by the development of osteodystrophia fibrosa, including lameness, enlargement of the facial bones, weakness, inefficient mastication, fracturing and loss of cheek teeth, and poor body condition. Maxillary bone enlargement may lead to narrowing of the nasal passages and obstruction to the flow of air. Angular limb deformities and physitis may be evident in young (growing) animals. Radiographic changes that are suggestive of osteodystrophia fibrosa include bony proliferation of the maxilla and the mandible and attenuation of the lamina dura that surrounds the cheek teeth. A reduction in the general density of bone may be radiographically evident in severe, protracted cases. Diagnosis of primary hyperparathyroidism is supported by the following clinicopathological abnormalities: hypercalcaemia, hypophosphataemia, phosphaturia (elevated fractional urinary clearance of phosphate) and increased plasma PTH concentration. Other possible causes of hypercalcaemia should also be considered, including hypervitaminosis D and neoplasia.

Endocrinopathies of the reproductive tract

Granulosa cell tumour

The granulosa cell tumour is the commonest equine ovarian tumour. Since the tumour can produce oestrogens, progesterone and androgens, the main presenting feature is often abnormal sexual behaviour (even stallion-like behaviour), persistent oestrus or anoestrus. Diagnostic techniques include rectal palpation, ultrasonography of the genital tract and plasma testosterone and inhibin assays. For details see Chapter 7: 'Genital diseases, fertility and pregnancy'.

Cryptorchidism

Retention of an inguinal or abdominal testis, or the remnants of testicular tissue following incomplete castration, may lead to stallion-like behaviour in an animal thought to have been castrated. This is not a true endocrinopathy, but it is considered here for convenience. Diagnostic techniques include external and rectal palpation, ultrasonography, laparoscopy and the human chorionic gonadotrophin (hCG) stimulation test. For details see Chapter 7: 'Genital diseases, fertility and pregnancy'.

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CHAPTER

Urinary diseases

Chapter contents

I. Practical techniques 101 Examination of the urinary tract per rectum 102 Kidneys 102 Ureters 102 Bladder 102 Urethra 102 Catheterization of the bladder 102 The male 102 The female 103 Cystoscopy, ureteral catheterization and urethroscopy 104 Cystoscopy 104 Ureteral catheterization 105 Urethroscopy 105 Ultrasonography of the urinary tract 105 Kidneys 105 Ultrasonography of the bladder 107 Renal biopsy 107

II. Clinical pathology 108

Analysis of urine 108 Specific gravity of urine 108

Chemical characteristics of urine 108 Sedimentary characteristics of urine 108 Assessing the glomerular filtration rate 109 Azotaemia 109 Clearance studies 110 Assessing renal tubular function 111 Urine concentration 111 Fractional excretion of electrolytes 111 Urinary enzymes 112 Urinary protein to creatinine ratio 113 Assessing plasma electrolyte concentrations in renal disease 113 Potassium 113 Sodium and chloride 113 Calcium 113 Renal failure and metabolic acidosis 113 Haematology in diseases of the urinary tract 113

Further reading 113

APPENDIX 6.1 114

Some applications of diagnostic techniques for the investigation of urinary tract diseases

I. PRACTICAL TECHNIQUES

Diseases of the urinary tract are usually indicated by a change in the horse's urination behaviour, such as frequent attempts to urinate (with or without discomfort), dribbling of urine and/or a tangible change in the quality or volume of urine passed. This chapter describes the practical techniques and the complementary clinical pathology that can be used for investigating urinary tract diseases.

Examination of the urinary tract per rectum

Kidneys

Only the left kidney is accessible per rectum. Its caudal pole is palpable in the roof of the abdomen to the left of the midline – usually at arm's length. It is normally smooth, pain-free and somewhat mobile. The right kidney is palpable only when it is grossly enlarged and/or displaced.

Ureters

The ureters are not usually palpable unless thickened by infection or urinary obstruction. Ureteral stones are most commonly palpated near the pelvis where there is a slight turn of the ureters towards the bladder at this position.

Bladder

The empty bladder is situated in the midline at the pelvic brim and is usually difficult to palpate. When distended with urine it becomes palpable just beyond the pelvic brim, although the overlying uterus may hinder exploration in mares. In chronic cystitis it is likely to be empty, but its wall is then palpably thickened and painful. The presence of a cystic calculus is best appreciated when the bladder is empty, at which time a firm mass, usually oval in shape, is felt at the pelvic brim. Often the calculus is felt when the clinician has only advanced the hand and wrist into the rectum. In cases of paralysis or obstruction the bladder is grossly distended and the wall and lateral ligaments feel taut. In cases of chronic paralysis, there may be a pendulous feel to the bladder after urine has been removed by catheter. This is due to the accumulation of crystalline sediment (mostly calcium carbonate). Bladder tumours may be palpated on rectal examination, but occur rarely.

Urethra

The pelvic urethra is difficult to identify in either sex. However, in the male a calculus that has become lodged in the pelvic urethra is palpable per rectum. If obstruction occurs in the distal urethra, the whole pelvic urethra is palpable as a result of urinary distension. There may be visible and palpable pulsations of the urethral muscles associated with obstruction.

Catheterization of the bladder

Passing a urethral catheter demonstrates the patency or otherwise of the urethra and enables collection of a urine sample from the bladder. Catheterized samples are preferable for bacterial culture since environmental contaminants are minimized. The technique is also useful to reduce bladder volume prior to rectal examination or endoscopy (cystoscopy). In addition, it may be required in recumbent males that will not urinate voluntarily.

The male

It is usually necessary to relax the penis for this procedure using a moderate dose of acepromazine if clinical circumstances permit (0.05–0.10 mg/kg i.m. or slow i.v.). In entires, where penile paralysis is considered a risk associated with the use of acepromazine, detomidine may be used (0.01 mg/kg slow i.v.), followed by butorphanol (25 mg/kg i.v.), but adequate relaxation of the penis is less predictable.

Once relaxed, the glans and external urethral orifice are cleansed in a warm solution of povidoneiodine. Using aseptic precautions, a horse catheter (Fig. 6.1) is lubricated at the tip with a water-based lubricant and passed into the urethra while the body of the penis is held gently in the other hand (Fig. 6.2). The catheter passes with ease through the length of the penile urethra but a slight increase in resistance is felt as it moves around the ischial arch, at which point the tail head is seen to rise. From this position the flexible stylet is gradually withdrawn, preferably by an assistant, as the catheter is advanced over the pelvis and into the bladder - failure to do so renders the stylet immovable once it is beyond the ischial arch. On entry to the bladder, air is often heard being drawn in at the catheter hub because of the negative pressure in the normal equine abdomen.

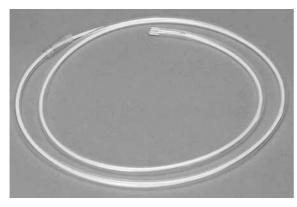


Figure 6.1 Eight millimeter horse urethral catheter (24 FG 137 cm), complete with stylet.

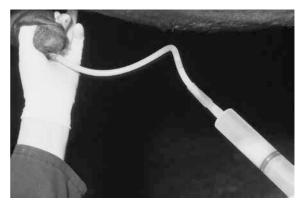


Figure 6.3 Using a catheter syringe to promote urine flow by applying suction.



Figure 6.2 Passing the catheter into the urethra.

In some horses noticeable resistance may be felt as the catheter arrives at the urethral sphincter prior to entering the bladder, and on rare occasions rectal examination and manual pressure on the bladder may be required to facilitate passage of the catheter into the bladder.

Unless urine is present under pressure, it is often necessary to start a syphon using a catheter syringe (Fig. 6.3). Even when the bladder is collapsed, it is usually possible to obtain 20–30 ml urine by syringe. If no sample is forthcoming, it is worth standing by with a sample container once the catheter is removed, since the passage of a small volume of urine is often stimulated by the presence of the aspirated air.

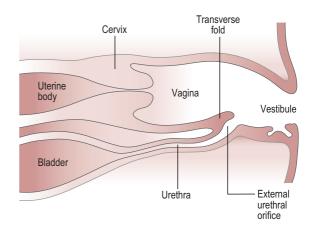


Figure 6.4 Relationship of the external urethral orifice to the transverse fold in the mare.

The female

The external urethral orifice of the mare or filly is highly distensible and is catheterized with ease. The tail is bandaged and the external vulva cleansed. Using aseptic precautions the urethral opening is found by advancing an exploratory finger along the floor of the vulva in the midline. In most cases the opening lies at a distance 10–12 cm from the ventral commissure of the vulval lips beneath the transverse fold (vestibulovaginal fold) that demarcates the entrance to the vagina (Fig. 6.4). The commonest mistake is to miss the transverse fold that overlies the opening and thus overshoot the site. The orifice

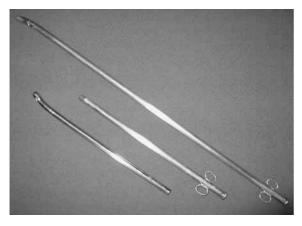


Figure 6.5 Mare catheters commonly used for catheterizing the adult female bladder.

is quite large and, once located, a lubricated catheter is fed through the vulva beneath the hand and directed under the exploratory finger. The urethra is very short (7–10 cm) and the bladder is soon entered. Aspiration by catheter syringe may be necessary to start a urine flow. Catheterization can also be performed without the need to place a hand into the vagina using a rigid mare catheter (Fig. 6.5).

Comments

- Following removal of the catheter the animal may adopt a urination stance and expel aspirated air.
- Complications are minimal, but poor technique could result in cystitis. This is extremely rare except in horses with neurogenic bladders. There is also a risk of 'knotting' the catheter if an excessive length is pushed into the bladder.
- Urine samples obtained by catheter are likely to show an increase in trace amounts of erythrocytes, transitional epithelial cells and protein.
- Flexible catheters are distorted by heat sterilization and rendered unsuitable for reuse.

Cystoscopy, ureteral catheterization and urethroscopy

Endoscopy is most appropriate to an examination of the bladder and urethra but can also be used to

catheterize the ureters in order to obtain individual urine samples from either kidney. This is particularly useful where renal lesions are thought to be unilateral. If necessary, the bladder should be drained by catheter prior to endoscopy.

Cystoscopy

In the female, cystoscopy is relatively easy using a standard fibreoptic instrument (1 m length, 1 cm outer diameter). The bladder is drained and, using aseptic precautions, an assistant introduces the endoscope into the urethra in the same manner as described for catheterization. Entrance to the bladder is at a distance of some 10 cm. It is then distended with air until the wall can be seen clearly. Air should not be forced into the urine or else large numbers of bubbles will form and obscure vision. Air will leak out around the endoscope and occasional repeated insufflation is required. Alternatively, the assistant may partially seal the urethra by placing a hand over the transverse fold at the vulvovaginal junction to gently compress the external urethral orifice. Overdistension with air will cause the mare to strain.

In the male the longer, narrower urethra may require a special endoscope 1.2–1.4 m in length with an ideal maximum outer diameter of 0.9 cm. The technique for passing the endoscope is essentially the same as that described for urethral catheterization in the male. Air leakage following bladder insufflation may be reduced by gently squeezing the body of the penis around the endoscope.

Orientation within the bladder is achieved by identifying the ventral pool of residual urine. The mucosal surface is then explored for anomalies of texture or structure. Inflammation, large calculi or sabulous sludge are easily identified. The volume of sludge (crystalline sediment) is greatly increased by chronic paralysis. The openings of both ureters are most easily evaluated by retroflexing the scope within the bladder, so that the scope itself is seen entering into the bladder, and both ureteral openings can then be observed simultaneously (Fig. 6.6). Tumours can be easily seen during endoscopic examination. Irregularities in the contour of the healthy bladder are common; these are due to the bowel pushing against the bladder.



Figure 6.6 Endoscopic (retroflexed) view of ureteral openings (arrows) and scope entering into the bladder.

Ureteral catheterization

The ureteral openings are located for catheterization by slowly withdrawing the endoscope from the bladder cavity until it is just inside the neck. The openings are then seen as small papillary-shaped structures either side of the midline in the dorsal wall, some 2 cm beyond the urethral opening. Frequent pulsatile squirts of urine identify the openings.

Sterile polyethylene tubing (2.0–2.5 mm outer diameter) is passed through the biopsy channel until seen in advance of the endoscope lens. The endoscope is then aligned so that the tubing can be advanced gently into the ureteral orifice for a distance of 5–10 cm. A urine sample is then carefully aspirated by syringe over 2–3 minutes. *Excessive force in either of these procedures will result in ureteral trauma*. Once sampling is complete and the tubing is withdrawn, the biopsy channel is flushed with sterile saline and the procedure is repeated on the opposite side using a fresh catheter.

Urethroscopy

The urethra is best examined as the endoscope is slowly removed from the bladder unless, of course, it is being examined for obstruction. The male urethra should be kept lightly inflated to give an optimal view of the mucosal lining as the endoscope is withdrawn. It should be noted that the initial forward passage of the endoscope causes the mucosa

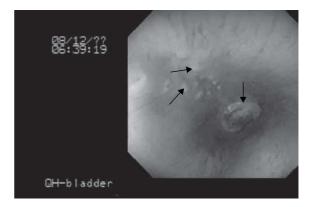


Figure 6.7 Endoscopic view of the normal male pelvic urethra with two rows of openings to the ducts of the bulbourethral glands (paired arrows) and the colliculus seminalis (vertical arrow).

to appear markedly hyperaemic at withdrawal. The entrances to the ductus deferens and the accessory sex glands can be seen in the pelvic urethra. These include two rows of openings of the ducts to the bulbourethral glands and, more proximally, the colliculus seminalis, which encompasses the openings of the prostate gland and the common openings of the ductus deferens and the seminal vesicles (Fig. 6.7). Distal to this area, but still in the pelvic urethra, is the site where urethral mucosal defects and spontaneous bleeding may occur in some males, particularly geldings. The female urethra is extremely short (7–10 cm).

Comment

 Transient stranguria may follow cystoscopy/ urethroscopy.

Ultrasonography of the urinary tract

Kidneys

In the normal horse both kidneys may be imaged or 'scanned' by percutaneous ultrasonography but usually it is only possible to scan the left kidney by the rectal approach. However, where renal disease is associated with enlargement of the right kidney, both kidneys may be scanned by rectal ultrasonography.

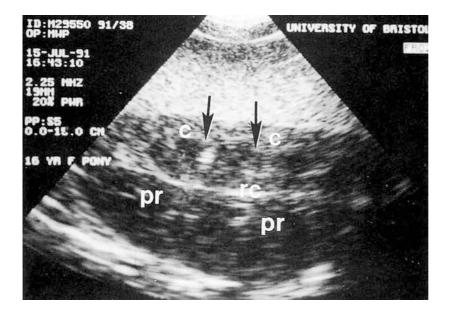


Figure 6.8 Ultrasonogram of a normal kidney showing the cortex (c), the corticomedullary junction (arrows), the hypoechoic pelvic recesses within the medulla (pr) and the hyperechoic renal crest (rc).

Percutaneous ultrasonography of the kidney

Careful skin preparation is essential for percutaneous ultrasonography and in most cases this involves clipping the hair, cleansing the skin with povidoneiodine and finally degreasing with spirit. Linear array or sector scanners may be used but, as intercostal views are required, the sector scanner is preferable since it enables a small transducer-patient contact area and a wide field of view.

Each kidney is scanned in the dorsal abdomen below the level of the transverse processes. The left kidney is approached through the 17th intercostal space and the paralumbar fossa using a 2.25– 3.5 MHz transducer. Here the kidney lies medial to the spleen, which is used as an acoustic window, so that a 20–26 cm depth of view may be needed. The right kidney is approached through the 15th, 16th and 17th intercostal spaces. In this position it lies immediately adjacent to the body wall and in young horses just caudal to the liver. It is best visualized with a 3–5 MHz transducer and a 15 cm depth of view is usually adequate.

In chronic renal conditions the scan may be expected to provide evidence of morphological change. As far as possible the entire kidney should be visualized from pole to pole and the scan should be performed in all planes to verify anomalies. Particular points to note are deviations of kidney position within the abdomen and the kidney's size, shape, surface contour and texture (i.e. relative brightness of tissues).

The overall size may be reduced by chronic disease or enlarged by hydronephrosis and, rarely, neoplasia. The cortex is more echogenic (brighter) than the medulla and a distinct corticomedullary junction should be identifiable lying 1–2 cm deep to the capsule. Within the medulla are the pelvic recesses, which are hypoechoic (darker) areas, approximately 1 cm in size, lying adjacent to the hyperechoic renal crest (Fig. 6.8). The renal pelvis may be identified as an anechoic area on the medial aspect of the kidney but both this and its associated ureter only become obvious when they are pathologically distended. Dilatations of the pelvis and recesses are seen in hydronephrosis.

Bright, hyperechoic reflections with marked acoustic shadows may indicate areas of mineralization. Small areas of mineralization occur commonly in the recesses of older horses, emphasizing the need to scan suspected lesions in multiple planes.

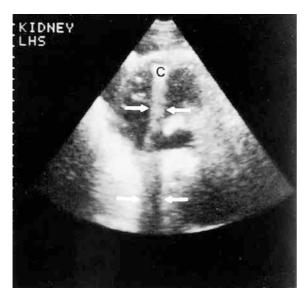


Figure 6.9 Ultrasonogram revealing renal calculi (c). Note the marked acoustic shadow (between arrows).

In the case of renal calculi the passage of ultrasound is totally blocked and an acoustic shadow is cast deep through adjacent tissues (Fig. 6.9).

Rectal ultrasonography of the kidney

Rectal ultrasonography of the left kidney is easily performed with a linear array transducer of 5–7.5 MHz. The transducer is applied to the medial side of the kidney and may be swept along the ventral aspect. The caudal pole is easily accessible and this is particularly useful where a gas-filled bowel prevents the percutaneous examination of this area. The left renal pelvis and both ureters are best examined by the rectal approach.

Ultrasonography of the bladder

The bladder can be visualized in the foal by percutaneous scan, but in the adult it is scanned per rectum using a 5 MHz transducer. The bladder wall is clearly identified as an echogenic structure with abnormalities appearing either as irregularities in the otherwise smooth contour, or as alterations in the wall thickness. Bladder size and wall thickness vary with the volume of urine present. Calcium carbonate crystals, normally found in adult horse urine, create a swirling echogenic appearance in the urine. Calculi are easily detected by the large acoustic shadow that they generate.

Renal biopsy

Renal biopsies are collected using a percutaneous needle technique, which may be performed 'blind' or with the aid of ultrasound guidance. Blind renal biopsy is not a safe procedure in the horse and is justified only if histopathology is likely to influence subsequent treatment significantly. The procedure may result in perirenal haematoma formation and has the potential to cause fatal haemorrhage. Ultrasound examination should immediately precede renal biopsy, such that the proper site and depth of the biopsy can be determined and marked on the horse. Alternatively, real-time ultrasound guided and visualized biopsy is much safer. This procedure is facilitated by the attachment of a sterile sleeve and biopsy guide to the transducer but it can be readily performed (with practice) using suitable triangulation between the scan plane and the biopsy instrument.

Biopsies are taken from the standing, sedated animal using a 14–18 gauge biopsy needle at least 15 cm in length. The skin overlying the kidney is clipped at the sites described for renal ultrasound (see above) and surgically prepared. Care should be taken to select a site where the path of the needle is parallel to the interlobular arteries and does not cross any of the arcuate arteries. When the left kidney is biopsied a trans-splenic route can be used. While the trans-splenic route does not appear to be associated with a markedly increased risk of haemorrhage, it is preferable to biopsy the right kidney if bilaterally symmetrical disease is expected.

Local anaesthetic is then infiltrated into the skin and underlying abdominal wall and a number 11 scalpel is used to make a stab incision through the skin at the selected site. The biopsy instrument is inserted through the skin followed by quick manual movement of the Tru-Cut needle into the kidney parenchyma, quickly followed by biopsy collection and removal. With the ultrasound guidance/ visualization method, the needle is inserted through

the skin and abdominal wall and directed so that the tip is pressing against the renal capsule. Taking care to avoid major blood vessels, the needle is operated and withdrawn. If the first attempt is unsuccessful, a second attempt may be made. Following this procedure the horse should be kept as still as possible for 2 hours to permit clotting. If bilateral renal biopsies are considered necessary, which is highly unlikely, they should not be performed simultaneously; 24 hours should be left between the two procedures.

Comments

- Trace haematuria is inevitable, but overt haematuria may also occur and these patients need careful observation. Ultrasonography may allow identification and subsequent monitoring of a subcapsular or parenchymal haematoma.
- Biopsy specimens examined by light microscopy may appear normal despite clinicopathological evidence of acute and severe dysfunction.

II. CLINICAL PATHOLOGY

Significant diagnostic information concerning diseases of the urinary tract can be obtained by the strategic analysis of blood and urine samples.

Analysis of urine

Urine analysis can provide evidence of upper and/ or lower urinary tract disease.

Sample containers should be clean for routine analysis and sterile for bacterial culture. Ideally, samples should be processed as soon as possible after collection to avoid spurious results. Certain urinary constituents are degraded by sunlight and the use of opaque or dark containers will prevent this. Containers with preservatives will inhibit bacterial multiplication but may interfere with some of the chemical tests. If culture of the urine is thought to be important, a colony count (number of organisms per millilitre of urine) should be requested.

When collecting a free-flow sample, try to catch a midstream aliquot. Avoid catching the first part of

the urine stream as it will contain cellular debris, leukocytes and exudate flushed from the urethra, prepuce and female genital tract. In addition, it will contain commensal bacteria flushed from the urethra. Similarly, an end-of-flow sample will contain bladder debris. Catheterized samples may show trace amounts of erythrocytes, transitional epithelial cells and protein.

Horses will not volunteer urine samples when required, but their behaviour patterns can be exploited to this end. A horse held in a bedding-free box for 2–3 hours will often void urine when transferred to a freshly bedded box after a short walk. This simple ploy may avoid the need for catheterization.

Specific gravity of urine

This is the only indicator of renal function in the urine analysis. The concentrating ability of healthy equine kidneys will produce a urinary specific gravity (SG) of between 1.025 and 1.050 when challenged by water deprivation and/or dehydration. Dehydration will cause decreased renal perfusion and a more concentrated urine of smaller volume (oliguria). However, if poor perfusion persists and/or a toxic insult occurs, intrinsic function is diminished and, unless it is reversed, there will be a loss of tubular resorptive capacity resulting in a urine of low SG that is independent of the osmotic pressure of the blood. The SG then assumes that of the glomerular filtrate (isosthenuria) and registers between 1.008 and 1.012. Persistent tubular dysfunction may lead to polyuria and polydipsia. Conversely, horses that consume excessive amounts of water, or those with diabetes insipidus (central or renal), pass dilute hyposthenuric urine (less than plasma osmolality).

Chemical characteristics of urine

The chemical characteristics of normal equine urine and the changes associated with disease are shown in Table 6.1.

Sedimentary characteristics of urine

The sedimentary characteristics of normal equine urine and the changes associated with disease are shown in Table 6.2.

Parameter	Normal range	Disease	
рН	Range 7.0–9.0 in normal urine Tends to acidity on concentrate feeds	Urine acidity is associated with metabolic acidosis or anorexia	
Protein	Usually <100 mg% in normal urine	Severe proteinuria is associated with glomerular lesions; the protein concentration is also raised by inflammatory lesions of the urinary tract	
Glucose	None present usually	Glycosuria occurs when hyperglycaemia exceeds the renal threshold, as in some cases of Cushing's disease, stress-related hyperglycaemia or rare forms of diabetes mellitus. Sedation with alpha-2 agonists may cause transient glycosuria Alternatively, glycosuria in the absence of hyperglycaemia is indicative of tubular dysfunction	
Ketones	None present usually	Ketosis is rare in horses; its presence suggests a nutritional stress associated with protein catabolism	
Bilirubin	None present usually	It is present during obstructive jaundice (only conjugated bilirubin is filtered)	
Haemoglobin	None present usually	Haemoglobinuria occurs during intravascular haemolysis; the serum will appear haemolysed*	
Myoglobin	None present usually	Myoglobinuria occurs during acute degenerative changes in skeletal muscle (rhabdomyolysis); the serum is usually not discoloured by myoglobin*	

Table 6.1 Chemical characteristics of normal urine and changes associated with disease

*It is difficult to differentiate haemoglobinuria from myoglobinuria without sophisticated laboratory procedures. However, serum discoloration is only apparent during haemolysis.

Assessing the glomerular filtration rate

There is a fall in the glomerular filtration rate (GFR) in renal failure and eventually this leads to an increase in the circulating concentration of nitrogenous waste products (azotaemia). A state of azotaemia is most conveniently defined in the laboratory by measuring the concentrations of urea and/or creatinine in plasma or serum. The fall in GFR may precede these biochemical changes in the blood and a single measurement of urea and creatinine should not be considered as a sensitive indicator of early renal dysfunction. However, more sensitive measures of GFR are often not readily available in the horse.

Azotaemia

The development of azotaemia reflects the loss of nephron function, which may be caused by:

- Prerenal factors that reduce vascular perfusion of the kidneys
- Intrinsic factors associated with damage to renal tissues
- Postrenal factors that hinder urine excretion.

Some 75% of glomerular function is lost before azotaemia becomes apparent. It is therefore an insensitive indicator of the onset of reduced GFR. However, once raised above baseline for the individual horse, subsequent increases in the serum concentration of urea or creatinine usually reflect further decreases in GFR and become useful monitors of disease progress.

Comment

• Small increases in the blood urea concentration alone (i.e. up to twofold, with creatinine remaining within normal range) frequently

Parameter	Normal content	Disease	
Erythrocytes	None present usually	Haematuria reflects: inflammation; trauma; neoplasia or coagulopathy in the urinary tract Trace amounts may be associated with catheterization or tubular necrosis	
Leukocytes	None present usually	Large numbers are associated with infection or inflammation of the tract (pyuria)	
Transitional cells	Few present usually	Large numbers reflect: inflammation; trauma or neoplasia of the bladder Numbers increase in endstream urine and catheterized samples	
Bacteria	None present usually	Bacteria are significant in the presence of large numbers of inflammatory cells Gram staining of a sediment smear is required and a moderate to heavy growth in culture may reflect pyelonephritis or cystitis	
Crystals	Usually calcium carbonate present – normal Occasionally, triple phosphate and calcium oxalate are seen	5	
Casts	No cellular casts are usually present but occasional hyaline (mucoprotein) casts appear	Cellular casts reflect tubular damage – the cells are bound together by protein exudate or leakage	

Table 6.2 Sedimentary characteristics of normal urine and changes associated with disease

accompany dehydration and/or wasting diseases associated with increased tissue catabolism. Feeds that are high in protein may also raise blood urea slightly. Decreases in urea without concurrent and comparative decreases in creatinine may occur with liver failure, diarrhoea and increases in body water, e.g. oedema.

 Increases in serum creatinine concentration alone can accompany severe myopathies such as exertional rhabdomyolysis. Breeds of horses with a greater percentage of muscle mass, e.g. Quarter Horses, often have serum creatinine concentrations greater than 'lean breeds', e.g. Thoroughbreds.

Clearance studies

The measurement of GFR by clearance studies is impractical in horses. Inulin, a starch that is excreted

in the urine at a rate equal to the GFR, is restricted to laboratory applications. An alternative is the measurement of endogenous creatinine clearance, but this requires long-term collection of urine using special harnesses. A simpler approach is the measurement of sodium sulphanilate clearance, which is performed using blood samples alone. This technique is outlined below for interest, but few commercial laboratories will undertake sulphanilate assays. The quickest clearance study method is a single injection of ^{99m}Tc-labelled diethylenetriaminepentaacetic acid, but this requires use of a gamma counter and is limited to specialist centres.

Sodium sulphanilate clearance

Following intravenous injection, sodium sulphanilate is rapidly distributed throughout fluid spaces and is then cleared, primarily by glomerular filtration, at a linear rate which can be measured. Sulphanilate clearance is not a true measure of the GFR, but does reflect it because renal excretion is a major factor influencing its decay curve.

The patient receives 10 mg/kg bodyweight of the sulphanilate preparation intravenously and heparinized blood samples are taken from the opposite vein at 45, 60, 75 and 90 minutes after injection. The concentration of sodium sulphanilate in blood samples is then determined from a standard curve using a colorimetric assay. The standard curve must be prepared from the batch of sodium sulphanilate used for injection.

The sulphanilate concentrations in the test samples are then plotted against their respective sample times on semilogarithmic coordinates. This should produce a linear clearance curve, providing an interval of at least 45 minutes has been allowed between the sulphanilate injection and subsequent blood samples.

The clearance rate of sulphanilate is defined as the time taken for 50% of the salt to be cleared from the blood ($t_{\frac{1}{2}}$). This can easily be calculated from the curve. In healthy horses and ponies the $t_{\frac{1}{2}}$ has been found to lie between 26 and 45 minutes. In cases of suspected early renal failure, the clearance times would be expected to lengthen. In cases of established renal failure, clearance times in excess of 200 minutes have been determined.

Comment

• The assessment of GFR offers no advantage in the diagnosis of renal failure once azotaemia is established. Its diagnostic potential lies in detecting and monitoring early renal failure, in advance of azotaemia, in patients suffering acute toxic or ischaemic renal insults, or chronic and potentially progressive renal disease.

Assessing renal tubular function

Urine concentration

The SG of urine is an indicator of the renal tubular ability to resorb or excrete water in response to changes in hydration. The presence of persistently dilute (hypotonic) urine in an azotaemic or dehydrated horse is therefore indicative of tubular dysfunction.

Horses with polyuria/polydipsia (PU/PD) usually pass urine of persistently low SG. Tubular function in cases of PU/PD can be assessed by water deprivation tests. However, it must be emphasized that these tests are both dangerous and pointless in patients that already have clinical or clinicopathological evidence of decreased GFR. Nevertheless, the majority of equine patients with PU/PD are unlikely to be suffering from renal disease; the most usual differential diagnoses being pituitary adenoma (pituitary pars intermedia dysfunction: equine Cushing's disease), psychogenic polydipsia or occasionally diabetes insipidus. Of these conditions, pituitary adenoma and psychogenic polydipsia are the commonest. The differential diagnosis of PU/PD, including the use of water deprivation tests, is detailed under: 'Polydipsia/polyuria (PU/PD) in the horse' in Chapter 5: 'Endocrine diseases'.

Fractional excretion of electrolytes

In the healthy kidney, the net urinary excretion of an electrolyte is governed by two factors: the GFR and the extent of tubular resorption. In contrast, endogenous creatinine is excreted by glomerular filtration alone and its rate of excretion thus approximates to the GFR, even during renal dysfunction. Creatinine clearance is therefore a useful standard against which the clearance of an electrolyte may be compared in health or disease.

The fractional excretion (FE) of an electrolyte is defined as the percent ratio of its clearance to the clearance of endogenous creatinine. In normal homeostatic balance FE values are very variable, but they are usually within a definable range. With a loss of tubular resorption, the excretion of an electrolyte is often increased and its FE rises above the normal range. The percent ratio is derived as follows.

 $\begin{array}{l} \mbox{Urinary concentration of electrolyte} [E]_u \\ \mbox{Plasma concentration of electrolyte} [E]_p \\ \mbox{Urine flow rate/min} \quad 100\% \end{array}$

divided by:

 $\frac{\text{Urinary concentration of creatinine}[Cr]_{\text{u}}}{\text{Plasma concentration of creatinine}[Cr]_{\text{p}}}$ Urine flow rate/min

which is simplified to:

$$\mathsf{FE} = \frac{[\mathsf{E}]_{u}}{[\mathsf{E}]_{p}} \quad \frac{[\mathsf{Cr}]_{p}}{[\mathsf{Cr}]_{u}} \quad 100\%$$

The FE of an electrolyte is therefore calculated once the urinary and plasma (or serum) concentrations of both the electrolyte and creatinine are known. This approach eliminates the need for protracted collection of urine but the urine and plasma samples must be obtained at the same examination time (within 30 min of each other). The following FE ranges, determined for healthy horses on a balanced electrolyte intake, serve as a normal guide:

- Sodium: 0.02–1.00%
- Potassium: 15-65%
- Inorganic phosphorus: 0.02-0.53%
- Chloride: 0.04–1.60%.

Urine should be submitted in capped, sterile containers to avoid artefactual changes in the phosphate and creatinine concentrations as a result of bacterial contamination. The plasma should be separated fairly soon, and both urine and plasma should be analysed as quickly as possible (certainly within 4 days). In cases of delay, high temperatures must be avoided.

In general terms a persistent increase in the FE of one or more electrolytes (frequently sodium and phosphorus) is indicative of tubular dysfunction.

Comments

- In health, the urinary concentrations of electrolytes and their rates of excretion vary between horses and within the same individual throughout the day. This is because clearance is highly influenced by dietary, hydration and endocrine factors. *Tests producing abnormal results should be repeated to confirm the trend*.
- Excessive phosphate intake, or a diet with an abnormally low calcium:phosphate ratio, can produce elevated FE phosphate values in animals with normal renal function.

- Despite the fact that calcium is precipitated in urinary crystals that may be lost to analysis, its FE value can be of use. However, the colorimetric methods used in most commercial laboratories are unsuitable for urinary calcium estimation and its FE value is not considered here.
- Urine samples delayed in transit and having abnormally low creatinine concentrations (<10000 mol/l) are probably contaminated and the FE results will be spurious.
- Measurements undertaken in horses following exercise or sedation, or those currently receiving intravenous fluids, will be spurious.
- Abnormal increases in FE values should never be used as the sole criterion for diagnosis of tubular failure; they are simply part of the cumulative clinicopathological evidence that indicates failure.

Urinary enzymes

Gamma glutamyltransferase (GGT) is found in the liver, pancreas and luminal brush border of the proximal renal tubular cells. This enzyme is not excreted by glomerular filtration, so its appearance in urine is indicative of acute tubular damage. It appears before azotaemia develops, thus offering a sensitive indicator of early tubular disease.

Urinary GGT concentrations are conventionally expressed as a ratio to urinary creatinine concentrations [Cr]_u. This approach allows for variations in urine flow rate at the time of sampling, thus standardizing comparisons:

$GGT\left(IU/I\right) \quad [Cr]_u \left(mmol/I\right).$

The normal urinary GGT: creatinine ratio should be less than 0.25.

Comment

• Urinary GGT values increase early in the course of treatment with most potentially nephrotoxic drugs, e.g. aminoglycosides, and values do not correlate to change in function, which limits the value of the test. Additionally, urinary GGT values fall once the acute insult has ceased, despite the persistence of tubular dysfunction.

6

The value of this assay in signalling progressive failure is therefore dubious.

Urinary protein to creatinine ratio

The quantitative measurement of urine protein and creatinine can be used to more accurately determine renal protein loss, as may be significant with glomerulonephritis. Urine reagent strips and even sulphosalicylic acid testing do not accurately quantify proteinuria in the horse. Ratios greater than 3:1 are highly suggestive of glomerular disease.

Assessing plasma electrolyte concentrations in renal disease

There is no totally consistent pattern to the changes in plasma electrolyte concentrations that occur as a result of renal disease in horses, although hyponatraemia and hypochloraemia are commonly found in both acute and chronic renal failure. Hypercalcaemia is common with chronic renal failure. The following comments are offered.

Potassium

Since the kidneys are the main site of potassium excretion, conditions of oliguria or anuria are likely to be associated with hyperkalaemia. However, progressive tubular damage and anorexia will eventually lead to hypokalaemia.

Sodium and chloride

Tubular damage will be associated with the loss of sodium and chloride and plasma concentrations often reflect this. At a stage when tubular damage is associated with polyuria, the plasma concentrations of sodium and chloride may fall. Additionally, horses with glomerulonephritis and oedema will have dilutional decreases.

Calcium

The horse is unusual in that the major site of calcium regulation is the kidney rather than the small intestine. Renal dysfunction may be associated with either hypercalcaemia or hypocalcaemia.

Renal failure and metabolic acidosis

In the healthy kidney, tubular cells conserve and generate bicarbonate for the blood alkali reserve but also excrete large amounts of alkali in the urine; hence the high urinary pH of roughage-fed healthy horses.

Comment

• Systemic pH is highly variable in horses with renal failure, being dependent on the degree of dysfunction, feed intake and strong ion differences.

Haematology in diseases of the urinary tract

Haematology provides non-specific information in cases of urinary tract disease. Increases in the packed cell volume indicate dehydration and in chronic disease a non-regenerative anaemia is to be expected. Leukocytosis may accompany gross inflammation of the urinary tract and an elevated plasma fibrinogen concentration is indicative of septic inflammation.

Chapter appendix

Appendix 6.1 suggests some applications of the diagnostic techniques covered in this chapter for the investigation of urinary tract diseases, based upon the presenting signs.

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APPENDIX 6.1 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF URINARY TRACT DISEASES

Observation	Possible cause	Aids to diagnosis	
		Preliminary investigation	Definitive investigation
Frequent attempts to urinate ± pain	Cystitis	Rectal examination Urinalysis Urine culture (catheter)	Cystoscopy/biopsy
	Pyelonephritis/cystitis	Assess cystitis (above) Measure blood urea and creatinine Examine kidney and ureters per rectum Renal ultrasonography	Assess tubular function Ureteral catheterization
	Urolithiasis	Rectal examination Urethral catheterization	Ultrasonography Endoscopy
	Pressure on the bladder, e.g. intestinal distension	Rectal examination Ultrasonography	Laparotomy
Persistent dribbling of urine	Retention overflow:		
	1) Bladder paralysis	Rectal examination	Evacuate bladder by catheter and reassess function
	2) Partial obstruction	Rectal examination Urethral catheterization	Endoscopy
	Ectopic ureter	Endoscopy	Excretory urogram
Anuria/oliguria	Acute renal failure	Measure blood urea and creatinine Urinalysis Palpate kidney per rectum	Assess tubular function Renal ultrasound Renal biopsy
	Obstruction	Rectal examination Urethral catheterization	Endoscopy
	Dehydration	Clinical examination; measure PCV and/or total serum protein and urine SG	
Polyuria/ polydipsia See also Ch. 5: 'Endocrine diseases'	Renal failure	Measure blood urea and creatinine Urinalysis – isosthenuria Palpate kidney per rectum	Assess tubular function Renal ultrasound Renal biopsy
	Pituitary adenoma (hyperadrenocorticism)	Measure blood glucose	Dynamic function test (see Ch. 5)

6

APPENDIX 6.1 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF URINARY TRACT DISEASES—cont'd

Observation	Possible cause	Aids to diagnosis	
	Diabetes mellitus	Measure urinary glucose	Eliminate pituitary adenoma (see Ch. 5)
	Diabetes insipidus (DI)	Hyposthenuria Modified water deprivation test (Ch. 5); test negative in DI	Exogenous ADH response (see Ch. 5)
	Psychogenic polydipsia (PP)	Hyposthenuria Modified water deprivation test; test may be positive in PP but can be confounded by medullary washout	
Azotaemia in biochemical profile of patient	Renal failure Bladder rupture	See above Measure creatinine in peritoneal fluid	See above Cystoscopy

CHAPTER

Genital diseases, fertility and pregnancy

Chapter contents

I. The mare 118 Examination for breeding soundness 118 Indications 118 Examination of the vulva and perineal region 118 Clitoral swabbing 120 Manual examination of the internal genital tract per rectum 121 Ultrasonography 122 Endometrial swabbing 124 Endometrial cytology 125 Vaginal examination 126 Digital examination of the vagina and cervix 126 Endometrial biopsy 126 Examination for breeding soundness – ancillary techniques 128 Chromosome analysis 128 Progesterone analysis 128 Endoscopy of the uterus (hysteroscopy) 128 Pregnancy diagnosis 129 Pregnancy diagnosis by transrectal palpation 129 Pregnancy diagnosis by ultrasonography 130 Hormone assays 131 Investigation of abortion, stillbirth or birth of a sick foal 132

Investigative approach 132 Infectious causes of abortion - diagnostic features 132 Non-infectious causes of abortion - diagnostic features 133 Differential diagnosis of genital diseases in the mare 133 Small ovaries 133 Enlarged ovaries 134 Uterine diseases 135 137 Cervical diseases Vaginal disorders 137 II. The stallion 138 Physical examination 138 Testicular measurement 138 Testicular consistency 138 Examination of the penis 138 Swabbing 138 Semen collection 139 Semen evaluation 140 Cryptorchidism 142 Diagnosis 142 Further reading 143

I. THE MARE

Examination for breeding soundness

Indications

Mares should be examined for breeding soundness in the following circumstances:

- Maiden mares entering a breeding programme
- If they have repeatedly failed to conceive after breeding
- If they are non-pregnant and behaviourally anoestrous during the breeding season
- If they require genital surgery
- Prior to purchase
- After embryonic or fetal loss.

It is important to adopt a systematic approach to this examination to avoid compromising the interpretation of later procedures by interference from an earlier one. Not all the diagnostic techniques described below are recommended in all the circumstances mentioned above. However, if a complete examination is required, the sequence of diagnostic techniques should be performed as follows:

- **1.** Examination of the vulva and perineal region
- **2.** Clitoral swabbing (as required by regulatory bodies
- **3.** Manual examination of the internal genital tract per rectum
- 4. Transrectal ultrasonography
- 5. Endometrial swabbing
- 6. Endometrial cytology
- 7. Vaginal examination
- **8.** Digital examination of the vagina and cervix
- 9. Endometrial biopsy.

Examination of the vulva and perineal region

The following anatomical relationships should be examined.



Figure 7.1 Normal perineal and vulval conformation displaying upright vulva with appropriate apposition of the vulval labia.

Seal of the vulvar lips

The integrity of the vulvar lips and their anatomical relation with the perineal area and anus are an essential component of a mare's fertility because they provide the first barrier to contamination between microorganisms in the external environment and the uterus. The vulvar lips should be closely apposed to each other to minimize contamination of the vestibule and possibly the vagina and uterus (Fig. 7.1). Mares with an ineffective vulvar seal may be predisposed to pneumovagina ('wind-sucking'), which facilitates the entry of debris and contaminants into the caudal reproductive tract. This contamination could variously result in acute or chronic endometritis, embryonic death or placentitis leading to abortion, stillbirth or neonatal sepsis.



Figure 7.2 Poor perineal conformation with sunken anus.

Vulvar vertical inclination

Normal perineal conformation is needed for maximal function of the vulvar lips as a physical barrier. The vulva should be vertical or have a cranial to caudal slope of no more than 10° from the vertical toward to the anus. Vertical inclination of more than 10° (sunken anus) may predispose the entry of faeces and contaminants into the vagina (Fig. 7.2).

Anatomical relation between the vulva and pelvic brim

At least two-thirds of the vulva should lie below the floor of the pelvis (Fig. 7.3).

The vestibule

The vestibule is the area that separates the vulva and clitoris from the vagina proper. At the cranial border of the vestibule, where it forms the vagina, lies the



Figure 7.3 Good perineal conformation with more than two-thirds of the vulval labia below the floor of the ischiatic arch.

vaginovestibular fold, which functions as a sphincter. This folded muscular membrane acts as the second physical barrier between the uterus and the external environment. Occasionally, a persistent hymen may be present in a maiden mare. Usually, manual examination of the vagina will be enough to rupture a persistent hymen.

'Windsucker test'

Listening for an inrush of air into the vagina when the vulvar labia are gently parted can test the adequacy of the vaginovestibular fold as a physical barrier to external contaminants. A noticeable sound of air inrushing into the vagina indicates that the vestibule fold is not adequately restricting the vagina proper from the outside environment. It is important to note that the windsucker test verifies how competent the vaginovestibular fold is, not the vulva. A mare with an inadequate vulvar seal but a strong vaginovestibular fold may not develop pneumovagina or ascending placentitis.

Pneumovagina

Improper functioning of the first barrier (vulva) and second barrier (vaginovestibular fold) may lead to the constant or frequent entry of air into the vagina. The condition can be exacerbated during oestrus, when the perineal body and the cervix are more

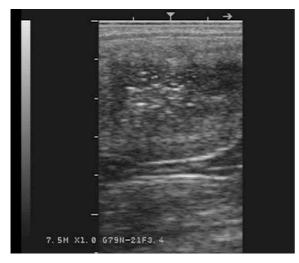


Figure 7.4 The presence of air in the uterus (pneumouterus) can be seen in this sonogram depicting the uterine body filled with hyperechoic particles (air). This mare had poor perineal conformation and pneumovagina.

relaxed than at other stages of the oestrous cycle. Accumulation of small amounts of a frothy fluid in the cranial vagina may be indicative of pneumovagina, as well as the presence of pneumouterus (air seen as hyperechoic particles between the endometrial folds during ultrasonographic examination: Fig. 7.4).

Clitoral swabbing

In some countries it is recommended that swabs should be collected routinely from all mares at the start of the breeding season. In the UK, the Horserace Betting Levy Board's *Code of practice for the control of contagious equine metritis and other equine reproductive diseases* recommends that a clitoral swab should be collected before moving the mare to the stallion stud farm. It also recommends that an endometrial swab should be taken on the stud farm at the oestrus prior to mating.

Technique

If there is gross contamination of the vulva, it should be wiped with a dry paper towel. The clitoris is exposed using a gloved hand to part the vulvar lips, and everted by placing the index finger below the



Figure 7.5 Clitoral area exposed to show the central sinus.



Figure 7.6 Clitoral area exposed to show the clitoral fossa.

vulvar lips. The central and, if present, lateral sinuses are swabbed with a paediatric-type narrow-tipped swab (Fig. 7.5). A standard-type swab is used to swab all areas of the clitoral fossa (Fig. 7.6). The swabs should be placed in Amies charcoal-based transport medium and sent to a 'designated' or 'approved' laboratory for the purpose of testing for the contagious equine metritis organism (*Taylorella equigenitalis*). The sample should preferably be kept at 4 °C and reach the laboratory within 48 hours.

Comments

• The lateral sinuses may be too shallow to harbour *T. equigenitalis*.

• For all procedures, any assistant holding the mare's tail out of the way should wear disposable gloves and change them between mares.

Manual examination of the internal genital tract per rectum

Following the perineal conformation inspection, a pregnancy diagnosis using palpation per rectum and transrectal ultrasonography should be performed to rule out pregnancy. *It is very important to withhold any vaginal, cervical or intrauterine examination (endometrial swabbing) until confirming that the mare is not pregnant.*

Restraint

The mare should preferably be restrained in stocks. Most mares will calmly walk through stocks; the back door should then be closed, with the operator staying away from the swing of the door in case the mare kicks at the door. Once the back door is shut, the front door is closed. Cross-ties can be used to secure the mare's head; it is recommended to have someone standing by the mare's head until the procedure is completed. Reassuring the mare with voice and touch may help some mares to walk to the stocks and to stand quietly during the procedure.

The mare can also be examined in her loose box. The handler should always stand on the same side as the clinician so that when the head is pulled towards the handler the hindquarters automatically move away from the clinician. A bridle with a Chifney bit gives the handler more control. Additional aids include lifting the foreleg on the same side as the examiner, or applying breeding hobbles.

Comment

• Whatever restraint is being used, the application of a nose twitch or intravenous administration of tranquillizers to uncooperative mares is useful. Clinicians should keep in mind that even heavily sedated mares may still kick out unexpectedly.

Preparation for examination

The mare's tail hairs can abrade the rectal mucosa and the tail should therefore be bandaged; neoprene

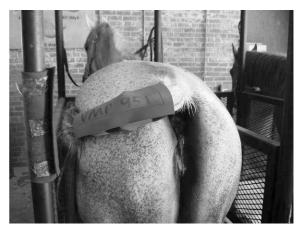


Figure 7.7 Velcro tail wrap is easily applied to prevent tail hairs entering the rectum during reproductive palpation.

tail wraps with Velcro[®] fasteners (Fig. 7.7) provide an easy and fast way to keep the tail hairs from getting into the rectum; alternatively, the tail can be placed into a disposable plastic palpation sleeve and secured with tape at the base of the tail.

Technique

The mare's rectum tears more easily than that of the cow. If the rectum fills with air ('ballooning'), the fingers should be kept behind a peristaltic wave and withdrawn. The clinician should never push against a peristaltic wave. Faecal balls should be removed as far as the reach of the clinician's arm. During the removal of faecal balls, an opportunity presents to palpate the cervix for its tone and size.

Cervix. The cervix is palpated by sweeping the hand from side to side over the floor of the pelvis near the pelvic brim. A thick cord-like structure will be identified, which can then be palpated in more detail by pressing downward with the fingertips. The objective is to assess the cervical tone, which can be classified into four levels: grade 1 would be the firmest tone, while grade 4 would be the tone felt typically around ovulation, a time when the mare experiences maximum relaxation of reproductive tract tissues.

Uterus. Once the rectum has been emptied, the examiner's arm is introduced into the rectum up to his/her shoulder. The hand is cupped and gently

Stage of cycle	Cervix	Uterus	Ovaries
Oestrus	Relaxed* Oedematous	Oedematous Flaccid	>25 mm follicle
Dioestrus	Firm Narrow	Increased tone Tubular	Multiple small follicles or a >25 mm follicle
Anoestrus	Moderately firm or thin and open	Flaccid	No palpable structures
Transitional	Not tightly closed until first ovulation	Flaccid	Multiple follicles can be >30 mm

Table 7.1 Changes in the mare's genital tract that are palpable per rectum

*The cervix may not relax at oestrus in maiden mares.

brought back sweeping the rectal floor; the intestinal loops are not caught by this sweeping motion and the examiner's hand invariably ends up against the bifurcation of the uterus. The examiner can then follow a uterine horn up to its ovary and repeat the procedure for the other side. At palpation, the uterus feels soft, flat and often flaccid. To confirm that it is the uterus, the tissue is slipped between fingers and thumb to palpate the longitudinal endometrial folds. A check is made for ventral enlargements, which can occur at the horn–body junction. These can be endometrial cysts or more usually lymphatic lacunae.

In addition to palpating the internal reproductive tract (cervix, uterus and ovaries), the examiner should palpate the floor of the pelvis and the shafts of the ilium in a sweeping motion toward the sacrum to detect any potential lesions (broken pelvis), which may cause dystocia. Specific structures are then located.

Ovary. The uterine horns can be followed up to the ovaries. The location of the ovaries can be quite variable. They are often located cranial and lateral to the mid-shaft of the ilium at '2–3 o'clock' and '9–10 o'clock'. They measure approximately 5–8 cm in length and 2–4 cm in width. The length of the mesovarium limits the extent to which the ovaries are movable.

The ovaries are kidney-shaped and orientated longitudinally. The free, concave border containing the ovarian fossa is located ventrally and medially, and should always be palpated during a reproductive examination. The ovaries are considerably mobile compared to the uterus and can be flipped for better examination of ovarian structures. If they lie laterally to the broad ligament then accurate palpation will not be possible unless the ovaries are first manipulated on to the craniomedial aspect.

As the preovulatory follicle matures, it protrudes from the ovarian surface, creating a shoulder. The follicle reaches an average size of 45 mm on the day before ovulation and usually starts to soften 2 days before ovulation. After ovulation, the crater becomes filled with blood to form a plum-like corpus haemorrhagicum (CH). The CH feels spongy and nonfluid-filled. The corpus luteum (CL) is formed 4–5 days later and blends into the ovarian stroma.

Palpable features of the cervix, uterus and ovaries vary with the stage of the oestrous cycle and are shown in Table 7.1.

Ultrasonography

The introduction of transrectal ultrasonography as a diagnostic aid in evaluating the mare's genital tract had a profound effect on the accuracy of detecting ovarian structures and uterine pathology, and facilitated the early diagnosis of pregnancy. Serial ultrasonography of the reproductive tract of the mare is very useful in detecting the stage of the oestrous cycle.

Most of the ultrasound scanners used for transrectal ultrasonography are of the B-mode linear array type. There are commonly three different transducer frequencies: 3.5, 5 and 7.5 MHz. Lowerfrequency transducers have greater penetration but poorer resolution. Thus 3.5 MHz transducers are better suited to examining the late pregnant or early postpartum uterus. The 5 MHz transducer is the most versatile transducer for reproductive examinations of the non-pregnant and early pregnant mare.

Technique and image interpretation

Faeces are evacuated from the rectum. Manual examination should always precede ultrasonographic examination to facilitate orientation of the genital tract and to assess the shape, tone and size of the individual components. A disposable plastic sleeve, containing lubricant as a contact gel, can be placed over the probe. The probe should be protected by the hand, which forms a cone shape during entry into the rectum. The hand should remain cupped around the transducer and protect the rectal wall during the scanning procedure. The investigation should always be systematic to avoid possible scanning errors. The transducer is held longitudinally with respect to the mare's body, so that the cervix and uterine body are seen sagittally with the cervix to the left of the screen. The uterine horns are then seen in cross-section.

The appearance of the uterus changes during the oestrous cycle. In oestrus, the uterine horns and body have a characteristic pattern of alternating echogenic and hypoechoic areas. This corresponds to oestrual oedema. The hypoechoic areas are thought to be the outer oedematous portions of the endometrial folds (Fig. 7.8). Oedema often, but not always, decreases or disappears within the 24 hours before ovulation. During dioestrus the uterus takes on a much more homogeneous appearance (Fig. 7.9). The uterine lumen is often identifiable by an echogenic line when the uterus is viewed longitudinally.

Ultrasonography of the ovaries is used to provide information on:

- Whether the mare is cycling
- The stage of the cycle
- The predicted ovulation time
- Double ovulations

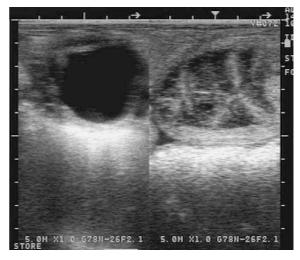


Figure 7.8 Ultrasound images. Right: uterine oedema indicative of low progesterone and physiological oestrus; left: preovulatory follicle filled with characteristic anechoic fluid.

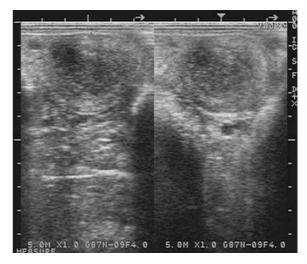


Figure 7.9 Ultrasound images of left and right uterine horns during dioestrus. Note the spherical contour of the cross-sections of uterine horns with their characteristic uniform echogenicity.

- Failure to ovulate
- Whether a follicle is of adequate size to induce ovulation by pharmacological means
- Ovarian pathologies (epithelial inclusion cysts, ovarian haematoma, hypoplasia, granulosa-cell tumours, paraovarian cysts, etc.).

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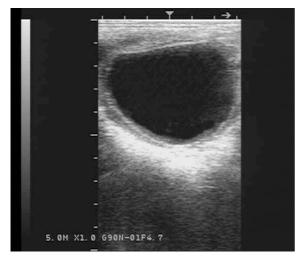


Figure 7.10 Ultrasound image of a mature preovulatory follicle showing slight distortion.

Follicles appear as black (anechoic) areas. Some follicles appear to be an irregular shape owing to compression from surrounding structures or, occasionally, because apposing walls are not detectable (Fig. 7.10). Follicular diameter can be measured by freezing the image and using the callipers on the machine. A 5 MHz transducer can detect follicles as small as 2–3 mm.

Preovulatory follicles attain a mean diameter of 45 mm on the day before ovulation in natural cycles. 85% of follicles change shape, developing a wedge shape or a neck-like protuberance pointing towards the ovulation fossa on the day before ovulation. The shape change, plus size and soft consistency, are currently thought to be the best predictors of imminent ovulation.

The occurrence of ovulation is easily diagnosed by ultrasonography based on the disappearance of a large follicle that had been recorded previously and the appearance of a newly forming corpus luteum, which in most mares is highly echogenic (Fig. 7.11). Sometimes the ovulation crater then fills with blood, forming a corpus haemorrhagicum, which appears as mottled hypoechoic areas of serum interspersed with echogenic fibrinous bands (Fig. 7.12).



Figure 7.11 Ultrasound image of a mature, uniformly hyperechoic corpus luteum.



Figure 7.12 Ultrasound image of a corpus luteum that is filled with pockets of serum and interspersed fibrinous strands.

Endometrial swabbing

Whenever indicated, the endometrium can be swabbed at the oestrus prior to breeding; if a positive culture results and therapy is instituted, the mare may be swabbed at subsequent oestruses, if necessary, to confirm that the infection has subsided. However, mares without clinical signs of



Figure 7.13 Double-guarded uterine culture swab with all three components exposed. Once inside the uterus, the second protective casing is pushed through the outer casing and then the swab is pushed into the uterus. Once uterine sampling is performed, the swab is then double-guarded in the reverse order.

endometritis (as determined by ultrasonography) should not be routinely swabbed. Swab samples are highly prone to contamination and therefore careful aseptic procedures are essential. Culture of cervical mucus is thought to be unreliable in reflecting the presence of bacteria in the uterus. Double-guarded swab techniques give the most reliable results (Fig. 7.13).

The perineum should be washed three times with a non-residual soap or povidone-iodine, rinsed thoroughly with warm, clean water and dried with clean paper towels. The clinician wears a clean plastic sterile sleeve and cups the end of the culture instrument in his/her hand. Sterile water-soluble lubricant is applied to the back of the hand and arm before entering the vestibule and vagina. The instrument is guided through the cervix by the index finger. The inner tube is then pushed through the opening at the tip of the outer tube and the swab is advanced to contact the endometrium. After 10 seconds the swab is retracted into the inner, and then the outer tube, and withdrawn from the uterus. Alternatively, a standard swab can be passed into the uterus on an extension rod via a sterile vaginal speculum. The swab should be placed in Amies transport medium, labelled clearly and forwarded to the laboratory where it should arrive within 48 hours of collection.

Endometrial cytology

Preparative cleansing of the perineum and vulva for endometrial cytology is the same as described above. There are some instruments available that can provide both culture swabs and cytology smears. More commonly, a second swab is used after the



Figure 7.14 Single-guarded uterine culture swab showing all components. Once inside the uterus, the swab is pushed through the outer casing to sample the uterus. Once the swab is guarded back into the protective outer casing, the device is rotated a few times to sample endometrial cells for uterine cytology. Endometrial cells are often successfully collected in the cap and then smeared on to a glass slide.



Figure 7.15 The use of a uterine cytology brush is an efficient method to sample endometrial cells for cytology.

initial swabbing to obtain a sample of endometrial cells. This swab can then be smeared on to a proprietary slide, which stains the cells (Testsimplets: Boehringer Ingelheim, UK), or on to an ordinary microscope slide (Fig. 7.14) that is then stained with Geimsa or Diff-Quik (Baxter Healthcare Ltd, Thetford, UK). Optimal cytology specimens are obtained when human cervical brushes are adapted for use in mares (Fig. 7.15). A cytology fixative may also be used once a smear is made on to a glass slide to prevent cellular distortions and artefacts.

Comments

 Manipulations involving entry into the uterus are best performed during oestrus. It is inevitable that microorganisms will gain entry to the uterus from the vestibule and vagina, which contain resident populations of microorganisms. Mares can best eliminate this contamination during oestrus. If an endometrial swabbing is performed during dioestrus, the mare should be given a luteolytic dose of prostaglandin (PG)F_{2α} to minimize the chances of possible contamination, resulting in endometritis. A dioestrus digital examination is indicated if there is concern about cervical integrity.

• Endometrial cytology should always be performed in conjunction with endometrial culture swabbing in order to aid in the interpretation of culture results.

Vaginal examination

Vaginal examination can be useful in identifying cycle stage and pathological and anatomical changes. This procedure can be performed before or after endometrial swabbing. The advantage of performing speculum examination before swabbing is that the cervix will not have been digitally manipulated prior to viewing. Furthermore, air contact quickly causes artefactual reddening. The disadvantage of performing it before swabbing is that there is an increased chance of contamination of the external os of the cervix, which will then be transferred into the uterus at the time of swabbing.

Various types of speculum have been developed for vaginal examination. A metal trivalve or duckbilled speculum has been in common use and is an essential instrument for examining postpartum mares because it provides a more detailed and broader view of the vaginal wall (especially important when looking out for tears, etc.). A disposable speculum provides good visibility of the cervix and cranial vaginal floor and can be discarded after each mare, which is ideal when more than one mare needs to be examined. Plastic tubes that fit over a metal template with an integral light source provide good visibility of the cervix and vagina but need to be sterilized between mares (Fig. 7.16).

Sterile, water-soluble lubricant should be applied to the speculum, which is then inserted into the vestibule at a craniodorsal angle of 45° with the vulval lips parted. Once the speculum passes through the transverse fold, it can be introduced horizontally. A moderate resistance at the vaginovestibular fold is commonly felt in reproductively normal mares, indicating the presence of a competent

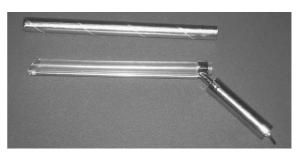


Figure 7.16 Disposable vaginal speculum (top) and autoclavable plastic speculum.

sphincter. The best technique is to apply constant gentle pressure and rotate the speculum as it advances until no resistance is suddenly felt (a sign that the speculum has reached the vagina proper). A pen torch or ophthalmic light source can be used to illuminate the cervix and vagina. Various changes in the appearance of the vaginal mucosa and cervix (including its position in relation to the vaginal floor) can be detected according to the stage of the cycle (Table 7.2).

Digital examination of the vagina and cervix

Lesions can be missed in the vagina and cervix if manual examination is not carried out. A clean plastic sleeve with a sterile glove should be worn. The lubricant used should be sterile and water-soluble, such as K-Y Jelly (Johnson and Johnson). The vagina and cervix should be palpated for tears and adhesions. Tears in the cervix are most obvious during dioestrus when good tone is present. Digital examination of the vagina and cervix can be conveniently performed during the endometrial swabbing procedure.

Endometrial biopsy

Examination of the mare's endometrium is important when investigating causes of reproductive failure. Mares with extensive endometrial fibrosis (endometrosis) or disseminated chronic endometritis may become pregnant, but the endometrial pathology invariably leads to fetal resorption or abortion. As well as mares with subfertility prob-

Parameter	Oestrus	Dioestrus	Anoestrus	Pregnancy
Number of fingers that can be passed	Three or more	One	One to three (or more)	One
Colour	Red	Pale grey or yellow	Pale white	White
Appearance	Glistening Oedematous Slit-like cervical os	Dry Closed	Dry Can be atonic and open	Dry Closed
Position	On floor of vagina	In mid-vagina	In mid-vagina	In mid-vagina

Table 7.2 Cyclical changes in the cervix detectable by speculum or digital examinations

lems, mares requiring genital surgery should be biopsied prior to surgery to give an indication of their ability to carry a foal to term. Biopsy can also be used to monitor the response to therapy in mares with uterine infections. In that case, if a biopsy is taken during dioestrus, it is recommended to administer PGF_{2α} to decrease the chances of reinfection. It is important to include a history and give the stage of the cycle at which the biopsy was obtained.

Technique

Preparative cleansing of the perineum and vulva prior to endometrial biopsy is the same as described above under 'Endometrial swabbing'. The biopsy procedure is as follows:

- Large alligator-jaw forceps are available for obtaining endometrial biopsies (Fig. 7.17). These are introduced into the vagina by a lubricated hand covered in a plastic sleeve under a sterile glove, or simply using a sterile plastic palpation sleeve. The forceps are guided through the cervix by the index finger and advanced until they reach the body of the uterus. The hand is then withdrawn and inserted into the rectum.
- The jaws of the forceps are kept closed until they are located in the uterus by the hand in the rectum. The jaws should be positioned sideways (horizontally), then opened, and the uterine endometrium is pushed down into the jaws, which are then closed. The bite should be taken by pushing the tissue down or sideways into the



Figure 7.17 A large alligator-jaw forceps is used for obtaining endometrial biopsies. To avoid tissue damage and artefacts, a fine needle (25G) is used to carefully remove the tissue within the basket.

jaws of the forceps rather than from in front, since this may cause penetration of the uterine wall.

- The forceps are then removed, keeping the jaws shut. If the tissue has not been completely severed it is sometimes necessary to give the forceps a gentle tug. Very occasionally slight bleeding is seen from the vulvar lips after biopsy, but recovery is typically uneventful. No further treatment is usually necessary; however, an intravenous injection of oxytocin (10–20 units) may be given to promote uterine contractions.
- The biopsy sample should be carefully removed from the jaws with a fine needle or forceps and placed in Bouin's fixative solution or an alternative fixative prescribed by the laboratory. If the biopsy is not likely to reach the laboratory within 24 hours, it should be transferred to 70% alcohol on the day after collection.

Interpretation of results

Morphology of the normal endometrium varies with the stage of the cycle. Thus, it is important to include a reproductive history and to give the stage of the cycle at which the biopsy was obtained.

During anoestrus the endometrial glands are inactive and the epithelium is cuboidal or low columnar. Anoestrous mares frequently have groups of closely associated glands due to coiling of gland branches. No oedema is present.

During oestrus the epithelium becomes columnar to tall columnar. Vacuoles are common in the basal cytoplasm of the luminal epithelium. Neutrophils are often seen in capillaries under the luminal epithelium and at the margin of blood vessels in the lamina propria, but not in the tissue. There may be considerable oedema. Glands appear relatively straight and non-tortuous.

In dioestrus the epithelium varies from cuboidal to columnar depending on the proximity to oestrus. The glandular branches appear highly coiled and tortuous, giving the appearance of a 'string of pearls'.

Examination for breeding soundness – ancillary techniques

Chromosome analysis

The incidence of chromosomal abnormalities in horses is not known, but in human beings chromosomal abnormalities have been associated with abortion, infertility and congenital defects. The most commonly reported chromosome problems are errors in structure or number of sex chromosomes in infertile or subfertile mares. Errors in sex chromosomes and other chromosomes can cause unthriftiness and growth retardation in either sex; some affected horses are healthy and grow to the standard size to their breed, but show reproductive abnormalities.

Karyotyping (chromosome analysis) can be performed on any tissue with dividing cells. Peripheral blood lymphocytes are normally used. Blood samples (10 ml) should be collected into heparin or acid citrate dextrose (ACD) and transported overnight to a specialist referral laboratory.

Progesterone analysis

Serum progesterone assay is a reliable monitor of ovarian function. Serial samples can confirm cyclical ovarian function. If three samples collected at weekly intervals are consistently low (<3 nmol/l), it is likely that the mare is anoestrous. If the samples are consistently above 6 nmol/l, it is likely that the mare has prolonged luteal function or has had a late-dioestrus secondary ovulation.

The progesterone assay is also useful in confirming that a mare is in oestrus if she is not displaying overt signs. Analysing a sample collected 2 days after injection of PGF_{2 α} will confirm whether the mare has experienced luteolysis and can be expected to return to oestrus. The progesterone concentration should be low at this time (<3 nmol/l).

Comments

- Progesterone is a poor indicator of pregnancy in the mare, with a high false-positive rate. However, basal progesterone concentrations (<3 nmol/l) at around 18–21 days after ovulation are thought to be 100% accurate in detecting *non-pregnancy*.
- Determination of serum progesterone may assist in assessing the end of the vernal transition, once the first ovulation of the year has occurred. It may also assist in confirming ovulation or determining the stage of the oestrous cycle if teasing, palpation or ultrasound findings are inconclusive.

Endoscopy of the uterus (hysteroscopy)

The use of endoscopy should be considered when a localized problem within the endometrium has been diagnosed by palpation or ultrasonography, or when the cause of infertility cannot be determined by other means. Flexible fibreoptic endoscopes should be used, at least 1 m in length, with an outer diameter of at least 10 mm. The endoscope should preferably be gas sterilized before use, but failing this the working end can be cleaned using disinfect-ants recommended by the manufacturer. It is essential that any chemical sterilization agents are actively flushed from the biopsy channel using sterile water.

The endoscope should then be rinsed in alcohol and allowed to dry prior to use.

Technique

- The mare's perineum and vulva should be cleansed as described previously under: 'Endometrial swabbing'.
- The endoscope is introduced into the uterus through the cervix by a lubricated, sterile-gloved hand.
- The lumen should be distended with a nonirritant gas such as CO₂, or using sterile saline. When infusing saline, 1–2 litres can be run through an embryo collection catheter once the endoscope is in the uterus.
- The normal endometrium appears pink and free of exudate. The endoscope is advanced to the uterine bifurcation and then down each horn until the oviductal papilla is seen at the end of the horn.

Comments

- This procedure is best performed in dioestrus when the cervix is closed. If the mare is susceptible to endometritis, it may be advisable to infuse antibiotics into the uterus after endoscopy. Just as with endometrial swabbing and uterine biopsy, if the procedure is performed during dioestrus, the mare should be treated with a luteolytic dose of $PGF_{2\alpha}$ once the procedure is completed.
- Hysteroscopic insemination utilizing low-dose sperm numbers deposited at the oviductal papilla is becoming increasingly common.

Pregnancy diagnosis

Pregnancy diagnosis by transrectal palpation

Rectal palpation for pregnancy diagnosis is accurate after about 28 days but is easiest to perform around 42 days of gestation.

Ovarian changes

Numerous follicles are palpable in the ovaries during the first 100 days of pregnancy. Supplemen-

tary corpora lutea comprise secondary corpora lutea (follicles that ovulate) and accessory corpora lutea (follicles that luteinize), which form on the ovaries after day 40.

Cervical and uterine changes

- *Day 1*. The cervix closes and uterine tone increases.
- *Day 14*. There is marked tubularity of the uterus.
- *Day 21*. The cervix is long and tightly closed. The endometrial folds are no longer palpable. The conceptus may be palpated as a ventral swelling (1.5–3 cm in diameter) at the base of one of the uterine horns.
- *Day 28*. The spherical embryonic vesicle (2–3 cm in diameter) is palpable ventrally at the base of the pregnant horn.
- *Day 42*. The enlargement is slightly oval, approximately 5 cm in diameter and occupies one half of the pregnant horn. The non-gravid horn remains tonic.
- *Day 60*. The conceptus measures approximately 12 cm in diameter and fills the gravid horn. The ovaries are now progressively pulled cranially and medially. They are no longer palpable by about day 150.
- *Day 80.* Most of the body and both horns are filled by the conceptus.
- *Day 90*. Decreased pressure in the allantois allows the conceptus to be detected by ballottement. The enlarged uterus begins to descend over the pelvic brim and cannot be retracted.
- *Day 200*. The descent of the uterus is complete. Fremitus in the uterine arteries is present from day 150.
- *Day 200 plus*. The uterus starts to ascend and the fetus is easily palpable.

Comments

- Early pregnancy diagnosis may be difficult in older mares that are bred at a foal heat since the uterus may still be enlarged.
- A filled urinary bladder (located ventral and/or cranial to the brim of the pelvis), or fluctuating pelvic flexure of the colon, can be mistaken for

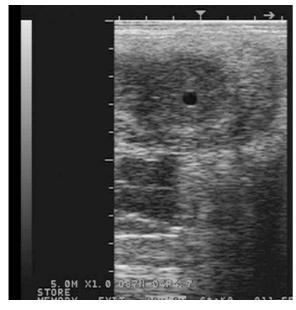


Figure 7.18 Ultrasound image of a 10-day conceptus measuring 4 mm in diameter.

a pregnant uterus. A systematic approach and identification of the cervix and uterus will allow correct diagnosis. The closed cervix can be followed to the dorsal bulge of the gravid uterine body.

Pregnancy diagnosis by ultrasonography

The embryonic vesicle is first detectable in the pregnant horn as a round, hypoechoic structure of approximately 4 mm around day 10 (Fig. 7.18). Detection of the vesicle is aided by the bright ultrasound echoes (specular reflection), which are often seen on the dorsal and ventral surfaces of the vesicle. The embryo 'fixes' on day 16 (Fig. 7.19) and changes from a round to a triangular ('guitar-pick') shape by day 18 (Fig. 7.20). On day 21 the embryo is first detected on the ventral surface of the vesicle, typically between the 5 and 7 o'clock positions. By day 25 a heartbeat is detectable and the fluid-filled allantoic sac is visible as a non-echogenic area beneath the embryo. At day 30 the allantois occupies the ventral half of the vesicle (Fig. 7.21) and by day 40 the yolk sac has regressed and the allantois fills most



Figure 7.19 Ultrasound image of a 16-day conceptus at time of embryo fixation.



Figure 7.20 Ultrasound image of a 22-day pregnancy. Note the 'guitar-pick' appearance of the whole conceptus.



Figure 7.21 Ultrasound image of a 30-day pregnancy.

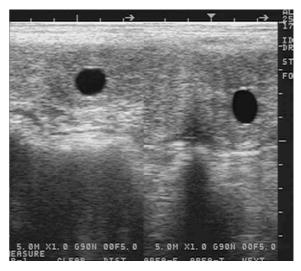


Figure 7.23 Ultrasound images of twin conceptuses located bilaterally.



Figure 7.22 Ultrasound image of a 50-day pregnancy.

of the vesicle. By day 50 the umbilical cord has lengthened and the fetus has descended to the floor of the allantois (Fig. 7.22).

Comments

• Twin conceptuses can be detected reliably at day 14 (Fig. 7.23). A thorough search of both

uterine horns and the body should always be performed. Intraluminal cysts can be confused with a conceptus but, since they do not move or grow, a recheck in 1–2 days can help to confirm the diagnosis. If there is uncertainty about diagnosis, the mare should be re-examined at around day 24 when the embryos are easily visible.

- Transabdominal ultrasonography requires a 3.5 MHz transducer and cannot be performed until about day 80. It is useful, but not 100% accurate, for diagnosing twins that are thought to have been missed earlier. Accuracy increases closer to term provided that a meticulous search of the ventral abdomen is performed.
- Although a 3.5 MHz transducer is preferred for abdominal ultrasonography, a 5 MHz transducer may be useful in examinations for pregnancies after day 80 and examination of the combined utero-placental thickness.

Hormone assays

Serum oestrone sulphate

This is produced by the fetoplacental unit after synthesis from precursors that are produced by the enlarged fetal gonads. Thus, high levels confirm the viability of the fetus. It is present in high concentrations from day 90 to late gestation.

Equine chorionic gonadotrophin

Equine chorionic gonadotrophin (eCG – formerly known as pregnant mare serum gonadotrophin (PMSG)) is present in high concentrations in serum between days 40 and 120. False positives can be recorded if the fetus dies, because the endometrial cups remain active until day 120–140 gestation. Therefore, high eCG concentration indicates the presence of endometrial cups, not a viable fetus. See also Chapter 1: 'Submission of samples and interpretation of results', under 'Pregnancy tests'.

Investigation of abortion, stillbirth or birth of a sick foal

Abortion rates for Thoroughbreds have been estimated to be greater than 12%. Strictly speaking, 'abortion' refers to fetuses lost before 300 days and those lost after 300 days are called 'stillbirths'.

Investigative approach

It is necessary to adopt a methodical approach to investigate a cause of abortion. The Horserace Betting Levy Board's Code of Practice (UK) recommends that veterinary intervention should take place in the case of any abortion, stillbirth or foal death that occurs within 14 days of birth.

Ideally, the whole fetus plus the fetal membranes should be sent to a specialist laboratory along with a serum sample from the mare and a detailed clinical history. Otherwise, the following procedure should be adopted:

- The crown-rump length is measured to assess fetal age and development.
- The placenta is examined. The recommended method is to lay it flat in an F shape with the cervical star at the bottom of the F, the pregnant horn forming the top arm and the non-pregnant horn the lower arm. A check is made to see whether the foal has exited through the cervical star. After normal expulsion, the

smooth allantoic surface of the allantochorion should be outermost. The length of the cord is measured and cord blood is collected. The placenta is then turned inside out and the chorionic surface is examined for avillous areas and for areas of discoloration.

- A gross general examination is made of the fetus noting the appearance of organs and any fluid in body cavities. The presence of fractured ribs, haemarthrosis of the shoulder joint or subcutaneous oedema of the head can indicate dystocia.
- Microbiological culture: the liver, stomach contents, lung, allantochorion (near the cervical star) and allantoamnion are sampled aseptically and put in sterile containers packed in ice.
- Virus isolation: the liver, lung and thymus are sampled and put in sterile containers packed in ice.
- Histological examination: the liver, lymph node, adrenal gland, lung, thymus, spleen, allantochorion and allantoamnion are submitted as tissue sections (1 × 2 × 2 cm) in phosphate-buffered formalin (10% formol saline) or Bouin's fixative.
- Blood is taken from the mare for serology preferably as paired samples.

If infection is suspected and the foal becomes unwell within 14 days of birth, isolate the mare and foal. Nasopharyngeal swabs and heparinized blood samples should be collected and sent to a specialist laboratory for virological examination.

Infectious causes of abortion – diagnostic features

Infectious causes of abortion are associated with viral, bacterial and fungal agents.

Equine viral rhinopneumonitis (EHV-1)

This highly contagious herpes virus abortion is not associated with concurrent maternal illness and tends to occur between 7 months of gestation and term. Abortion at an earlier stage is possible.

Gross examination of the fetus will reveal:

Genital diseases, fertility and pregnancy

7

- Recent death (not autolysed)
- Petechiation on mucosae
- Jaundice
- Enlarged liver and spleen
- Focal areas of necrosis in the liver
- Subcutaneous oedema
- Serosanguineous fluid in body cavities.

Where facilities allow, the best diagnostic test is the fluorescent antibody test, performed on chilled or frozen samples of lung, thymus, lymph node, spleen and adrenal cortex. Second best is virus isolation, followed by detection of viral inclusion bodies in histopathology of the liver, lung and thymus.

Equine viral arteritis (EVA)

In the dam, clinical signs range from asymptomatic to severe systemic illness. The typical clinical presentation is fever, lethargy, depression, conjunctivitis, nasal discharge, urticarial rashes and oedema. The fetus shows no specific lesions but is autolysed.

Paired blood samples from the mare should be submitted for serology. The stallion should also be tested. Virus isolation is possible from:

- Nasopharyngeal swabs (mare)
- Heparinized blood (mare)
- The stallion's semen
- The stallion's urine.

If the stallion is found to be seropositive, he has either been vaccinated or has previously been infected with the virus. Because a proportion of stallions become persistent 'shedders', it is important to ascertain whether virus is present in their semen.

Bacterial infections

Placental examination. Inflammation of the allantochorion is usually most severe around the cervical star (the ascending route of infection). The placenta is oedematous and the chorionic surface tends to be brown with a variable amount of fibrinonecrotic exudate.

Microbiology. Samples of the fetal organs and stomach contents, together with the placenta, should be submitted for bacteriology.

Fungal infections

Placental examination may reveal extensive chorionic oedema and necrosis, originating at the cervical star.

Histopathology of the placenta demonstrates fungal hyphae.

Microbiology. Samples of the fetal organs and stomach contents, together with the placenta, should be submitted for fungal culture.

Non-infectious causes of abortion – diagnostic features

Twinning: one twin is frequently small and autolysed, whereas the other is fresh. Placental examination should reveal that areas of placenta–placenta contact are without a normal villous structure.

Placental insufficiency: in these cases the fetal crown-rump length is less than predicted for the gestational length and the fetus appears emaciated. Endometrial biopsy may reveal endometrial fibrosis or cysts.

Uterine body pregnancy: the fetal membranes are underdeveloped and the fetus shows retarded growth.

Premature placental separation: the fetus is full term. There is an incomplete or complete tear in the middle of the body of the allantochorion. The placenta is often thickened and the detached areas are dry and brown.

Umbilical cord damage: If the cord is more than 84 cm in length, it is predisposed to excessive twisting. Once the blood supply is occluded, the fetus dies and autolyses.

Differential diagnosis of genital diseases in the mare

The various conditions outlined in this section are associated with a failure to conceive or embryo loss. The applied diagnostic techniques that are indicated have already been described above under: 'Examination for breeding soundness'.

Small ovaries

The differential diagnoses for small ovaries include: hypoplasia (congenital); atrophy (acquired) and winter anoestrus.

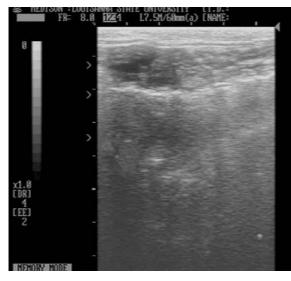


Figure 7.24 Ultrasound image of ovaries of a mare with gonadal hypoplasia. Note the absence of follicular activity.

Diagnostic techniques

Palpation: the ovaries are small and smooth; the uterus and cervix are flaccid.

Ultrasonography: no obvious follicles are present (Fig. 7.24).

Karyotype: the most common abnormality is 63XO.

Enlarged ovaries

The differential diagnoses include:

- Pregnancy (first trimester)
- Spring transition
- Haematoma
- Tumour (most commonly the granulosa cell tumour; Fig. 7.25)
- Anovulatory haemorrhagic follicle
- Paraovarian cyst
- Abscess (Fig. 7.26).

Diagnostic techniques

Palpation: in general, mares with a granulosa cell tumour usually have one large ovary and one that is very small and hard. The large ovary can be uni-

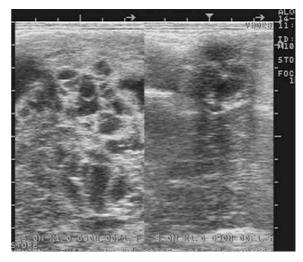


Figure 7.25 Granulosa cell tumours are often unilateral (left) with the contralateral ovary small and inactive (right). Granulosa cell tumours with a large single cavity are less common than those with honeycomb appearance (left).

formly smooth, knobbly and hard, or soft and fluctuating. The typical indentation at the ovarian fossa of the affected ovary is not palpable as tumoral cells fill the area.

Transrectal ultrasonography: Postovulatory haematomas are seasonal and have a mottled appearance. The fluid-filled areas probably represent pockets of serum. Serial examinations by transrectal ultrasonography may reveal tissue organization changing from fluctuant fluid to a more solid ('organized') appearance. Classic granulosa cell tumours are described as having a honeycomb appearance with distinct multilocular cysts. However, the appearance of affected ovaries can vary from being uniformly echogenic to having one (or a few) large fluid-filled cysts.

Anovulatory follicles can grow to 100 mm. The follicle may be filled with clear follicular fluid, or some eventually become progressively filled with blood, and fibrin strands can be seen (Fig. 7.27).

The diagnosis of paraovarian cysts is relatively easy with the aid of ultrasonography. The folliclelike fluid-filled structure may be confused with an ovarian follicle but careful examination will reveal the absence of its association within the ovary.

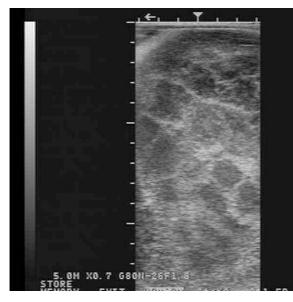


Figure 7.26 Ovarian abscess. Exudates from the ovary leaked into surrounding abdominal tissues causing adhesions in the ipsilateral uterine horn and in the gastrointestinal tract. The mare was euthanized after a colic episode.

Ovarian abscesses are very uncommon; they usually cause extensive adhesions of tissues surrounding the ovary and may include the intestines; colic is a typical sign of gastrointestinal involvement in a mare with an ovarian abscess. Ultrasonographically they appear as thick-walled structures with an echogenic centre.

Hormone assay: approximately 60% of mares with a granulosa cell tumour will have elevated concentrations of testosterone in the plasma (basal concentration: 0.02–0.5 nmol/l). Inhibin is elevated in approximately 90% of mares with a granulosa cell tumour and it is the single most important diagnostic hormone for this ovarian abnormality.

Uterine diseases

Diagnostic techniques for the following uterine diseases are indicated below:

- Acute endometritis
- Endometrial cysts

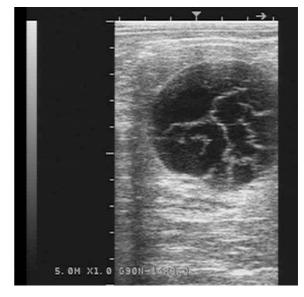


Figure 7.27 Ultrasound image of an anovulatory follicle filled with fibrin strands.

- Endometrial transluminal adhesions
- Endometriosis.

Diagnosis of acute endometritis

Ultrasonography: fluid of variable echogenicity can be found in the uterus and its presence during dioestrus is highly suggestive of endometritis (Fig. 7.28). Pneumouterus (secondary to wind sucking) can be seen as hyperechoic reflections caused by air in the uterus (Fig. 7.4).

Vaginal speculum examination: there may be evidence of vaginitis and/or cervicitis with exudate visible in the vagina or coming through the cervix, especially during oestrus. If the fluid is suspected to be urine, its creatinine and urea concentrations should be analysed and calcium carbonate crystals may be seen on cytology. The finding of debris and air bubbles indicates that the mare is a windsucker. If the speculum passes readily into the vagina without resistance at the vaginovestibular sphincter, it suggests that the transverse fold is not forming an effective seal.

Microbiology: the growth of known pathogens from an endometrial swab is diagnostic only if

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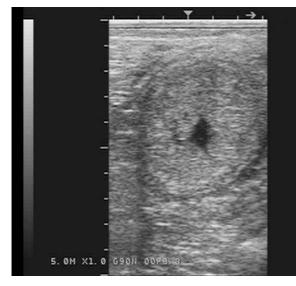


Figure 7.28 Ultrasound image of intraluminal fluid present during dioestrus: indication of endometritis.

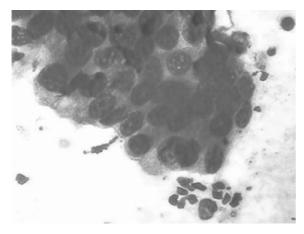


Figure 7.29 (Plate 4 in colour plate section) Cytology of the endometrium showing epithelial cells and polymorphonuclear cells.

corroborated by examination of endometrial cytology. Pathogens include: haemolytic streptococci, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Taylorella equigenitalis, yeasts and fungi.

Endometrial cytology: the presence of large numbers of neutrophils is evidence of endometritis. The exact proportion of neutrophils to epithelial cells depends upon the method of collection. In general,

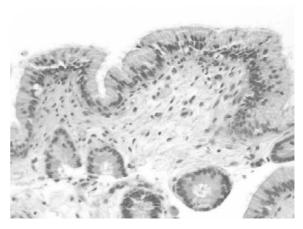


Figure 7.30 (Plate 5 in colour plate section) Endometrial biopsy showing diffuse inflammation of the stratum compactum.

if more than 2% of the cells are neutrophils, it is likely that the mare has an endometritis (Fig. 7.29 (Plate 4)).

Biopsy: infiltration of the biopsy tissue with neutrophils indicates an acute endometritis. These cells are usually found in the stratum compactum (the superficial layer of the lamina propria) and migrating between luminal epithelial cells. In more severe cases, neutrophils will be present in the stratum spongiosum (the deeper layer of the lamina propria). Glands may be dilated and degenerated neutrophils may be present in the lumen (Fig. 7.30 (Plate 5)).

Diagnosis of endometrial cysts

Palpation: cysts must be large and/or widespread before they will interfere with pregnancy. It is thought they may be significant if the whole uterus feels spongy. Palpation of a single cyst or a cluster of small cysts may be difficult to ascertain. Transrectal ultrasonography is the method of choice.

Ultrasonography: cysts can be classified as intraluminal (Fig. 7.31) or intramural. They can arise either from endometrial glands (<10 mm) or from lymphatics (>10 mm). They should be differentiated from the early embryonic vesicle by failure to grow, compartmentalization, failure to move and irregular appearance.

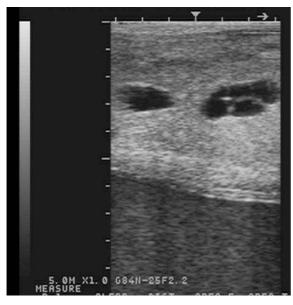


Figure 7.31 Ultrasound image of multiple uterine cysts.

Endoscopy: intraluminal cysts can be seen as shiny, fluid-filled structures that are often pedunculated.

Diagnosis of endometrial transluminal adhesions

Endoscopy: these appear as bands, sheets or tunnels of fibrous tissue.

Ultrasonography: adhesions are not usually visible. If one uterine horn is completely occluded, infusion of saline into the uterus will enable this to be demonstrated.

Diagnosis of endometriosis by biopsy

Endometriosis is characterized by a number of chronic, irreversible changes that can be defined by histopathological examination of uterine biopsies. A good correlation exists between the severity of histological endometrial lesions and the ability of a mare to carry a foal to term. However, the interpretation of biopsy results should be modified according to the age of the mare; increased age is associated with decreased fertility. The number of years barren also appears to have a significant effect on foaling probability in mares with mild to moderate inflammatory and fibrotic changes.

Cervical diseases

Cervicitis and vaginitis

These conditions are diagnosed at speculum examination (see above under: 'Vaginal examination').

Cervical laceration

Cervical laceration should be diagnosed by manual examination of the cervix per vaginam. This should be undertaken during dioestrus when the cervix is likely to have good tone. The index finger is passed into the cervical canal and the integrity of the muscular tissue is determined by palpation with the thumb, moving in a clockwise manner such that the entire circumference is evaluated.

Vaginal disorders

Apart from vaginitis, the differential diagnoses of vaginal disorders include pneumovagina, urovagina, persistent hymen and varicose veins. Pneumovagina and urovagina (urine pooling) are generally related to problems with perineal conformation. A persistent hymen is usually easily broken by vaginal examination with a speculum or by hand. Varicose veins are relatively more common in older pluriparous mares; these veins become more prominent during pregnancy or during oestrus. When significantly enlarged, thrombosis and ulceration of these varices cause bleeding that usually does not require treatment and regresses once pregnancy or oestrus ends. If bleeding is excessive, ligation of vessels, resection of submucosa or cautery by laser may be indicated.

Diagnosis of pneumovagina

Conformation: the perineum and the vulval seal should be examined (see above under: 'Examination of the vulva and perineal region').

Rectal palpation: in severe cases the uterus may be distended with air, making palpation of the reproductive tract difficult. Gentle pressure applied centrally to the rectum (dorsal to vaginal wall) may help to release the excessive air. Palpation per rectum should precede vaginal examinations to avoid causing artefactual pneumovagina.

Ultrasonography: echogenic air bubbles may be visible if pneumouterus is present. A large hypo-echoic or anechoic area caudal to the bladder may indicate pneumovagina.

Speculum examination: frothy exudate may be seen in the vagina.

Diagnosis of urovagina

Speculum examination: may reveal urine in the vagina.

Laboratory analysis: fluid assay for creatinine and urea may indicate urine. In addition, the fluid deposit should be examined for calcium carbonate crystals.

Diagnosis of persistent hymen

Speculum examination: may reveal a membrane visible in the region of the transverse fold. If not ruptured by the speculum, many persistent hymens will break upon digital examination with a sterile-gloved hand.

Transrectal ultrasonography: may indicate mucoid secretions accumulated behind the hymen.

II. THE STALLION

An examination of the stallion for breeding soundness should be performed routinely in the following circumstances:

- Before the start of the breeding season
- Before purchasing a stallion to be used as a stud horse
- In stallions with a history of subfertility.

Physical examination

Testicular measurement

The length, width and height of each testis should be measured and recorded in any breeding stallion's medical record. Measurements are best performed after collection of the first ejaculate because most stallions will not be as excited as before semen collection and will remain tractable during the examination. Testicular size is highly correlated with daily sperm output.

Calliper method

Tuberculin callipers can be used to measure testicular size. It is necessary to place the callipers on the testis without distorting the surface and therefore decreasing the diameter. Minimum total scrotal width should be 80 mm before a stallion should be passed as a satisfactory prospective breeder. Some commercially available callipers are designed to measure the total scrotal width. However, this measurement relates to horses; there is little data on smaller pony breeds. When measuring the length of the horizontally orientated equine testis, the clinician must ensure that neither the head nor tail of the epididymis is included in the measurement.

Ultrasonography

More accurate measurements of testis size can be made by ultrasonography using a 5 or 7.5 MHz transducer (see 'Further reading' for more detail). Distension of the central vein may be indicative of venous congestion associated with other pathological features (haematocele, orchitis, trauma, tumours that cause compression of testicular parenchyma, etc.).

Testicular consistency

Each testis is examined by passing it between the thumb and fingers after pushing the contralateral testis out of the way. The testes should be freely movable within the vaginal tunic. The epididymis, particularly the tail, should also be palpated. Soft or hard testes may indicate some degree of degeneration. If the tail of the epididymis is hard and small it is likely that fibrosis is present, which will reduce the capacity of the tail to store sperm. If any localized lesions are suspected, ultrasonography can be used for further investigation.

Examination of the penis

The penis is best examined when erect, during the washing procedure prior to semen collection.

Swabbing

Stallions are passive carriers of venereal diseases. They are classified as infected if they harbour one or more of the following: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (capsule types 1, 2 and 5) and *Taylorella equigenitalis*. In the UK, the Horserace Betting Levy Board's Code of Practice recommends that all stallions and teasers be swabbed after 1 January but before the start of the breeding season, on two occasions not less than 1 week apart.

Technique

The best way to collect swabs is to tease the stallion with an oestrous mare until he has a full erection. The stallion should be backed away from the mare, preferably into a padded corner. The penis can then be deflected with a gloved hand and the swabs collected. The regions to be swabbed before washing include the urethral fossa and penile shaft. The penis should then be rinsed in clean warm water, with particular attention to removing debris from the glans, including the diverticulum of the fossa glandis, which may contain a 'bean' consisting of inspissated smegma. The penis should be dried with clean, soft paper towels. The washing procedure usually stimulates the release of copious quantities of pre-ejaculatory fluid, which can be collected for culture. A swab is then inserted 3-5 cm up the urethra to obtain a urethral sample prior to semen collection. Semen can also be cultured, but it is prone to external contamination. On cytological examination, a positive culture in the absence of neutrophils is suggestive of contaminants.

If inflammatory processes are suspected further up the genital tract, samples for culture are as follows:

- *Urethra*. A post-ejaculatory urethral swab is obtained. The urethral swab is taken immediately after the penis is withdrawn from the artificial vagina. Normally there should be no growth from this swab.
- Vesicular glands. The stallion should be teased to distend the vesicular glands with fluid. A 1 × 100 cm sterile catheter with an inflatable cuff can then be passed up the urethra to the level of the colliculus seminalis (the origin of the excretory ducts of the vesicular glands). The

cuff should then be inflated and fluid in the vesicular gland can be expressed manually per rectum for bacteriological and cytological study.

- *Prostate, ampullae, ductus deferens.* The first jet of semen contains secretions that are largely from these sites. An open-ended artificial vagina can be used to collect the first jet and the rest of the ejaculate can be collected separately.
- *Infections in the epididymis and the testis.* These usually cause changes that can be identified on palpation.

Swabs should be placed in Amies transport medium and transported to the laboratory within 48 hours of collection.

Semen collection

Semen from stallions can be collected using an artificial vagina (AV). Several models are available. The most commonly used are the CSU model (Animal Reproduction Systems, Chino, CA, USA) and the Missouri (Arnolds Veterinary Products, UK). The CSU model holds its temperature well and is easy to assemble but is relatively heavy. The Missouri loses its temperature more quickly but is light and inexpensive. Both models can be set up with latex or disposable plastic liners (Fig. 7.32).



Figure 7.32 Assembled CSU model (top) and Missouri Artificial Vagina (middle) with plastic disposable liner (bottom).

A disposable plastic liner is preferred for use with the AV as the quality of semen is improved and the chances of transferring infection are reduced. Unfortunately, some stallions do not work well with plastic liners. In these cases the rubber liner needs to be sterilized after use. The liner should be submerged in 70% alcohol for 20 minutes and hung up to dry. Before use it should be rinsed in sterile water to remove any traces of alcohol (which is spermicidal) and dried.

The AV should be filled with water to give an internal temperature of approximately 45-48°C. At the start of the breeding season or in slow stallions the internal temperature can be increased to around 50°C. Because of the sensitivity of sperm to high temperatures, it is important that the stallion's penis fully enters the AV and that semen does not have to run down a length of hot liner. The semen can be collected in a non-spermicidal plastic bottle or bag, which should be insulated from external cold temperatures. After the AV is assembled a warm, sterile, water-soluble and non-spermicidal lubricant such as K-Y Jelly (Johnson & Johnson, UK) should be applied to the upper two-thirds of the internal surface of the AV with a plastic sleeve. The hand is then withdrawn leaving the sleeve in place to prevent the lubricant from drying out and to help maintain the temperature. The lubricant should be applied just before the semen collection to avoid having lubricant running down into the collection bottle; some stallions' semen appears to be very sensitive to osmotic changes caused by lubricants.

The semen can be collected using a teaser mare or a 'phantom mare'. If a teaser mare is being used, she needs either to be in oestrus, or ovariectomized and oestrogen-treated. Her tail should be bandaged or enclosed in a plastic sleeve so that the stallion's penis is protected from abrasion by tail hairs. If several stallions are using the same mare, her perineal region should be washed with povidone-iodine solution between each stallion. Alternatively, a blanket, which is changed between stallions, can be placed over her hindquarters.

The stallion should be allowed to tease the mare to check that she is receptive and to allow his penis to be rinsed with clean warm water at 42°C. The penis should be dried thoroughly with clean paper towels. Long plastic disposable sleeves should be worn by the person washing the penis and collecting semen from the stallion, in order to avoid transfer of infection. While the stallion's penis is being washed, the mare can be positioned with a twitch applied.

The stallion can then be allowed to mount the mare. The loose plastic sleeve in the AV should be removed before the penis is deflected into the AV. Pushing the AV upwards against the stallion's abdomen is usually more stimulatory than pushing it down on the penis. The free hand should be placed on the base of the stallion's penis so that the pulsations of ejaculation can be felt. These pulsations will occur at the same time as the tail is seen to 'flag' (move rhythmically up and down). After two or three pulsations the anterior end of the AV should be lowered gradually. Once ejaculation is completed, the stallion will slide off the mare and the collector should follow him and let the penis leave the AV. Moving the AV into a vertical position permits the semen to flow down into the collecting vessel. If working with Colorado-type AVs, the cap of the filler spout should be removed to drain the water immediately after the stallion 'dismounts' the AV. This allows any potentially trapped semen to run into the collection bottle; the AV should be held upright in a slight angle to drain the water. A frothy seminal fluid can be noticed in the stallion's glans penis as the penis undergoes detumescence; this typically indicates ejaculation has occurred.

The seminal gel must be removed from the semen before evaluation. This can be done either using a filter built into the AV liner (preferable) or after collection. Nylon filters are more effective than filters used for milk and are readily available for either type of AV. It is important that semen and all equipment that comes into contact with it be kept at 37 °C until the semen is 'extended' with diluent. Semen evaluation should be performed as soon after collection as possible.

Semen evaluation

The following parameters should be assessed:

Volume. The mean is 60–70 ml. Extended teasing time may result in greater volumes of gel and/or seminal plasma.

Colour. Normally whitish.

Motility. One drop of semen is placed on a warm microscope slide and covered with a clean coverslip. To estimate motility accurately, a phase-contrast microscope with a heated stage is essential. If a microscope with a heated stage is not available, have the slide on a slide warmer until it is taken to the microscope. An estimate should be made of both the total percentage that is motile and the percentage that is progressively motile. For sperm to be classified as progressively motile they must be moving rapidly across the field and, with each lash of the tail, the head must rotate 360°. If the head does not rotate, many of the sperm will move in circles as 50% of stallion sperm have abaxial heads. Ideally, at least 60% should be progressively motile. A more accurate assessment of motility can be obtained if the semen is extended to a concentration of $25-50 \times 10^6$ /ml.

pH. Normal range = 7.2–7.6. If the pH is raised, it is possible that the semen is contaminated with urine, or that infection is present.

Concentration. The number of sperm per millilitre must be determined. The quickest way to perform this is with a densimeter or calibrated spectrophotometer. However, a haemocytometer is commonly used. The semen is diluted 1:100 (10 µl semen + 990 µl formol-saline). The haemocytometer chamber is filled with diluted semen and analysed after a few minutes such that the sperm can settle to the bottom of the chamber. The sperm heads in the 5×5 squares (total of 25 squares) surrounded by triple lines (large central square) in the Neubauer chamber are counted. Only the heads on the left and top lines of each square are counted. Those on the right and bottom lines are excluded. The number obtained is multiplied by 10⁶ to give the number of sperm per millilitre. For samples of very low concentration, the semen is diluted 1:10 rather than 1:100, and the correction factor is ×10⁵/ml. In very concentrated samples (e.g. $>500 \times 10^6$ /ml), only the sperm heads in five small squares in the large central square of the Neubauer chamber are counted. The number obtained is then multiplied by 5×10^6 to give the number of sperm per millilitre.

The total sperm number in an ejaculate averages 8.0×10^9 but is subject to seasonal variation and

will vary with frequency of ejaculation. Daily sperm output (DSO) from the testes can be estimated by using the testicular measurements to calculate testicular volume (see 'Further reading' for more detail). An accurate determination requires that the extragonadal reserves are stabilized by daily collections over a period of 5–6 days. The DSO is then based on the average sperm content from ejaculates obtained on a further 3 consecutive days.

Morphology. This examination can be performed using buffered formol-saline (one drop in approximately 2-3 ml) and phase contrast microscopy, or by staining with eosin-nigrosin (commercially available already premixed as one solution). To stain with eosin-nigrosin, a drop of stain is mixed with a drop of semen on the end of a slide. The resulting drop is then smeared along the slide using a second slide (as for a blood film) and allowed to dry on the slide warmer. Since the solution is moderately hypotonic, artefacts can be created if the smear dries slowly. Morphology should be assessed under the oil immersion lens of the microscope. 200 sperm should be counted and specific types of abnormal morphology are recorded. There should be more than 60% of sperm that are morphologically normal.

Total number of morphologically normal, progressively motile sperm. This number is derived from the total number of sperm in the ejaculate multiplied by the percentage that are progressively motile (PM) times the percentage that are morphologically normal (MN). This figure is much more important than individual figures for motility, morphology, volume or density. The minimum acceptable number of usable sperm is 1 billion in the second ejaculate. This figure should be approximately 50% of that of the first ejaculate.

NB: This figure probably represents an underestimate of usable sperm. Many of the morphological defects (e.g. midpiece) will negatively impact upon progressive motility and thus some 'double jeopardy' is inherent in the % PM $\times \%$ MN calculation.

Longevity of motility. A small vial containing a portion of the ejaculate placed in semen extender (approximately 1:2) and another vial containing only raw semen are kept in airtight, dark conditions at room temperature. Motility should be evaluated hourly for 6 hours and then evaluated at 24 hours. At least 10% sperm should be progressively motile at 6 hours in the raw semen sample and at 24 hours in the extended semen. The value of this test may be questionable as a means of reflecting how long sperm live in the female tract. If a stallion is being evaluated for a chilled, transported semen programme, it would be essential to test longevity of motility at 4°C in different extender/antibiotic combinations at 24 and 48 hours.

Cryptorchidism

This is a fairly common condition in which one or both of the testes fail to descend into the scrotum. If the testis has passed through the vaginal ring but not the external inguinal ring, the horse is referred to as an inguinal cryptorchid ('high flanker'). Subcutaneous testes that cannot be displaced into the scrotum are termed ectopic. If the testis and epididymis are retained within the abdomen the horse is termed a complete abdominal cryptorchid. If the testis is within the abdomen, but a portion of the epididymis is in the inguinal canal, the horse is called a partial abdominal cryptorchid.

Diagnosis

Palpation

The presence of only one scrotal testis (occasionally none) can be detected on palpation of the scrotal contents and external inguinal ring. Tranquillization may relax the cremaster muscles making subcutaneous or inguinal testes more accessible. The scrotum should be palpated and inspected visually for the presence of a scar that would indicate previous surgery.

If the testis cannot be found by external palpation, rectal palpation can be performed. However, this should only be undertaken if adequate facilities for restraint are available. Abdominal testes are small, flabby, mobile and difficult to identify. It is usually more informative to palpate the vaginal ring on the appropriate side; if this is identifiable it is likely that the testis or the epididymis has descended into the inguinal canal. The vaginal ring is located by placing the wrist laterally on the brim of the pubis near the pubic symphysis. The fingertips are then pressed against the abdominal wall. By flexing and extending the middle finger in a cranioventral direction it is possible to enter the slit-like vaginal ring.

Ultrasonography

Scanning is started at the pubic brim and then continued cranially using lateral movements between the midline and the lateral abdominal wall. *This procedure is more useful than palpation in locating inguinal and abdominal testes and estimating their size*. The retained testis tends to be less echogenic than a descended testis.

Blood (serum or plasma) hormone tests

The human chorionic gonadotrophin (hCG) test can be performed to stimulate testosterone production by retained testicular tissue, if the contralateral testis has been surgically removed. A blood sample is collected and then 5000–10000 IU hCG is administered intravenously. A second blood sample is typically collected 1–2 hours later. A significantly elevated concentration of testosterone in the second sample is diagnostic of the presence of testicular tissue. This test is considered to be approximately 95% accurate.

Serum oestrone sulphate

Serum oestrone sulphate can be measured in a single sample from horses more than 3 years old and is reported to be 95-96% accurate. The test should not be used on horses under 3 years of age or in donkeys, as these give a high incidence of falsenegative results. A single baseline concentration of oestrone sulphate is thought to be a more reliable indicator of cryptorchidism than a single testosterone value. Horses with low baseline oestrone sulphate (<50 pg/ml) and testosterone concentrations (<40 pg/ml) should be considered geldings, whereas high concentrations of oestrone sulphate (>400 pg/ml) and testosterone (>100 pg/ml) indicate the presence of testicular tissue. Concentrations between the low and high values cited above are not diagnostic and an hCG stimulation test is recommended. See also under 'Cryptorchidism' in

Chapter 1: 'Submission of samples and interpretation of results'.

FURTHER READING

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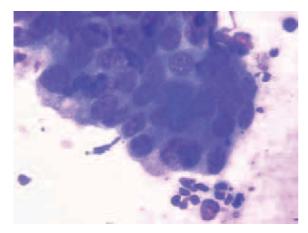


Plate 4 (Fig. 7.29) Cytology of the endometrium showing epithelial cells and polymorphonuclear cells.

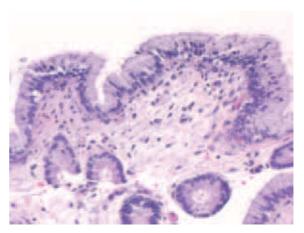


Plate 5 (Fig. 7.30) Endometrial biopsy showing diffuse inflammation of the stratum compactum.





Plate 7 (Fig. 8.3) Icterus of the oral mucous membranes.

Plate 6 (Fig. 8.2) Haemoglobinuria.

CHAPTER

Blood disorders

Chapter contents

I. Diagnosis of anaemia 146 Blood loss anaemia 147 Acute haemorrhage 147 Chronic haemorrhage 147 Haemolytic anaemia 148 Diagnostic characteristics of haemolysis 148 Infectious causes of haemolytic anaemia 149 Immune-mediated haemolytic anaemia 151 Microangiopathic haemolytic anaemia 153 Heinz body anaemias 154 Decreased red cell production 154 Chronic inflammatory disease 154 Deficiency anaemias 155 Neoplasia 155 Toxicity 155 Bone marrow aspiration/biopsy 156 Sternal aspiration of marrow 156 Rib aspiration/biopsy 157 Interpretation of sample analysis 158 II. Diagnosis of coagulopathies 158 Vascular disorders 159 Purpura haemorrhagica 159 Equine viral arteritis 159 Equine infectious anaemia 160 Thrombocytopenia 160 Coagulation disorders 161 Disseminated intravascular coagulation 161

Liver disease 162 Vitamin K deficiency 162 Coagulation tests 162 Prothrombin time or one-stage prothrombin time 163 Partial thromboplastin time or activated partial thromboplastin time 163 Bleeding time 163 Clot retraction time 163

III. Blood tumours 164

Lymphosarcoma 164 Erythrocytosis (polycythaemia) 164 Primary absolute erythrocytosis 164 Secondary absolute erythrocytosis 164 Myelogenous leukaemias 164 Plasma cell myeloma 165

IV. Blood culture 165

Technique 165

Further reading 166

APPENDIX 8.1 *167* Diagnostic approach to anaemia in horses

APPENDIX 8.2 168 Diagnostic approach to coagulopathies

APPENDIX 8.3 169

Some applications of diagnostic techniques for the investigation of blood disorders

This chapter covers the diagnostic techniques for investigating blood disorders and includes clinical examination, the interpretation of haematology and any associated clinical pathology (e.g. coagulation tests), and bone marrow aspiration/biopsy.

I. DIAGNOSIS OF ANAEMIA

Anaemia is a decrease in the oxygen-carrying capacity of the blood, which is caused by an absolute reduction in the number of circulating red cells. The associated clinical signs vary with the onset and severity of the condition. In general terms these include one or more of the following:

- Depression and weakness
- Pale mucous membranes
- Tachycardia and tachypnoea at rest
- Reduced exercise tolerance.

Confirmation of anaemia is usually based on the evaluation of red cell parameters in a peripheral blood sample (EDTA). However, the interpretation of results must take into account the normal physiological variations of individuals, which are associated with breed, excitability and fitness (see 'Interpretation of haematological results' in Ch. 1: 'Submission of samples and interpretation of results'). In particular, the clinician should be aware that a state of anaemia could be masked if excitement at sampling causes elevation of the packed cell volume (PCV) as a result of splenic contraction. In

addition, anaemic patients suffering dehydration may show erythrocyte parameters that appear normal. Table 8.1 shows typical erythrocyte parameter ranges for different groups of horses. Values below the normal ranges are indicative of anaemia.

Once it has been established that a state of anaemia exists, the underlying cause needs to be determined. This will be one or a combination of the following events (see also Appendix 8.1):

- Absolute blood loss
- Haemolysis
- Decreased red cell production.

Of these, decreased red cell production (dyserythropoiesis) is the commonest cause of anaemia in horses.

Before considering the diagnosis of these causes of anaemia, it is important to realize a limitation of haematology in the horse. In the blood samples of other domestic species, regenerative changes (such as reticulocytosis, polychromasia, macrocytosis, anisocytosis and the appearance of nucleated red cells) are indicative of increased erythropoiesis associated with blood loss or haemolysis. These are termed 'regenerative' or 'responsive' anaemias, in contrast to 'non-regenerative' anaemias, which are associated with decreased erythropoiesis. In contrast, juvenile red cells mature within the bone marrow of the horse and are rarely found in the circulation. Because of this, erythrocyte indices are not particularly useful for characterizing anaemia in the horse and erythropoietic activity is best defined by bone marrow aspira-

Table 8.1 Typical erythrocyte parameter ranges for different groups of adult horse					
Parameter	Thoroughbred	Hunter	Pony		
PCV%	40–46	35–40	33–37		
RBC 10 ¹² /I	7.2–9.6	6.2–8.9	6.0–7.5		
Hb g/dl	13.3–16.5	12.0–14.6	11.0–13.4		
MCHC g/dl	34–36	34–36	33–36		
MCV fl	48–58	45–57	44–55		
MCH pg	14.1–18.1	15.1–19.3	16.7–19.3		

Source: adapted from data supplied by the Clinical Pathology Diagnostic Service, Department of Clinical Veterinary Science, University of Bristol.

tion or biopsy (see later). However, a moderate increase in mean corpuscular volume (MCV) is usually apparent during the course of a regenerative anaemia and an associated anisocytosis may be seen on occasion.

Blood loss anaemia

Blood loss may be acute and potentially life threatening, or chronic and barely detectable.

Acute haemorrhage

The blood volume of a healthy horse represents 6-10% of its body weight. An acute loss of 25-30% is tolerated, but greater losses induce hypovolaemic shock. This situation must be judged on clinical grounds alone since the early haematological parameters will appear normal despite impending shock. Assuming survival, there is a compensatory increase in plasma volume by 12-24 hours, causing a dilution of the total protein concentration and a drop in the PCV, red blood cell count (RBC) and haemoglobin concentration.

Diagnosis

The clinical signs reflect hypovolaemic shock and include tachycardia, tachypnoea, weak pulse, dry, pale mucous membranes, cold extremities, colic, anxiety, profound weakness and, eventually, cardiovascular collapse.

A decrease in both the PCV and total plasma protein concentration reflect acute blood loss 12-24 hours after the event. A PCV less than 20% suggests that red cell reserves have been depleted. However, a stable PCV between 12 and 20% does not usually warrant transfusion.

The possibility of internal abdominal or thoracic haemorrhage may be investigated by ultrasonography (Fig. 8.1), or by abdominal paracentesis (see Ch. 2: 'Alimentary diseases') or thoracocentesis, respectively. In such circumstances a defibrinated (non-clotting), platelet-free, bloody fluid is obtained.

A proliferative response by the bone marrow is usually demonstrable after 4-7 days.

Mild icterus may develop 3-5 days after intense internal haemorrhage when extravasated erythro-

fluid in the peritoneal cavity consistent with haemoabdomen.

Figure 8.1 Ultrasonographic appearance of free echogenic

cytes that are not reabsorbed are destroyed by phagocytosis.

Chronic haemorrhage

Chronic haemorrhage can be quite protracted before the problem is recognized because the bone marrow has a chance to regenerate erythrocytes as they are lost. Anaemia becomes apparent only when regeneration falls behind loss.

Early on there is an erythropoietic response by the bone marrow that continues until the iron reserves are depleted by persistent blood loss. After this, there is the potential for an iron-deficiency anaemia to develop. However, assuming the horse continues to eat, the quantity of dietary iron usually compensates the loss.

Diagnosis

Because of the physiological adaptation to chronic blood loss, clinical signs are generally masked until the PCV falls below 10-15%. However, signs will appear earlier if the animal is required to exercise.

Chronic blood loss is indicated by long-term depression of PCV, RBC count and haemoglobin concentration, together with evidence of bone marrow regeneration.

Unless a site of blood loss is obvious, investigation should include analysis of urine, faecal and

BLOOD





Figure 8.2 (Plate 6 in colour plate section) Haemoglobinuria.

peritoneal fluid samples for detection of blood. A number of coagulopathies, the diagnoses of which are covered later in this chapter, can also be associated with chronic blood loss.

Severe and persistent strongyle infestation can result in chronic blood loss. Faecal egg counts are indicative.

Haemolytic anaemia

Haemolysis or destruction of red blood cells can occur either within the vascular space (intravascular haemolysis) or extravascularly by mononuclear phagocytosis. Whether the mechanism of haemolysis is intravascular or extravascular, the released haemoglobin is converted to unconjugated bilirubin and hence the development of icterus within hours to days of the haemolytic event. However, with intravascular haemolysis, haemoglobin is first directly released freely into the vascular space and will first cause red discoloration of the plasma before the onset of icterus. If the renal threshold for free haemoglobin is surpassed, haemoglobinuria ensues and can be a helpful diagnostic clue that distinguishes the presence of intravascular haemolysis (Fig. 8.2 (Plate 6)). With extravascular haemolysis,

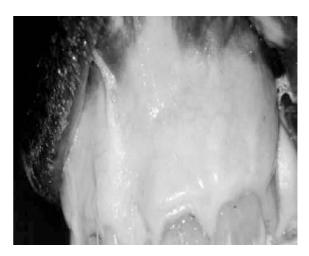


Figure 8.3 (Plate 7 in colour plate section) Icterus of the oral mucous membranes.

haemoglobin is contained within the extravascular space and thus haemoglobinaemia and haemoglobinuria do not develop. Haemolytic anaemia can occur in acute or chronic forms and can be caused by infectious agents but it is most usually the result of an immune-mediated disease (immune-mediated haemolytic anaemia). More rarely, it is associated with erythrocyte damage within lesions involving the microcirculation (microangiopathic haemolytic anaemia). Haemolysis is also a component of the terminal stages of liver failure.

Diagnostic characteristics of haemolysis

An intravascular haemolytic crisis is characterized by discoloration of the plasma with haemoglobin (haemoglobinaemia) and, if the renal threshold is exceeded, haemoglobinuria. Because haemoglobin is rapidly cleared from the plasma and converted to bilirubin, jaundice (icterus) may be the only obvious clinical evidence of recent haemolysis. Jaundice first appears in the mucous membranes approximately 12 hours after the initial crisis (Fig. 8.3 (Plate 7)), and is most pronounced in the sclerae.

The absence of jaundice does not preclude haemolysis since a more gradual destruction of red cells may be matched by the clearance of bilirubin by the liver. This is often the case in 'extravascular haemolysis', which is probably the commonest form of haemolysis in horses.

In haemolytic patients haematologic evaluation reveals subnormal PCV, RBC count and haemoglobin concentration. In an acute intravascular haemolytic crisis the plasma is discoloured red by liberated haemoglobin. An increase above ranges in the mean corpuscular haemoglobin (MCH) or mean corpuscular haemoglobin concentration (MCHC) also indicates the presence of free haemoglobin and a state of intravascular haemoglobin, MCH or MCHC could reflect haemolysis as a result of sample spoilage and should be interpreted with this caution in mind.

Serum biochemistry reflects haemolysis by demonstrating raised unconjugated (indirect) bilirubin concentrations. However, hyperbilirubinaemia is not of itself pathognomonic for haemolysis. Anorexia is probably the commonest cause of hyperbilirubinaemia (and jaundice) in horses. Likewise, significant liver disease can also cause hyperbilirubinaemia. Unlike the anaemia of acute haemorrhage, the total plasma protein concentration is maintained during haemolysis, despite a fall in erythrocyte parameters.

Infectious causes of haemolytic anaemia

These are rare in horses:

- *Equine infectious anaemia* is a viral disease transmitted by biting flies (or contaminated needles). The disease has a worldwide distribution but most outbreaks occur in warm, wet areas where the blood-sucking vectors are abundant.
- *Ehrlichia equi* is a rickettsial organism believed to be transmitted by ticks.
- *Babesia caballi* and *B. equi* are protozoal parasites transmitted by ticks.
- Several bacteria such as *clostridia*, *staphylococci* and *leptospira* are capable of producing haemolysins during the infective process, but these are seldom encountered in practice.

Equine infectious anaemia (EIA)

Equine infectious anaemia is a notifiable disease in many countries, including the UK and others in the EU, Canada, Sweden and some Australian states. Although it is not endemic to these countries it is liable to importation and the clinician should be aware of its diagnosis. It is caused by a virus that is transmitted by biting flies. The anaemia is the result of immune-mediated haemolysis associated with the attachment of viral particles to erythrocytes and it is therefore a form of secondary autoimmune haemolytic anaemia. The red blood cell destruction that occurs with EIA is primarily extravascular.

Diagnosis. Signs of extravascular haemolysis provide initial presumptive evidence.

In the acute form there is depression, fever and petechiation of the mucous membranes. At a later stage this is followed by jaundice, anaemia and oedema of the lower abdomen and legs. Any horse showing these clinical signs should be viewed with suspicion and tested for EIA, especially if there is a history of importation from an infected area or contact with imported horses.

Infection is confirmed by an agar gel immunodiffusion test (the Coggins test) or enzyme-linked immunosorbent assay (ELISA), which identifies EIA virus neutralizing antibodies in a serum sample.

Other clinical pathology is non-specific. Haematology will show anaemia with possible neutropenia and lymphocytosis. Serum biochemistry will show hyperbilirubinaemia.

Comment

• The petechiation and oedema associated with EIA are thought to be the result of a hypersensitivity vasculitis. In terms of petechiation and oedema the clinical expression may be similar to ehrlichiosis, purpura haemorrhagica and equine viral arteritis (see below). In terms of haemolytic anaemia, the clinical signs may be similar to ehrlichiosis and immune-mediated haemolytic anaemia (see below). The importance of excluding EIA from these differential diagnoses is self-evident.

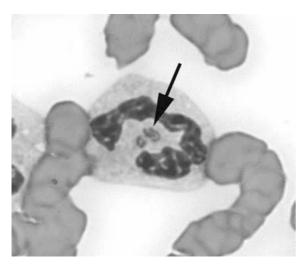


Figure 8.4 (Plate 8 in colour plate section) *Ehrlichia equi* morulae (arrow) in a peripheral blood neutrophil.

Equine ehrlichiosis

Ehrlichiosis is caused by a rickettsial organism, *Ehrlichia equi*, which parasitizes the white cells of the blood and is believed to be transmitted by ticks. Isolated cases of *E. equi* have been reported in the UK, but it is believed to be rare. Anaemia is extravascular and is the result of secondary autoimmune haemolysis.

Diagnosis. The clinical signs are of pyrexia, depression, jaundice (mild haemolytic anaemia), mucosal petechiation and oedema of the extremities.

Definitive diagnosis is based on demonstration of *E. equi* morulae in the cytoplasm of neutrophils (Fig. 8.4 (Plate 8)) and eosinophils in a peripheral blood smear. In the laboratory, routine staining with Giemsa or Wright's stains reveals blue-grey inclusions that have a 'mulberry-like' appearance. Polymerase chain reaction (PCR) analysis of the blood and/or a positive serologic titre also provide diagnostic evidence.

Other clinical pathology is non-specific. Haematology may reveal a transient, mild to moderate anaemia as a result of haemolysis. Leukopenia, including thrombocytopenia, is seen during the period of pyrexia.

Babesiosis

Babesiosis is an intra-erythrocytic parasitic disease of horses that is distributed in parts of the Americas and parts of eastern and southern Europe. The distribution probably reflects the suitability of habitat for the tick vector. Despite the occurrence of ticks in the UK, it is not thought to be a problem in the indigenous horse population. The anaemia results from intravascular haemolysis that occurs during completion of the life cycle as replicated organisms emerge from infested erythrocytes. A component of secondary autoimmune haemolytic anaemia ensues as the host mounts an immune attack on the infested red blood cells.

Diagnosis. The clinical signs are of pyrexia, depression and evidence of both intra- and extravascular haemolysis (jaundice and haemoglobinuria). In endemic areas the indigenous horses often carry *Babesia* without showing clinical signs, but newly introduced stock can be expected to succumb.

Parasitized erythrocytes may be seen in smears during the febrile period, but these are often absent once haemolysis develops. In consequence, diagnosis may have to be confirmed serologically by complement fixation or indirect fluorescent antibody tests or by PCR analysis of the blood.

Leptospirosis

Leptospirosis is known to be associated with a variety of overt clinical signs in horses including uveitis, intermittent pyrexia and abortion. Some *Leptospira* spp. produce potent haemolysins that directly induce intravascular haemolysis. Serological evidence indicates that leptospiral infection is relatively common in the horse but clinical signs of infection are often unapparent. Moreover, the horse does not seem to harbour a specific serotype, but rather reflects those of the wildlife in its environment. Consequently, the association of infection with a specific disease process is often uncertain.

Diagnosis. Signs of intravascular haemolysis, icterus and anaemia serve as initial presumptive evidence.

Culture of leptospires is difficult, costly and rarely attempted. The organisms can be demonstrated

directly by dark-field microscopy or PCR of urine, blood or aqueous humour. A fourfold rise in paired serological titres also provides sufficient diagnostic evidence of recent disease.

In the UK, the Central Veterinary Laboratory at Weybridge assays serum antibodies to mixed leptospiral antigens. Titres greater than 1 : 100 are regarded as suspicious.

Immune-mediated haemolytic anaemia (IMHA)

Immune-mediated haemolytic anaemia is not common in horses but it remains the commonest cause of equine haemolytic anaemia in the UK. IMHA is usually secondary to some event that alters the surface of erythrocytes, making them susceptible to immune recognition. The subsequent attachment of antibodies or complement components results in haemolysis. The effect is either lysis within the circulation by activation of the complement cascade ('intravascular haemolysis') or removal from the circulation and gradual destruction by the mononuclear phagocytic system of the spleen ('extravascular haemolysis'). Both forms of haemolysis can occur concurrently. An immune-mediated haemolytic anaemia occurs in neonatal foals and is termed 'isoimmune haemolytic anaemia' or 'neonatal isoerythrolysis'. During pregnancy the dam becomes inadvertently sensitized to 'foreign' paternal antigens on the foal's erythrocytes and antibodies are produced. Because there is no transplacental antibody exchange during gestation, the foal is healthy at birth but suffers haemolysis following the passive transfer of colostral antibodies. The diagnosis of neonatal disease is outside the scope of this book and the reader should consult appropriate texts.

The clinical history in cases of IMHA may be diagnostically useful since the condition is usually secondary to an earlier disease or treatment regimen or, alternatively, to some chronic intercurrent disease. Predisposing causes of immune-mediated haemolysis in adult horses are thought to include the following mechanisms:

• Antigenic components of bacteria, viruses, parasites and tumours coat red cells and provoke an immune response that results in

erythrocyte destruction. Examples of infectious agents that cause secondary autoimmune haemolytic anaemia include equine infectious anaemia and equine ehrlichiosis

- The erythrocyte surface may be altered by drug components acting as haptens. This provokes an antibody response to the 'foreign' component with the same results as above. Examples of drugs that most commonly have been implicated in haemolytic anaemia in horses are phenylbutazone and penicillin
- During or after an acute infection, circulating immune complexes may be formed from the antigenic remnants of the infectious agent coupled to the antibodies they generated. On rare occasions these immune complexes may bind non-specifically to erythrocyte surfaces and activate the complement cascade, thereby lysing the cells as 'innocent bystanders'. *Streptococcus equi* infection can induce this form of haemolytic anaemia
- In some cases of lymphosarcoma, a clone of lymphocytes is activated that produces an immunoglobulin with a non-specific binding capacity for red cells, i.e. it acts as a weakly bound 'antibody'.

On occasion, 'idiopathic' forms of IMHA occur in the absence of any apparent causation and are thereby regarded as a true (primary) autoimmunity. However, this diagnosis is somewhat arbitrary since some form of predisposition is difficult to disprove. In horses, antibodies of the IgG class are most often involved and these are usually associated with extravascular destruction of red cells by the mononuclear phagocytic system. In this case the cells are sequestered and destroyed in the spleen. Less frequently, antibodies of the IgM class are involved. These readily fix complement and activate the cascade, resulting in rapid intravascular haemolysis.

Diagnosis of intravascular immune-mediated haemolysis

This is the most aggressive form of the disease in which there is an acute intravascular haemolytic

crisis. The clinical signs of acute haemolysis may be accompanied by fever.

Autoagglutination in anticoagulant (EDTA) is often seen in the blood of IMHA patients shortly after sampling. Resuspension of the sample by gently turning the tube is much more difficult than usual. This can be confused with the normal phenomenon of 'rouleaux' formation in which the red cells stack like a pile of coins. In addition, autoagglutination of red cells can occur non-specifically in any severe inflammatory state. To differentiate, dilution of the sample 1:4 in isotonic saline will disperse rouleaux and prevent non-specific agglutination but it will not disrupt immune-mediated haemagglutination. In the latter instance, scanning a diluted smear under low-power microscopy will reveal groups of erythrocytes bound together at their circumference.

Haematology during the haemolytic crisis reveals a drop in PCV, RBC count and haemoglobin concentration, as well as transient discoloration of the plasma by haemoglobin. Haematology reports may indicate erythrocyte abnormalities such as anisocytosis (abnormal variations of size), and the presence of spherocytes (red cells in the process of lysis: small, globular, hyperchromic cells lacking the usual central pallor).

A high proportion of the erythrocytes left in the circulation following intravascular haemolysis have an increased fragility, i.e. their cell surface is damaged or compromised and they are prone to haemolysis. This tendency can be measured in the laboratory using the osmotic fragility test. Samples of blood are pipetted into a series of tubes containing decreasing concentrations of saline and the sequential haemolysis is measured by spectrophotometer. Compromised cells suffer 100% lysis at significantly higher concentrations of saline than healthy cells. A particularly useful application of the test is monitoring the return of normal fragility values to circulating erythrocytes once treatment is under way. Despite its simplicity, the osmotic fragility test is not available at commercial veterinary laboratories. However, this probably reflects the low incidence of intravascular haemolysis in horses.

The definitive diagnosis of immune-mediated haemolytic anaemia is detection of erythrocyte-

bound immunoglobulin by the *Coombs' antiglobulin test.* Specialist veterinary laboratories will undertake this test on samples submitted in anticoagulant (EDTA). Essentially, the cells are washed in isotonic solution and reacted with an anti-equine immunoglobulin reagent. The reagent binds to erythrocyte-bound immunoglobulin and by cross-linking causes agglutination of the patient's cells (direct Coombs' test). Healthy erythrocytes do not react in this way. By using class-specific antisera the immunoglobulin class can be identified.

Specialist laboratories can also confirm the presence of anti-erythrocytic antibodies with the use of species-specific fluorescent-labelled antiglobulin antibodies and flow cytometry. This technology is more sensitive than the Coombs' test.

Comments

- Because the endpoint of the Coombs' test is agglutination, the assay is inappropriate if immune autoagglutination has already been demonstrated in the blood sample (see above).
- The Coombs' test may be negative following a severe haemolytic episode in which all the affected RBCs have been destroyed. Falsenegative results are also likely during periods of corticosteroid treatment.
- The reactivity of the erythrocyte-bound immunoglobulins is often temperature-sensitive and laboratory tests conducted at inappropriate temperatures can produce false-negative results. For this reason and those given immediately above, a negative Coombs' test does not preclude a diagnosis of immune-mediated haemolytic anaemia.
- Because EIA induces autoimmune haemolytic anaemia, the Coombs' test can also be positive in the acute phase of EIA. In cases of uncertainty, the clinician must rule out the possibility of EIA by submitting serum for an antibody test.

Diagnosis of extravascular autoimmune haemolysis

Extravascular haemolysis is the commoner of the two forms of immune-mediated haemolytic

8

anaemia in the horse and presents the greater diagnostic challenge. This is because the splenic sequestration of erythrocytes is not associated with a haemolytic crisis; they are instead destroyed gradually by cells of the mononuclear phagocytic system. The clinical consequence is anaemia without a preceding haemoglobinaemia or obvious mucosal jaundice. Rectal examination will reveal a greatly enlarged spleen, but splenic enlargement is not of itself pathognomonic for extravascular haemolytic anaemia. Diagnostic pointers are as follows:

- As in the case of intravascular IMHA, blood samples in anticoagulant may show autoagglutination. Dilution of the sample 1:4 in isotonic saline will indicate whether the agglutination is immune-mediated or not (see above under: 'Diagnosis of immune-mediated intravascular haemolysis').
- Haematology reveals a reduction in PCV, RBC count and haemoglobin concentration. This can often be quite marked and in the absence of clinical signs of hypovolaemia it indicates a gradual rather than a sudden removal of erythrocytes from the circulation. A laboratory report of profound anaemia is not diagnostic of extravascular haemolytic anaemia but, coupled with the finding of a persistently enlarged spleen, it is at least suspicious.
- A blood sample will not reveal haemoglobinaemia, but serum biochemistry may show an increase in total bilirubin concentration as a result of gradual erythrocyte destruction. However, such an increase will not be apparent if the rate of production is matched by the rate of hepatic clearance.
- Definitive proof of extravascular haemolytic anaemia requires the demonstration of erythrocyte-bound immunoglobulin using the Coombs' test. However, in our experience the conventional test often fails to show positive haemagglutination in anaemic patients that subsequently respond to treatment for extravascular IMHA. There are three possible reasons for this:

- The bulk of affected cells are sequestered in the spleen and are unavailable to peripheral blood sampling
- The test is conducted in the laboratory at a temperature that is inappropriate to the immunoglobulin involved
- The immunoglobulins are weakly bound to the erythrocytes and are too few in terms of surface concentration to be identified by the conventional Coombs' test.

Comment

- In the absence of a positive Coombs' test, a diagnosis of extravascular haemolytic anaemia is indicated by profound anaemia, hyperbilirubinaemia, persistent splenic enlargement and the elimination of other causes of haemolysis.
- If an immune-mediated haemolytic anaemia has been diagnosed, but the cause is not apparent, then investigation must turn to identifying an occult infection or neoplasia before the condition can be judged 'idiopathic'. In addition, the possibility of EIA should be ruled out by antibody test (see above under 'Equine infectious anaemia').

Microangiopathic haemolytic anaemia

This form of haemolysis is secondary to some other disease process in which erythrocytes are physically damaged during their journey through abnormal vasculature or turbulence of flow. As such, the resultant low-grade haemolysis is likely to be masked by the enormity of the primary disease. Examples are disseminated intravascular coagulation, hypersensitivity vasculitis (e.g. purpura haemorrhagica) and arteriovenous shunts (e.g. laminitis).

Diagnosis

Clinical pathology is non-specific. Haematology may be unremarkable or reveal irregularly shaped red cells (schistocytes), but these can be a feature of any haemolytic condition. Gross anaemia is unlikely. Serum biochemistry may show hyperbilirubinaemia, even in the absence of positive haematological

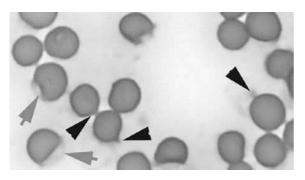


Figure 8.5 (Plate 9 in colour plate section) Heinz bodies on the surface of red blood cells (black arrows) and eccentrocytes (light arrows) in the peripheral blood of a horse with red maple leaf toxicity.

findings, but it is not of itself pathognomonic of haemolysis.

Heinz body anaemias

Oxidative injury to erythrocytes can result in the formation of Heinz bodies (Fig. 8.5 (Plate 9)), which are denatured deposits of precipitated haemoglobin found on the surface of RBCs. In the horse, ingestion of wilted or dried leaves from the red maple (Acer rubrum), wild onions or certain phenothiazine anthelmintics can cause Heinz body formation. Heinz bodies are most easily seen with new methylene blue or brilliant cresyl green and appear as small, dark, round 'dots' on the edge of erythrocytes when these stains are used. In addition, direct oxidative damage to the RBC membrane can result in the formation of eccentrocytes (Fig. 8.5 (Plate 9)), which have a pale area on one side of the cell. Heinz body formation makes the erythrocyte membrane fragile, thus rendering it more susceptible to intravascular haemolysis.

Diagnosis

Signs of intravascular haemolysis, icterus and anaemia are apparent. The presence of Heinz bodies in the peripheral blood provides clear diagnostic evidence in animals with known or presumptive exposure to red maple leaves (Fig. 8.6 (Plate 10)).

Toxicity can be seen within 1–3 days after ingestion of dried red maple leaves. Large amounts of wild onions have to be ingested to result in Heinz body formation. Signs of intravascular haemolysis



Figure 8.6 (Plate 10 in colour plate section) Red maple leaves.

following idiosyncratic reaction to phenothiazine anthelmintics usually develop within 1 week of ingestion.

In addition to Heinz body anaemia, ingestion of red maple leaves may also cause or exclusively cause the formation of methaemoglobin, producing a characteristic brown discoloration to the mucous membranes and whole blood. Methaemoglobin forms when the iron moiety of the haem groups has been oxidized from the ferrous (+2) state to ferric (+3) state, which is no longer capable of binding oxygen.

Decreased red cell production

Decreased red cell production or dyserythropoiesis is the most common form of anaemia in equine practice and is usually secondary to some other condition such as:

- Chronic inflammatory disease
- Nutritional or other deficiency
- Neoplasia
- Toxicity.

Of these, chronic inflammatory disease is the most usual cause.

The slow onset of anaemia usually allows plenty of time for physiological adaptation by the patient and it may not be clinically apparent until exercise intolerance develops.

Chronic inflammatory disease

Long-standing inflammatory, infectious or malignant diseases depress erythropoiesis. Because of the lengthy life span of the equine erythrocyte (some 140–155 days), there is a prolonged time lapse before clinical anaemia is apparent. In consequence, signs of mild anaemia are frequently seen to follow in the wake of a chronic disease process.

Diagnosis

The anaemia is generally mild to moderate with a PCV of 20–30%. There is often clear clinical or diagnostic evidence of a primary disease process.

Comment

• Inflammation associated with tumour necrosis depresses erythropoiesis, but there are other mechanisms by which neoplasia can cause chronic anaemia. These include: blood loss secondary to ulceration of a tumour; immunemediated haemolytic anaemia (which is sometimes associated with lymphosarcoma); bone marrow neoplasia (see below); and microangiopathic haemolytic anaemia.

Deficiency anaemias

Theoretically, deficiencies of iron, cobalt, copper or folate will cause anaemia, but in reality they are exceedingly rare in horses; particularly if there is access to grazing.

The commonest is probably iron deficiency anaemia secondary to chronic external blood loss. In these cases the serum iron concentration is decreased and the total iron binding capacity (TIBC) may be increased.

Diagnosis

The clinical signs of iron deficiency anaemia are the same as those of chronic blood loss. Blood should be taken into iron-free tubes for estimation of serum iron and TIBC and the serum must be separated before submission to the laboratory. Haemolysed samples are quite unsuitable for analysis. The referral laboratory should be consulted before taking samples for these specialized procedures. Bone marrow aspiration/biopsy reveals arrested maturation of erythrocytes and reduced iron stores (see below).

Neoplasia

Primary myeloproliferative diseases and secondary neoplastic invasion of the bone marrow result in proliferation of abnormal cells at the expense of normal cell lines (myelophthisis). Because of this the anaemia is usually accompanied by leukopenia and thrombocytopenia.

Diagnosis

Clinical signs may include spontaneous haemorrhages (due to thrombocytopenia) and local or systemic infections (due to leukopenia), as well as lethargy and pallid mucous membranes. Haematology indicates pancytopenia, with anaemia, leukopenia (specifically neutropenia) and thrombocytopenia. Leukopenia and thrombocytopenia precede anaemia because the life span of these cells is shorter than that of red blood cells. Bone marrow aspiration/ biopsy reveals neoplastic cells and depressed erythropoiesis.

Toxicity

Toxic depression of bone marrow activity may be caused by: the persistent use of drugs at high dosage (e.g. phenylbutazone; potentiated sulphonamides); heavy metals (e.g. chronic lead ingestion) and insecticides. The usual result is depression of erythropoiesis without effect on leukocyte production.

Diagnosis

Bone marrow aspiration/biopsy reveals depressed erythropoiesis. Toxic concentrations of lead may be reflected in a blood assay, but not invariably. Estimations in topsoil from the grazing or, in fatalities, liver and kidney assays, are more indicative.

Comment

• On rare occasions an *aplastic anaemia* is described in which the development of all marrow cell lines is inhibited. This is reflected in the peripheral blood as a pancytopenia. This rare disease may be idiopathic rather than the result of toxic depression. Bone marrow aspiration/biopsy shows generalized hypoplasia and replacement by fatty tissue.

Bone marrow aspiration/biopsy

As indicated at the beginning of this chapter, nonregenerative anaemias are difficult to differentiate from regenerative anaemias in the horse because juvenile red blood cells are confined to the bone marrow and do not usually enter the circulation. In consequence, both types of anaemia usually have a similar appearance in a blood smear and erythropoietic activity is best judged on examination of a bone marrow aspirate/biopsy.

Bone marrow aspirates/biopsies may be obtained from a number of skeletal sites in the horse but the most accessible are the sternum and the ribs. Aspirates for cytology or biopsy can be obtained from both sites; however, core biopsies are more readily obtained from the ribs.

The marrow sample must be correctly processed as soon as it is obtained and accurate histological interpretation requires considerable experience. For these reasons it is best to have an experienced haematologist available at aspiration/biopsy and the technique tends therefore to be confined to specialist centres. However, providing the clinician has experience of preparing good air-dried smears, the technique can be used in the field.

Sternal aspiration of marrow

Technique

- A small area of skin in the ventral midline is clipped at the crossing point of an imaginary line drawn between the two points of the elbow. This is scrubbed with povidone-iodine, rinsed and swabbed with spirit.
- 2–3 ml of local anaesthetic is placed subcutaneously at the midline site between the deep pectoral muscles.
- A bone marrow collection needle, or a 3.5 inch 18G (90 1.2 mm) disposable spinal needle with stylet, is inserted through a small stab incision in the skin and advanced until its tip reaches the bone. The tip is then rotated using firm upward pressure against the hub while the body of the needle is steadied with the fingers of the other hand (Fig. 8.7). The sternal cortex



Figure 8.7 Placement of needle at the sternum for bone marrow aspiration.



Figure 8.8 Suction applied to aspirate bone marrow at the sternum.

is very thin and there is no obvious loss of resistance as the medullary cavity is entered.

• Once the needle is self-retaining within the sternum, the stylet is removed and a 20 ml syringe is attached. Clotting of the sample may be prevented by including a few drops of 15% tripotassium EDTA in the syringe. While the needle is steadied with one hand, the other rapidly pulls the plunger down to the 10 ml mark and releases it (Fig. 8.8). This is repeated two or three times until blood is seen at the hub of the syringe. The purpose of this activity is to break apart marrow stroma without allowing free blood to enter the site. As soon as blood is seen at the hub, suction must be released immediately.

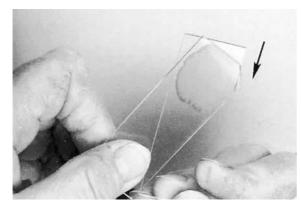


Figure 8.9 Creating a bone marrow smear.

- The needle and syringe are removed together and the sample is transferred through the syringe hub to a glass slide. Marrow has a granular, fatty appearance and does not flow in the manner of peripheral blood. If marrow is not obtained, the sampling process may be repeated at a slightly different site.
- Thin smears must be prepared immediately to preserve cellular morphology. A drop of marrow is placed at the end of a clean glass slide and overlaid with another, causing the material to spread in a thin layer between them. The slides are then pulled apart to create a thin smear on each (Fig. 8.9). Suitable samples have a gritty feel on smearing owing to the presence of marrow spicules. Several smears may be produced from one sample to increase the chances of one good specimen.
- The specimens are promptly air-dried and should be fixed in methanol for 20 minutes. Fixing should be undertaken within 12 hours of preparation in order to preserve cell morphology. The slides can then be stained after arrival at the laboratory (Wright's stain for cell morphology; methylene blue for identifying reticulocytes and Prussian blue for semiquantification of iron storage).

Rib aspiration/biopsy

Rib aspiration or biopsy requires the use of a larger bone marrow collection needle. A 2-inch 13G



Figure 8.10 Placement of bone marrow aspiration/core biopsy needle at the rib.

(50 2.2 mm) bone marrow needle is suitable. In general, core biopsies allow a more complete histopathological assessment of bone marrow activity. Specially machined needles are available for this purpose.

Technique

- The hair over the upper third of a rib, between ribs 9 and 15, is clipped and the skin is scrubbed with povidone-iodine, rinsed and swabbed with spirit.
- 2–3 ml of local anaesthetic is placed subcutaneously over the middle of the rib width.
- The biopsy needle is introduced at right angles to the rib through a small skin incision. Once touching the bone, pressure is applied to the needle while turning it with a to-and-fro movement (Fig. 8.10)
- There is a slight release of resistance as the marrow cavity is entered. At this point either a biopsy or an aspirate may be obtained.
- *For biopsy*: the stylet is removed and the needle is advanced further into the bone. Once a core



Figure 8.11 Suction applied to aspirate bone marrow at the rib.

of tissue has been cut, the needle is rocked to break the core attachment and is then removed. Some designs of needle have particular requirements for manipulation of the core and the manufacturer's instructions should be followed closely. The core is pushed out with a probe and can be rolled on a microscope slide to produce an impression smear. The remainder is placed in 10% buffered formalin for histopathology.

• *For aspiration*: the stylet is withdrawn and a 20 ml syringe is attached. As with sternal aspirates, clotting of the sample is prevented by including a few drops of EDTA in the syringe. The plunger is rapidly pulled down to the 10 ml mark and released (Fig. 8.11). If no marrow appears in the syringe after two or three attempts at suction, the needle should be withdrawn slowly to check that it has not re-entered bone after passing through the marrow cavity. If this manoeuvre fails and a decision is taken to advance the needle again, the stylet must be replaced to prevent the

needle blocking with bone. If necessary, a further attempt to obtain a sample may be made at an adjacent rib, repeating all the sterile precautions. An air-dried smear is prepared immediately as described above under 'Sternal aspiration of marrow'.

Comments

- Contamination of core samples with free blood is difficult to avoid but the marrow portion can still be useful for interpretation providing it is not excessively diluted.
- Pleural puncture is a potential hazard of rib aspiration/biopsy but is unlikely to cause complications.

Interpretation of sample analysis

Normal erythropoiesis is indicated by a myeloid to erythroid cell ratio of less than 1.5 and a regenerative response often produces a ratio less than 0.5. The haematologist will also report on cell morphology and reticulocyte counts within the marrow. Reticulocyte counts greater than 2% are indicative of erythroid regeneration.

A report of megakaryocytes in marrow is indicative that platelets are being adequately produced by the bone marrow, despite any peripheral evidence of thrombocytopenia.

II. DIAGNOSIS OF COAGULOPATHIES

Coagulopathy may be associated with either obvious or occult bleeding and often results in petechial (Fig. 8.12 (Plate 11)) or ecchymotic (Fig. 8.13 (Plate 12)) haemorrhages, or both.

In the healthy horse, haemorrhage is controlled at three fronts:

- The reaction of a blood vessel to limit injury
- The formation of a platelet plug at the site of injury
- Coagulation of the blood.

Accordingly, an investigation of coagulopathy (Appendix 8.2) must consider:

Vascular disorders



Figure 8.12 (Plate 11 in colour plate section) Oral petechiation.

- Thrombocytopenia or platelet function defects
- Coagulation disorders.

Vascular disorders

Coagulopathies resulting from vascular disorders are characterized by petechiation of the mucous membranes, dependent oedema, lethargy and occasionally fever. The usual causes are infectious agents and the vascular inflammatory response (vasculitis) may be immune-mediated. Potential causes are as follows:

- Purpura haemorrhagica
- Equine viral arteritis
- Equine infectious anaemia.

Purpura haemorrhagica

This is probably the most commonly recognized vasculitis of horses and is believed to be a hypersensitivity reaction within blood capillaries to antigenic remnants from a previous bacterial or viral infection. Where implicated, *Streptococcus equi* infection ('strangles') precedes clinical signs of purpura by 2–3 weeks.

The presentation and severity of clinical signs varies greatly between cases, but consistent features are some form of predominantly symmetrical dependent oedematous swelling with petechiation of the mucous membranes. The oedematous swell-



Figure 8.13 (Plate 12 in colour plate section) Scleral ecchymotic haemorrhage.

ings can vary from diffuse urticarial plaques to a marked oedema of each limb that terminates as an abrupt ridge in the upper leg ('bottle neck leg'). In some cases there is oedema of the muzzle and face. There is often, though not invariably, a history of recent infection.

Diagnosis

Diagnosis is largely based on the clinical signs of oedema with petechiation. If there is no obvious petechiation of the oral mucous membranes, the clinician should carefully check the membranes of the intranasal septum and, in mares, the vulva.

Haematology is non-specific and usually reveals mild progressive anaemia with neutrophilia and a left shift. Thrombocytopenia is not a feature of purpura, but platelet numbers may be reduced as a result of extravasation. The plasma fibrinogen concentration is raised within 48 hours of onset.

Skin biopsies of acutely oedematous areas reveal vasculitis.

Equine viral arteritis (EVA)

EVA is a highly contagious viral disease spread by the respiratory and venereal routes. The virus replicates in the tunica media of small arteries throughout the body and the resultant vascular damage can lead to lung oedema, pleural effusion, limb swelling, conjunctivitis ('pink eye') and placental separation. The result is respiratory disease with widespread tissue inflammation, which causes abortion in mares. The causal virus has a worldwide distribution and entered the UK in 1993.

Clinical diagnosis can be difficult because the presenting signs vary greatly in their severity; infection is more common than clinical disease. As with other viral respiratory infections in horses, infection may be obvious or inapparent.

The arteritis is associated with petechiation of the nasal mucosae and conjunctivae, but as the only clinical sign this could be confused with purpura haemorrhagica (see above). However, most clinical cases of EVA feature a marked keratoconjunctivitis and photophobia, with swelling of the eyelids – this is not usual in purpura.

Diagnosis

In acute disease, PCR detection or viral isolation is possible from:

- Nasopharyngeal swabs
- Heparinized blood (the virus is isolated from buffy coat cells)
- Urine/semen
- The spleen and lung of an aborted fetus (if possible the entire fetus and membranes should be submitted).

Viral antibody can be detected in serum but the EVA vaccination status of a horse must be determined and reported to the laboratory when the sample is submitted. Rising titres within 10–14 days of the onset of illness are diagnostic. In the UK, laboratory diagnosis is undertaken by the Animal Health Trust in Newmarket. The disease became notifiable in 1995.

Equine infectious anaemia

EIA was considered earlier in this chapter under causes of haemolytic anaemia. In the chronic, recurring condition the virus can stimulate a continuous hyperimmunity that may result in hypersensitivity vasculitis as well as recurrent immune-mediated anaemia. In terms of petechiation and oedema, EIA is therefore a differential diagnosis for other causes of vasculitis, in particular purpura haemorrhagica and EVA. It also has clinical similarities to ehrlichiosis (see under: 'Haemolytic anaemia' above).

Thrombocytopenia

Thrombocytopenia is uncommon in horses and is usually associated with the excessive consumption of platelets by coagulation processes (e.g. disseminated intravascular coagulation). More rarely, neoplastic infiltration of the bone marrow (e.g. lymphosarcoma) may also cause thrombocytopenia. In both cases the clinical presentation reflects the primary disease and thrombocytopenia is often an incidental finding in a sample submitted for haematology.

Another well documented cause is immunemediated destruction of platelets by the mononuclear phagocytic system, but the exact aetiology is unknown and it is frequently termed 'idiopathic thrombocytopenia'. In this case the affected animal is bright and alert but shows petchiation of the mucous membranes. If the platelet count is sufficiently low (<20 000/ 1), spontaneous haemorrhage (e.g. epistaxis) may occur and haematomas may be noticed following minor trauma.

Diagnosis

In all forms of thrombocytopenia, haematology indicates depression of the platelet count (<90 000/ l).

Clinical signs of petechiation and/or haemorrhage in an otherwise healthy horse suggest a tentative diagnosis of idiopathic thrombocytopenia.

In idiopathic cases the bleeding time is prolonged and clot retraction is abnormal but the coagulation times and plasma fibrinogen concentrations are normal (see below under: 'Coagulation tests').

A definitive diagnosis of immune-mediated destruction in idiopathic cases requires the demonstration of immunoglobulin or complement components bound to platelets, and/or the presence of plasma antiplatelet activity. These tests are typically only available in speciality diagnostic centres.

In cases of bone marrow pathology, aspiration/ biopsy will reveal a low megakaryocyte count. However, the megakaryocyte count is normal or increased in idiopathic thrombocytopenia. Comments

- In summary, the criteria for a diagnosis of immune-mediated (idiopathic) thrombocytopenia are: (1) persistent thrombocytopenia in the presence of a normal (or increased) number of megakaryocytes in bone marrow; and (2) the exclusion of other disorders associated with the consumption of platelets, e.g. disseminated intravascular coagulation. A subsequent response to corticosteroid therapy will also support the diagnosis.
- The discovery of thrombocytopenia in the blood sample of a horse that shows no signs of petechial haemorrhage should be viewed with caution. Spuriously low platelet counts may be associated with poor sampling technique, inadequate anticoagulant in the sample, or the phenomenon of 'platelet clumping'. Platelet clumping is peculiar to EDTA samples and if in doubt, a fresh sample should be submitted in sodium citrate.

Coagulation disorders

Coagulation disorders are uncommon in the horse. The commonest is probably disseminated intravascular coagulation, which can occur secondary to a number of diseases that promote hypercoagulable states. Less common coagulation disorders of adults are liver disease and vitamin K deficiency.

Disseminated intravascular coagulation (DIC)

DIC is characterized by widespread deposition of fibrin in the microcirculation, with subsequent ischaemic injury to many body tissues. As a consequence, defensive fibrinolytic mechanisms are triggered to restore vascular patency and fibrin degradation products (FDPs) are released into the circulation to act as potent anticoagulants, preventing further fibrin formation. This fibrinolytic effect, together with the excessive consumption of platelets and clotting factors, results in widespread haemorrhages. Fibrinolysis is thus activated concurrently with coagulation and the patient may present clinical signs within a spectrum of two extremes: a thrombotic crisis and/or a haemorrhagic disease.

DIC is always secondary to any severe systemic disease in which a hypercoagulable state has been reached. It is associated most frequently with disease causing endotoxaemia, such as septic processes and, in particular, acute gastrointestinal disorders. These diseases promote DIC by generating excessive procoagulant activity within the blood and by endotoxic injury to vascular endothelium. The state of DIC can also be initiated by neoplastic diseases.

DIC is a state of affairs that tends to be inferred on the basis of the clinical circumstances, i.e. the patient is seen as being 'at risk' of DIC. Unfortunately, the early signs of microvascular thrombosis are not sufficiently specific for diagnosis and are more difficult to recognize than the later development of haemorrhage. In consequence, DIC is not usually diagnosed until late in its course.

Diagnosis

There is no definitive test for ante-mortem diagnosis of a state of DIC. Only post-mortem examination with histopathology can define disseminated fibrin thrombi in the microcirculation of organs. However, the cumulative clinical evidence can suggest that a state of DIC may be developing. Any clinical condition where endotoxaemia is suspected and there is a tendency to thrombosis following intravenous treatments (usually at the jugular veins) is highly suspicious. As the syndrome develops, there is a tendency for haemorrhage, characterized by petechial or ecchymotic haemorrhages in the mucosae and sclerae, and a tendency to bleed after venepuncture or minor trauma. At this stage the prognosis is poor.

Platelet counts are usually lowered in acute DIC as a result of excessive consumption. Serial analysis will reveal decreasing numbers during the course of the disease.

Coagulation tests (prothrombin time and/or partial thromboplastin time) tend to be extended and antithrombin III activity is reduced. Unlike other species, fibrinogen concentrations rarely decrease significantly in horses in DIC. The concentration of fibrin degradation products or D dimer increases in the circulation once DIC is established and fibrinolysis has commenced. FDP detection is a service offered by a limited number of veterinary laboratories. Special blood collection tubes are available from referral laboratories, which should be consulted before samples are taken.

Comment

• Most coagulation tests are likely to be abnormal during DIC, but no single test can specifically provide a definitive diagnosis. Although a consensus of agreement is not established for the diagnosis of DIC in horses, typically, as DIC progresses, multiple abnormal coagulation parameters are expected. A careful clinical appraisal to identify the hypercoagulable patient that is at risk is often very helpful.

Liver disease

All coagulation factors except III, IV and VIII are produced by the liver. In conditions of terminal liver failure a coagulopathy develops.

Diagnosis

The clinical observation is usually haematoma formation following venepuncture. Coagulation tests (prothrombin time (PT), activated partial thromboplastin time (APTT)) will demonstrate coagulopathy, but the clinical and clinicopathological evidence of liver failure will be obvious by the time this stage is reached (see Ch. 4: 'Liver diseases'). Because factor VII has a short circulating half-life, the prothrombin time is typically the first coagulation test affected in liver failure.

Vitamin K deficiency

Vitamin K is essential to the production of coagulation factors II, VII, IX and X. In horses, deficiency is usually associated with the therapeutic use of warfarin, a synthetic derivative of coumarol, as an antithrombotic in navicular disease or by accidental ingestion of rodenticides containing coumarol or dicoumarol. Coumarol inhibits the synthesis of the vitamin-K-dependent coagulation factors. Ingestion of some forms of improperly silaged or cured sweet clover hay can also result in signs of warfarin toxicity. Sweet clover contains coumarin, which can be converted to dicoumarol in the presence of certain moulds.

Clinical signs are of spontaneous haemorrhage: epistaxis; haematoma formation following minor trauma; ecchymoses in mucous membranes; haematuria; gastrointestinal bleeding; anaemia. Treatment with warfarin is uncommon these days but, if undertaken, should be monitored carefully.

Diagnosis

Known exposure to warfarin, warfarin-containing rodenticides or mouldy sweet clover in horses exhibiting signs of haemorrhage provide initial presumptive evidence. Factor VII has the shortest half-life of all vitamin-K-dependent clotting factors and its deficiency leads to an abnormality in the extrinsic clotting pathway. The PT is a measure of extrinsic pathway integrity and provides the earliest indication of warfarin toxicosis.

Comments

- PT should be monitored routinely during warfarin therapy in order to prevent toxicity.
- Toxicity is potentiated in conditions of reduced vitamin K intake, hypoalbuminaemia or concurrent use of other protein-bound drugs such as phenylbutazone.

Coagulation tests

Blood coagulation is generated by an enzymatic cascade of reaction steps that culminate in fibrin formation. The reaction pathways can follow alternate routes depending upon the initiating factor (Appendix 8.2).

The *extrinsic pathway* of coagulation is initiated by tissue thromboplastin (factor III), which is released from damaged tissues. The coagulation test used to evaluate this pathway is the prothrombin time, also known as the one-stage prothrombin time (OSPT). The *intrinsic pathway* is initiated by contact of blood with subendothelial collagen. The coagulation test used to monitor this pathway is the partial throm-

8

boplastin time (PTT), also known as the activated partial thromboplastin time.

Both pathways lead to the activation of factor X and proceed along the *common pathway* to the formation of a fibrin clot.

Prothrombin time or one-stage prothrombin time

The PT assesses the integrity of the extrinsic pathway and detects a deficiency of one or more of the specific coagulation factors II (prothrombin), V, VII, X and fibrinogen. Because factor VII has a short halflife in circulation, the PT is often affected before the PTT in diseases that affect both the intrinsic and extrinsic pathways.

A blood sample must be taken in the correct proportion of sodium citrate (9:1) for submission to the laboratory. Citrated Vacutainers (Becton Dickinson) are available; alternatively, the referral laboratory should be able to supply suitable sampling tubes. Ideally, the sample should be delivered to the laboratory within 4 hours, but postal samples should be satisfactory providing the delay does not exceed 3 days. As a laboratory control, it is advisable to send a sample from a clinically healthy horse, together with the patient's sample, in order to assess any artefacts induced by transit delay.

In the laboratory, tissue thromboplastin is added to plasma and the mixture is recalcified to assess clotting time. The laboratory procedure and the reagents used will have a marked bearing upon the clotting time. The result must therefore be judged against the laboratory's reference range, which should be reported with an in-test control for 'normal prothrombin time'.

Prothrombin time will be extended in:

- Vitamin K deficiency
- Advanced liver failure
- Reduced factor VII or common pathway factor deficiencies (e.g. DIC).

Partial thromboplastin time or activated partial thromboplastin time

PTT assesses the intrinsic clotting activity of whole blood and detects deficiencies of the specific

coagulation factors II, VIII, IX, X, XI, XII and fibrinogen.

As with PT, a sample in sodium citrate is submitted to the laboratory, together with a healthy control sample, as rapidly as possible. In the laboratory, a source of partial thromboplastin is mixed with the plasma and the time to clotting after the addition of calcium is recorded. Once again, the laboratory's reference range and a suitable in-test control should be reported.

An extended PTT indicates a coagulation defect of whole blood, such as DIC, liver failure, or vitamin K deficiency. In particular, it indicates a deficiency of one of the factors listed above and modifications of this test may be used to identify a specific factor deficiency, e.g. factor VIII deficiency in foals (haemophilia A).

Bleeding time

This simple though imprecise test can be used to evaluate the capillary–platelet aspect of haemostasis. A relatively hairless area is chosen and a small, deep skin puncture is made with a medical lancet. A stopwatch is then started as the first drop of blood appears. As drops accumulate, they are removed every 30 seconds with a filter paper, which should not touch the skin. When blood no longer appears, the endpoint is reached. The normal bleeding time in horses is 2–5 minutes.

Bleeding time will be prolonged in:

- Vascular disorders
- Thrombocytopenia or platelet function defects
- Advanced liver failure
- Vitamin K deficiency.

Clot retraction time

Clot retraction time can be used as a crude indication of thrombocytopenia or a platelet defect. Normal blood drawn into a plain glass tube will form a clot, which draws away from the vessel wall in 1–2 hours at room temperature. This retraction is a function of *thrombosthenin*, a protein released by platelets. Extended retraction time (or poor retraction) suggests thrombocytopenia or a platelet defect.

III. BLOOD TUMOURS

Tumours of the haematopoietic system are rare in horses. The commonest is lymphosarcoma, but this rarely takes a leukaemic form. Much rarer conditions include erythrocytosis, myelogenous leukaemia and plasma cell myeloma.

Lymphosarcoma

Lymphosarcoma is the commonest haematopoietic tumour in the horse and is probably the commonest internal tumour. The clinical presentation depends upon the organs affected and techniques for its diagnosis are considered in detail under 'Investigating specific diseases' in Chapter 10: 'Lymphatic diseases'.

A definitive ante-mortem diagnosis of lymphosarcoma requires identification of neoplastic lymphocytes in the peripheral blood, bone marrow, pleural or peritoneal fluids, or in the biopsy sample of a tumour mass. However, neoplastic cells are rarely found in the peripheral blood of horses with lymphosarcoma and, although the total lymphocyte count can be variable, it is often normal.

Erythrocytosis (polycythaemia)

On occasion, haematology may show an increase above the expected values for PCV, RBC count and haemoglobin concentration. Most usually this is the result of a 'relative erythrocytosis' associated with dehydration, endotoxaemia or splenic contraction. Much more rarely it indicates 'absolute erythrocytosis' in which there is persistent elevation of red cell parameters.

Primary absolute erythrocytosis

Primary absolute erythrocytosis, or polycythaemia vera, is a myeloproliferative disease of red cell precursors in bone marrow.

Secondary absolute erythrocytosis

Secondary absolute erythrocytosis is a nonmyeloproliferative disease resulting from an increase in erythropoietin production. This can be a normal response to chronically decreased arterial oxygen tension, or an inability to deliver oxygen normally to tissues. Non-physiological increases in erythropoietin production may be associated with neoplasia or some forms of chronic renal disease.

Diagnosis

Clinical signs of erythrocytosis are non-specific, but include erythema of the mucous membranes. Relative erythrocytosis is usually associated with clinical conditions causing dehydration and/or endotoxaemia.

Absolute erythrocytosis is indicated by a persistent elevation of PCV, RBC count and haemoglobin concentration, which is unresponsive to treatments for dehydration or endotoxaemia. If necessary, a suspicion of relative erythrocytosis caused by splenic contraction at sampling can be investigated by resampling after sedation with xylazine.

A diagnosis of primary absolute erythrocytosis is finally derived by eliminating the causes of secondary absolute erythrocytosis, i.e. by careful evaluation of cardiopulmonary, renal and hepatic systems.

Comment

• Bone marrow biopsy is unlikely to be abnormal in cases of erythrocytosis.

Myelogenous leukaemias

Myelogenous leukaemias are extremely rare in horses and are classified on the basis of the predominant neoplastic cell type that is present in the bone marrow.

Clinical signs are non-specific, and include depression, weight loss, mucosal petechiation, hindlimb oedema and pyrexia. Anaemia and thrombocytopenia may accompany this marrow neoplasia.

Diagnosis

Haematology may reveal pleomorphic, poorly differentiated leukocytes in a peripheral blood smear.

8

Anaemia and thrombocytopenia may also be noted. Definitive diagnosis requires critical examination of bone marrow cytology.

Plasma cell myeloma

Plasma cell myelomas are primary diseases of the marrow and are extremely rare in horses. Those recorded are usually *multiple myelomas* in which the myeloma cells invade bone and other organs such as liver, spleen and lymph nodes.

The uncontrolled proliferation of a clone of plasma cells characteristically produces a plasma protein (often termed a 'paraprotein') in excessive amounts. On analysis, the protein is found to consist of a whole immunoglobulin molecule or its constituent fragments.

Clinical signs are variable and bizarre, reflecting tissue infiltration by neoplastic cells or the systemic effects of the immunoglobulin produced. For example, weight loss and anorexia are usual, but lameness and neurological deficits are reported secondary to osteolysis caused by an osteoclastactivating factor produced by the myeloma cells. Chronic infections can follow the obliteration of cellular defences by neoplastic cells in the bone marrow. This bone marrow pathology also results in chronic anaemia.

Diagnosis

Haematology may reflect the bone marrow pathology, e.g. anaemia, a reduced total white cell count and the presence of mature plasma cells.

Serum protein biochemistry demonstrates an elevated total globulin concentration and serum protein electrophoresis reveals a monoclonal gammaglobulin peak that characterizes the myeloma. Most commercial laboratories can demonstrate this peak but characterization of its protein content requires reagents that are only available in specialist laboratories. Bone marrow aspirates reveal an abnormal increase in plasma cells.

In patients displaying lameness, radiography of long bones may reveal 'punched-out' areas of radiolucency.

IV. BLOOD CULTURE

Blood culture is a useful diagnostic test in patients with suspected bacteraemia, although it is rarely indicated in adult horses. The technique offers identification of the organism and production of a sensitivity profile. However, in most cases the number of organisms within the circulation is likely to be transient and of a low order. Consequently, a relatively large volume of blood is required for the inoculum (e.g. 10 ml) and samples should be taken on at least three occasions to increase the chances of isolation. *Strict aseptic precautions must be used to avoid contaminating the sample*.

Technique

- A site over the jugular vein is clipped, cleansed with povidone-iodine, rinsed and swabbed with spirit. The skin should be allowed to dry before taking blood.
- A sterile syringe and needle are used to withdraw an appropriate volume of blood in an aseptic manner.
- The protective covering is removed from the blood culture bottle and the injection port is swabbed with spirit. If the bottle has been removed recently from cold storage, it should be warmed to ambient temperature before use.
- The sample in the syringe is injected into the medium using a new sterile needle to avoid contamination of the bottle with organisms from the skin. Both aerobic and anaerobic bottles should be inoculated (Fig. 8.14). Some manufacturers produce a single bottle system suitable for both aerobe and anaerobe recovery.
- The bottle contents are then gently swirled or inverted several times to mix the blood and medium.

In the laboratory, positive results are usually recognized within 24 hours by turbidity or haemolysis in the medium.



Figure 8.14 Aerobic and anaerobic blood culture bottles suitable for use in horses.

Comments

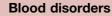
• A negative culture could be the result of a fluctuating bacteraemia rather than the absence

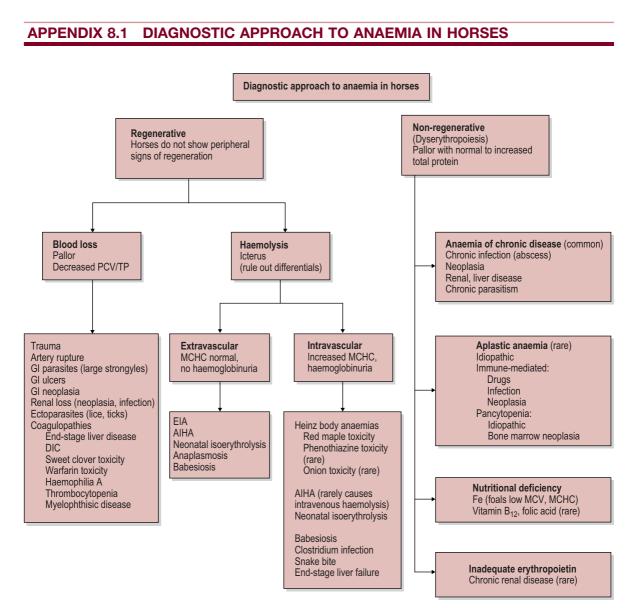
of bacteraemia. If the disease is associated with fluctuating pyrexia, the patient should be resampled as the rectal temperature rises.

- Repeat cultures should be undertaken if the condition appears refractory to treatment.
- If the patient is on a course of antimicrobial treatment, blood samples for culture should be obtained before a treatment is due. Use of cation exchange resins to remove antimicrobials from the blood prior to culture may increase the likelihood of a positive culture.

FURTHER READING

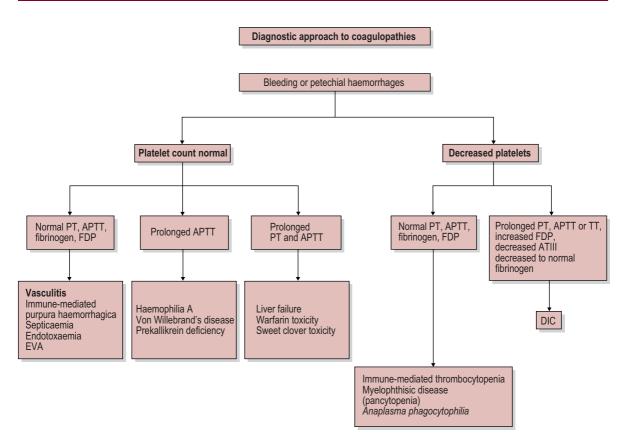
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AlHA, autoimmune haemolytic anaemia; DIC, disseminated intravascular coagulation; EIA, equine infectious anaemia; GI, gastrointestinal; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; TP, total serum protein.

APPENDIX 8.2 DIAGNOSTIC APPROACH TO COAGULOPATHIES



APTT, activated partial thromboplastin time; ATIII, antithrombin III; DIC, disseminated intravascular coagulation; EVA, equine viral arteritis; FDP, fibrin degradation products; PT, prothrombin time; TT, thromboplastin time.

8

APPENDIX 8.3 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF BLOOD DISORDERS

This appendix shows a number of clinical situations that suggest an associated blood disorder: anaemia; the presence of petechial or ecchymotic haemorrhages; thrombocytopenia; and jaundice. Further investigations are suggested, details of which are available within the text of this chapter.

Possible cause			Aids to diagnosis
Anaemia			
Haemorrhage	Acute external loss		Assess cardiovascular parameters; check haematology 12–24 hours later
	Acute internal loss		Assess cardiovascular parameters; abdominocentesis; thoracic auscultation; abdominal and thoracic ultrasound
	Chronic occult loss		Check urine, faeces and peritoneal fluid for blood; investigate parasitism
	Coagulopathy	,	Check for haematomas and petechial/ecchymotic haemorrhages (see differentials below)
Haemolysis	lmmune- mediated haemolysis	Intravascular	Assess cardiovascular parameters including jaundice; check blood for autoagglutination and haemoglobinaemia; Coombs' antiglobulin test
		Extravascular	Check blood for autoagglutination; Coombs' antiglobulin test; rectal examination (persistent splenic enlargement)
	Infection	EIA	Coggins' test/ELISA
		Leptospirosis	Serum antibody
		Ehrlichiosis	Cytoplasmic inclusions in neutrophils and eosinophils; PCR; serology
Dyserythropoiesis	Chronic inflam	nmatory disease	Investigate chronic infection/inflammation/tumour; bone marrow aspirate/biopsy shows dyserythropoiesis
	Deficiency disease		Bone marrow aspirate/biopsy shows dyserythropoiesis
		Iron deficiency	Check serum iron concentration and total iron binding capacity; iron stores in bone marrow reduced
	Bone marrow neoplasia		Check blood for pancytopenia; bone marrow aspirate/ biopsy shows infiltration by neoplastic cells (myelophthisis)
	Toxicity		Bone marrow aspirate/biopsy shows dyserythropoiesis, check recent drug therapy, lead poisoning, exposure to insecticides

Continued

APPENDIX 8.3 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF BLOOD DISORDERS—cont'd

Possible cause		Aids to diagnosis
Petechial/ecch	ymotic haemorrhage	
Purpura haemorrhagica		Check for associated oedema
Equine viral arteritis		Virus isolation (nasopharyngeal swab/buffy coat); serum antibody
Ehrlichiosis		Cytoplasmic inclusions in neutrophils and eosinophils
Equine infectious anaemia		Coggins' test/ELISA
Thrombocytopenia		Check platelet count; see other investigations below
Disseminated intravascular coagulation		Investigate predisposing disease (endotoxaemia in association with thrombosis)
Terminal liver failure		Check liver enzymes and function test (Ch. 4)
Vitamin K deficiency		Check concurrent warfarin treatment; prothrombin and bleeding times extended
Thrombocytop	enia	
Platelet clumping in sample		Resubmit in sodium citrate
Platelet consumption	DIC	Investigate predisposing disease (endotoxaemia in association with thrombosis)
	Loss in haemorrhage	See above under 'Haemorrhage'
Neoplastic infiltration of the bone marrow		Check blood for pancytopenia; bone marrow aspirate/ biopsy shows infiltration by neoplastic cells (myelophthisis)
'Idiopathic' thrombocytopenia		Check bleeding time and clot retraction; bone marrow aspirate/biopsy is normal or shows an <i>increase</i> in megakaryocytes
Jaundice		
Haemolysis		See under 'Haemolysis' above
Reduced feed intake		Check food consumption
Liver disease		Check liver enzymes (Ch. 4)

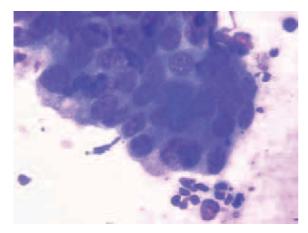


Plate 4 (Fig. 7.29) Cytology of the endometrium showing epithelial cells and polymorphonuclear cells.

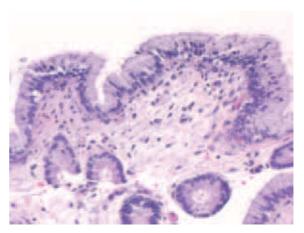


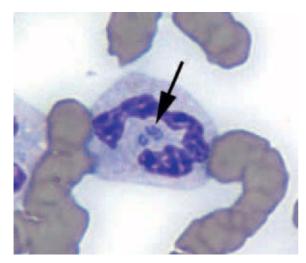
Plate 5 (Fig. 7.30) Endometrial biopsy showing diffuse inflammation of the stratum compactum.





Plate 7 (Fig. 8.3) Icterus of the oral mucous membranes.

Plate 6 (Fig. 8.2) Haemoglobinuria.



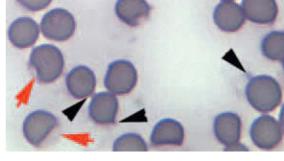


Plate 9 (Fig. 8.5) Heinz bodies on the surface of red blood cells (black arrows) and eccentrocytes (red arrows) in the peripheral blood of a horse with red maple leaf toxicity.

Plate 8 (Fig. 8.4) *Ehrlichia equi* morulae (arrow) in a peripheral blood neutrophil.



Plate 10 (Fig. 8.6) Red maple leaves.



Plate 11 (Fig. 8.12) Oral petechiation.



Plate 12 (Fig. 8.13) Scleral ecchymotic haemorrhage.

CHAPTER

Cardiovascular diseases

Chapter contents

I. Examination techniques 172 General clinical examination 172 Mucous membranes 172 Arterial pulse 172 Jugular distension 173 Jugular pulse 173 Dependent oedema 173 Respiratory sounds and pattern 173 Palpation of the apex beat 174 Auscultation 174 Equipment 174 Heart sounds 174 Heart rate 175 Cardiac rhythm 175 Cardiac murmurs 175 The practice of auscultation 176 Electrocardiography 176 Principles 176 Equipment 177 Techniques 177 Interpretation of ECGs 178 Holter monitoring 179 Echocardiography 179 Principles 179 Equipment 180 Technique 180 Radiography 182 Exercise tests 182 Exercise and arrhythmias 182 Exercise and murmurs 182

Exercise and heart rate 183 Standardized exercise tests using a high-speed treadmill 183

II. Murmurs and valvular disease 183

Murmurs 183 Systolic murmurs 183 Diastolic murmurs 184 Presystolic murmurs 185

III. Arrhythmias 185

Supraventricular arrhythmias 185 Second-degree atrioventricular block 185 Sinus arrhythmia 185 Sinoatrial block/arrest 186 Third-degree atrioventricular block 186 Atrial premature complexes 186 APCs and tachycardia 187 Atrial fibrillation 187 Paroxysmal atrial fibrillation 188 Ventricular arrhythmias 188 Ventricular premature complexes 188 Ventricular fibrillation 189

IV. Other heart diseases 189

Congestive heart failure 189 Congenital lesions in the growing/adult animal 190 Ventricular septal defect 190 Myocardial disease 190 Bacterial endocarditis 191 Pericarditis 192 Pericardiocentesis 192

V. Vascular diseases 192

Ultrasonography of peripheral vasculature 192

Aortic or iliac thromboembolism 192 Arteriovenous fistulae 193 Venous thrombosis 193 Identification of moving fluid 193 Major vessel rupture 193

Further reading 194

I. EXAMINATION TECHNIQUES

This section describes the techniques that can be used to aid diagnosis and prognosis in horses with suspected cardiovascular disease. It centres on cardiac disease and those techniques that are of most practical value in the field. The principles of each technique, suitable equipment and a guide to its practical use will be described.

Techniques for evaluation of the cardiovascular system include:

- General clinical examination
- Auscultation
- Electrocardiography
- Echocardiography
- Radiography
- Phonocardiography
- Exercise tests.

General clinical examination

Irrespective of whether an animal is known to have cardiac disease, a general examination is essential because it provides useful information about cardiovascular function and ensures that no other abnormalities are missed.

Mucous membranes

Examination of the mucous membranes should include an assessment of their colour and the capillary refill time. The oral mucosa is the most easily examined. The mucous membrane colour may be pale (e.g. in animals with anaemia) or injected (dark red; e.g. in animals with a septicaemia or toxaemia). Cyanosis due to cardiac disease is a rare finding, more often shades of grey or a bluish tinge are found in animals with circulatory collapse owing to endotoxaemia.

The capillary refill time (CRT) can be measured by blanching the mucous membranes with light digital pressure. The refill time is usually around 1.5–2.5 seconds, but this is a somewhat subjective measurement. It is a guide to peripheral perfusion and is dependent on cardiac output and local factors affecting the peripheral distribution of blood.

The most likely findings of significance in horses with cardiac disease are: 1) pale mucous membranes and a slow capillary refill time in patients with congestive heart failure (CHF) and 2) injected mucous membranes in rare patients with endocarditis. However, colour and CRT are crude indicators of cardiac disease.

Arterial pulse

The arterial pulse should be palpated in order to assess its rate, regularity and quality. The facial artery is the most easily assessed. Some experience is required in order to identify abnormal findings from the wide range of normality. Apart from arrhythmias, in which pulse quality is often reduced following a short diastolic interval, severe heart disease is usually present before any changes in pulse quality can be detected. However, a weak pulse may be found in animals with reduced cardiac output such as those with CHF due to mitral regurgitation (left atrioventricular valve regurgitation) or severe myocardial disease. In horses with aortic regurgitation, pulse quality is a very useful guide to severity. If aortic regurgitation is severe, there is a strong initial pulse quality because of a large stroke volume; thereafter the aortic diastolic pressure declines rapidly because of valvular insufficiency and the pulse pressure is not maintained, leading to a sharp, hyperkinetic or 'water hammer' quality.

It may be helpful to palpate the median artery at the same time as auscultating the heart in order to detect pulse deficits (i.e. contraction of the heart without a palpable pulse). However, pulse quality is difficult to assess from the median artery and the facial artery is preferable. The digital pulse quality should not be used because it is affected by many factors other than cardiac disease.

Jugular distension

Jugular vein distension can be caused by obstruction to flow (e.g. a thoracic mass), raised intrathoracic pressure (e.g. severe pleural effusion) or raised central venous pressure due to CHF. If the horse's head is raised to a normal upright position, the veins should not fill for more than a few inches at the thoracic inlet.

A raised central venous pressure is found in rightsided CHF. However, many horses will be presented with signs of right-sided CHF even when the primary cardiac abnormality is present on the left side of the heart, because pulmonary hypertension is a common sequel to left-sided heart failure. Raised central venous pressure is often associated with dependent oedema formation. Distension of the jugular veins is a useful guide to the presence of significant heart disease: the more marked it is, the higher the central venous pressure and the more severe the CHF. However, many horses have significant heart disease without jugular distension.

Jugular pulse

Changes in central venous pressure during the cardiac cycle result in pressure changes in the jugular vein at the thoracic inlet. The result is an apparent pulsation of the vein; however, some pulsation is normal and should not be termed a 'jugular pulse'. A true jugular pulse results from blood being ejected in a retrograde fashion from the right atrium into the jugular veins. This can be due to severe right atrioventricular (AV) valve insufficiency and also occurs with some uncommon arrhythmias. Most commonly, a jugular pulse is found when severe CHF has resulted in distension of the veins and right AV valve incompetence. A distended vein can also appear to pulsate as a result of transmission of impulses from the underlying carotid artery.

Dependent oedema

There are many causes of dependent oedema, including hypoproteinaemia, local obstruction to flow and right-sided CHF (see above). Even if there is evidence of heart disease, it is wise to rule out hypoproteinaemia as a potential cause. Dependent oedema owing to CHF usually extends along the ventral abdomen and includes the penile sheath in males; however, it may be present only at the brisket in mild cases. Distal limb oedema as an isolated clinical finding is very unlikely to reflect primary cardiac disease.

Respiratory sounds and pattern

The most common presenting sign of both cardiac and pulmonary disease is exercise intolerance. It is therefore very important to examine both systems in detail, even if cardiac disease is known to be present. The entire lung field should be auscultated. A rebreathing bag is essential for full evaluation of lung sounds (see 'Auscultation' in Ch. 12: 'Respiratory diseases'). Coarse crackles may be auscultated in horses with left-sided CHF and fluid sounds may be heard in those with frank pulmonary oedema. However, increased respiratory rate is the most reliable indicator of pulmonary oedema in horses with left-sided heart failure. Detailed examination of the respiratory tract may also be justified in these cases (see Ch. 12: 'Respiratory diseases'), as secondary bacterial infection of the lower airways can occur due to compromise of mucociliary

clearance and the presence of oedema fluid in the alveoli. The clinical signs that accompany pulmonary oedema, tachypnoea and hyperpnoea, may mimic those associated with primary pulmonary disease. However, although coughing is common in horses with marked pulmonary disease, it is uncommon in congestive heart failure, unless secondary bacterial infection occurs.

Palpation of the apex beat

Palpation of the apical impulse (the point at which the left ventricular free-wall is in contact with the chest wall, rather than the true cardiac apex) is helpful because it gives some indication of the force of ventricular contraction, and because it allows identification of the position of the heart in relation to external landmarks. The strength of the impulse is normally greater in fit, thin Thoroughbreds than in horses such as fat cobs, but it can also be weak in horses with myocardial disease or a pericardial effusion. Identification of the position of the apex acts as a reference point for auscultation (see below). It is usually found in the 4th or 5th intercostal space on the left side of the chest, but may be displaced caudally in horses with marked cardiomegaly or intrathoracic masses.

Auscultation

Auscultation is the basis of the diagnosis of cardiac conditions and careful use of the technique is essential before considering further diagnostic aids. There is no substitute for time spent practising this important technique and every effort should be made to auscultate the chest carefully in suitable surroundings and to record the findings accurately.

Auscultation allows the detection of the normal heart sounds that mark the mechanical events in the cardiac cycle and abnormal sounds, such as murmurs, that result from turbulent blood flow. In addition, identification of the heart sounds allows the clinician to appreciate heart rhythm.

Auscultation should be performed in a quiet environment, preferably indoors to avoid wind noise.

Equipment

A good-quality stethoscope is essential for auscultation. There is little point spending a great amount of money on sophisticated ultrasound equipment but compromising auscultation by the use of a cheap and unsuitable stethoscope.

Both a bell and a diaphragm are essential; the bell for listening to low-frequency sounds and the diaphragm for listening to high-frequency sounds. A standard diaphragm should always be used since a paediatric size is unsuitable for equine auscultation. A relatively flat bell is helpful because bulky heads are difficult to position sufficiently far forward in the axilla to auscultate over the entire heart area. Sound transmission is compromised once the length of the tubes exceeds 35 cm and extra length is not necessary for examining the horse. Double tubes are somewhat better at transmitting sound but should be fastened together to avoid movement artefact.

Heart sounds

The beginning of systole is marked by the closure of the atrioventricular valves (mitral and tricuspid), and to a lesser extent by the opening of the semilunar valves (aortic and pulmonary), which follows closely. The deceleration and acceleration of blood associated with this process results in the generation of a low-frequency sound designated S1. Because this sound is principally caused by deceleration of blood as the AV valves close, it is best heard in the area of the apex beat.

The second heart sound (S2) marks the end of systole and is higher-pitched in nature. Because it results from the deceleration of blood in the aorta and pulmonary artery, S2 is best heard over the semilunar valves at the heart base.

The third heart sound (S3) is a low-frequency sound associated with the deceleration of blood in the ventricles at the end of early diastolic filling. It is best heard over the apex on the left side and is often more noticeable in fit, athletic horses or in animals with volume overload.

The fourth heart sound (S4) is heard towards the end of diastole and marks atrial contraction. It is sometimes difficult to distinguish it from S1 if the P–R interval (i.e. the conduction period from the beginning of the P wave to the beginning of the QRS complex) is short. Because it is perceived as being the first of the sounds on auscultation, the term S4 is sometimes confusing and the term atrial contraction, or 'A', sound is often used. A single combined diastolic sound (gallop) is often heard following fast exercise and is a normal finding.

Heart rate

Heart rate is one of the primary factors governing cardiac output. When demands for increased output are present, the heart rate will rise above the normal range of 24–40 beats per minute (bpm). If cardiac disease is so severe that forward stroke volume is reduced (i.e. the heart is not pumping enough blood per beat), the resting heart rate will be elevated in order to maintain cardiac output. Measurement of heart rate is therefore an important part of examination of the cardiovascular system.

When a raised heart rate is detected, other causes of tachycardia must be discounted before the change is interpreted as resulting from cardiac disease. Pain, fever, toxaemia and anaemia are examples of other potential causes; however, excitement is by far the most common cause. It is therefore very important to allow a horse time to settle and become used to the presence of the examiner before a meaningful measurement of heart rate can be made.

Cardiac rhythm

Assessment of cardiac rhythm is an important part of auscultation that is often overlooked. Some minutes should be devoted to identification of heart sounds with the stethoscope positioned at the left cardiac apex, in order to establish the cardiac rhythm and identify intermittent arrhythmias. One of the most important findings is identification of S4. This indicates that atrial contraction has occurred and helps to identify the most common arrhythmias. In second-degree atrioventricular block (2° AVB), S4 can usually be identified during the long diastolic interval as an isolated 'bu' sound. In atrial fibrillation the absence of coordinated atrial contraction means that S4 is absent. Other identifying features of arrhythmias are discussed later.

Cardiac murmurs

Cardiac murmurs are abnormal sounds that are heard during a usually silent period of the cardiac cycle. They are caused by turbulent blood flow and the vibrations that result. *In horses audible vibrations are often detected in association with normal blood flow*. This is because of the substantial stroke volume and size of the heart and large great arteries. It is important that the distinguishing features of these murmurs are understood so that those associated with pathological change can be identified. To help this process, murmurs are characterized according to a number of different criteria:

- Timing and duration
- Character (change in intensity, pitch, quality)
- Intensity
- Point of maximal intensity (PMI) and radiation.

Timing and duration

Murmurs should be identified as being systolic or diastolic. The duration of the murmur should also be noted. Murmurs may be early systolic, early–midsystolic, late systolic, holosystolic (from the end of S1 to the beginning of S2) or pansystolic (from the beginning of S1 to the end of S2). Diastolic murmurs can be classified as early diastolic (between S2 and S3), presystolic (between S4 and S1) or holodiastolic (between S2 and S1).

Character

The character or quality of a murmur refers to the change in intensity during the murmur, the pitch of the murmur and other descriptive terms such as 'harsh' or 'musical'.

Intensity

The intensity of a murmur is graded on a scale of 1-6. Each grade should be given in relation to the range used (e.g. grade 3/6). The grades are:

• Grade 1: A quiet murmur that can be heard only after careful auscultation over a localized area

- Grade 2: A quiet murmur that is heard immediately the stethoscope is placed over its localized point of maximum intensity
- Grade 3: A moderately loud murmur
- Grade 4: A loud murmur heard over a widespread area, with no palpable thrill
- Grade 5: A loud murmur with an associated precordial thrill
- Grade 6: A murmur sufficiently loud that it can be heard with the stethoscope raised just off the chest surface.

Point of maximal intensity and radiation

The PMI helps to identify the source of a murmur. It must be remembered that vibration from the turbulent source is best transmitted to the body surface by more rigid tissues. Therefore murmurs associated with the AV valves are often transmitted down the ventricular walls and are best heard over the apex beat area where the walls are in closest contact with the body surface. In addition, the murmur may radiate over a localized area or more widely. The areas of auscultation are shown in Figure 9.1.

The practice of auscultation

A methodical technique is required to avoid missing important findings:

- Place the stethoscope over the apex beat area on the left side. S1 is loudest at this point
- Measure the heart rate and ensure that it is a true resting rate
- Evaluate the heart rhythm, it may be helpful to palpate an arterial pulse if an arrhythmia is detected
- Identify systole and diastole (diastole long and systole short at resting heart rates, identify S1 and S2)
- Identify S3 and S4 (if present)
- Listen for heart murmurs. It may help to concentrate first on systole and then on diastole, and then on sounds of different pitch
- Gradually move the stethoscope cranially and dorsally, listening to changes in the heart sounds. Identify the PMI and radiation of murmurs

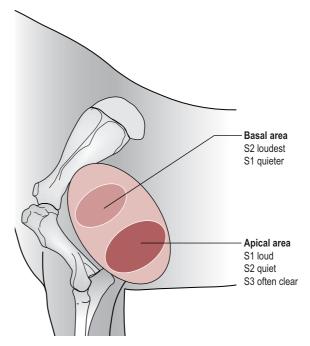


Figure 9.1 The areas of auscultation.

- Identify the heart base area. In this region S2 will be heard relatively louder than in other areas
- Repeat the process on the right side.

NB: It may help to draw the leg forward on the right side so that the stethoscope can be pushed well into the axilla.

Electrocardiography (ECG)

Principles

In humans and small animals, the *Einthoven limb lead system* is widely used to provide useful information about cardiac chamber size and rhythm. In these species the process of depolarization spreads through the myocardium in characteristic wave fronts. The contribution of these wave fronts to the surface ECG depends on the amount of myocardium that is being depolarized. As a consequence, characteristic changes in the size of complexes are seen in the different limb leads when there is enlargement of the cardiac chambers as a result of an increase in muscle mass or chamber size. Unfortunately, the pattern of ventricular depolarization is different in horses because the Purkinje network, which carries electrical impulses to the myocardium, is much more extensive than in humans or small animals. The vast majority of the equine ventricular myocardium therefore depolarizes almost simultaneously and all the resultant small wave fronts cancel each other out such that vectors are not recorded for individual anatomical regions of myocardium. The exceptions to this are the apical region of the septum, which forms the early part of the equine QRS complex, and the basal region of the heart, which makes up the latter portion. The end result is that overall the QRS complex shows little change, even when the mass of the ventricles alters radically in disease. Consequently, the Einthoven limb lead system and multiple recording channels should not be used for equine ECGs, as these techniques have no value in detecting chamber enlargement. More importantly, limb leads are more prone to movement artefact in horses and are generally very poorly tolerated. A number of systems, such as vector electrocardiography and heart score, have been developed to try to derive further information about the size of equine heart chambers, but they have no advantages in general equine medicine when the ECG is used exclusively for rhythm diagnosis.

In order to record the cardiac rhythm all that is required is a clear trace in which the P, QRS and T waves are easily seen (Fig. 9.2). Only one lead is required, with a positive and negative electrode (bipolar lead).

Equipment

Because a bipolar lead is all that is required for equine electrocardiography, a single-channel machine is ideal. Affordable digital recording units are now readily available and are ideally suited to equine work, being battery-operated with the added facility for both remote and prolonged recordings. Importantly, the digital format of the data allows them to be reviewed at leisure or to be e-mailed easily to others for review.

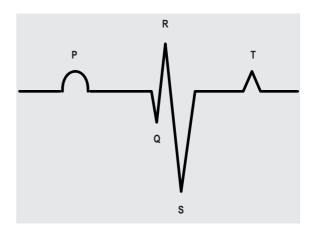


Figure 9.2 Illustration of the typical P wave, QRS complex and T wave in a normal equine ECG (base–apex leads).

Techniques

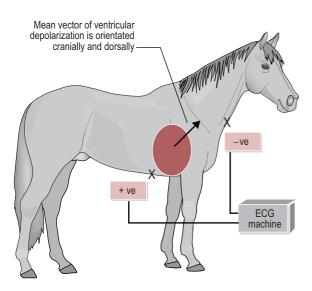
Resting recordings

For a clear bipolar lead trace all that is required is that one lead is placed above and in front of the heart and the other below and behind it. Most single-channel machines are labelled for use with the Einthoven lead system and a bipolar lead can be created by using the right arm (RA) and left arm (LA) leads and switching the selector to lead I. The polarity of the leads is unimportant but it is helpful to use a consistent lead. The base–apex lead configuration is the most universally used lead system. To achieve this, a positive electrode is attached (LA lead) over the cardiac apex and a negative electrode (RA lead) over a basal position such as the right jugular furrow. If the RA and LA leads are used, the ECG recorder should be switched to lead I (Fig. 9.3).

For mains powered machines, a neutral lead must also be used to earth the machine and can be attached to any convenient point on the animal. The skin in front of the scapula is usually most convenient.

Electrical contact between the leads and the skin is established using electrode gel. Ideal electrodes are disposable, pre-gelled, adhesive silver/silver chloride electrodes. Crocodile clips should be avoided, as they are poorly tolerated and very prone to movement artefact. It is rarely necessary to clip

Diagnostic techniques in equine medicine



Lead 1 records potential difference between right arm lead and left arm lead. The lead system used above is a base–apex technique and produces a negative QRS complex

Figure 9.3 Base–apex lead configuration: position of electrodes. This configuration produces a negative QRS complex.

horses for resting traces, provided that, in hairy individuals, the hair is parted to allow the coupling gel in the centre of the adhesive pad to make contact directly with the skin. A surcingle can be used to secure the smaller battery-operated recorders and prevent any redundant leads dangling.

Recordings during exercise

Arrhythmias that develop during exercise can be an important cause of poor athletic performance and can be difficult to detect and diagnose. Auscultation and ECG recording is possible immediately after exercise, but interpretation of heart sounds can be difficult at elevated and changing heart rates, particularly when S3 and S4 merge to create a 'gallop rhythm'. The heart rate falls very rapidly in the first 120 seconds after exercise; consequently it is very difficult to record an ECG before the heart rate has returned to near normal values. Additionally, vagally mediated physiological arrhythmias, such as sinus arrhythmia and second-degree atrioventricular block, often appear during the immediate post-exercise period as the heart rate slows. These arrhythmias are not associated with any significant problems but they can create confusion when detected during this period, particularly in horses suspected of being affected by cardiac disease. As a result ECG recordings during recovery are an entirely inadequate substitute for monitoring heart rhythm during exercise itself. The new generation of digital ECG machines all allow ECG recordings during exercise, but slight modifications to the standard base–apex lead system are needed in order to ensure high-quality recordings.

To reduce movement artefacts the electrode positions are modified slightly. For four-lead systems (three recording electrodes and one earth) designed for small animal and human applications, the positive or left-arm electrode (usually green) is positioned at the left cardiac apex and the right-arm, negative electrode (usually red) is placed on the left shoulder area. The third Einthoven lead, the left leg (usually yellow) is also placed caudal to the cardiac apex. This allows two identical tracings to be obtained from lead 1 and lead 2 on a three-lead system, allowing for a 'spare', should one of the ventral leads be displaced. The recording from lead 3, which is recorded between the left arm and left leg, will be of no diagnostic value because of the close apposition of these electrodes at the cardiac apex. However, a rhythm trace only is required from a horse, so this is not a serious limitation. The fourth earth electrode, when present (usually black), is then attached on the shoulder close to the negative, right-arm electrode (Fig. 9.4).

This vertical modification of the familiar baseapex lead system will still produce large 'QRS' deflections, but the atrial deflection ('P' wave) will be slightly smaller in amplitude than that of a true base-apex configuration. Nevertheless the P wave will still be clearly visible.

Interpretation of ECGs

A methodical technique is essential for the accurate interpretation of an ECG. A recording should be made at a paper or horizontal sweep speed of 25 mm/s, and also at 50 mm/s if the heart rate is



Figure 9.4 Silver/silver chloride adhesive electrodes and digital recorder positioned on a horse to obtain an exercising ECG using a four-lead system.

high or the complexes are of an unusual shape. Many digital units will allow variation of the sweep speed to optimize interpretation. If intermittent arrhythmias are present, recording for a prolonged period is helpful. A suitable interpretation technique is as follows:

- Assess the quality of the ECG trace. Check the calibration of the amplitude of deflection, the paper or sweep speed and whether the AC interference filter was on or off. Look for artefacts
- Calculate the heart rate. Establish whether it is fast, slow or normal, and whether it is variable
- Assess the overall rhythm. Establish whether changes in rhythm are intermittent or persistent and whether they are induced or terminated by excitement
- Assess each wave/complex in turn. Measure the duration and amplitude of the P wave and QRS complex and the duration of the intervals. Establish whether all the complexes are similar or not
- Examine the relationship between the complexes. Check whether each P wave is

followed by a QRS complex, and whether there is a P wave before each QRS complex

• Define the heart rhythm and plan further diagnostic investigations and treatment if necessary.

Holter monitoring

Twenty-four-hour 'Holter' ECG recordings are useful in selected cases in which arrhythmias are intermittent and may be missed during the relatively brief period over which a standard, resting or exercising ECG is recorded. An example is the animal with occasional atrial or ventricular premature beats and clinical signs that are thought to be cardiovascular in origin. The newer digital ECG equipment usually has the facility to allow long-term recordings to be made in individuals if required.

Echocardiography

Principles

Echocardiography is now a key part of the evaluation of the horse with suspected heart disease. It is particularly useful for assessing the clinical significance of cardiac murmurs suspected to be related to pathological change. However, its usefulness depends upon access to a suitable ultrasound machine/sector probe combination and development of practical skills in the manipulation of the transducer and the interpretation of findings. Meticulous attention to detail is necessary when measurements are made.

The principles of ultrasound are now well established but are largely outside the scope of this book. It is important to appreciate that the best images can be obtained only with appropriate equipment. M-mode and two-dimensional echoes (2DE) are strongest when the beam is perpendicular to the interface between structures of different acoustic impedance, principally between myocardium and blood.

The sternum, ribs and lung prevent transmission of ultrasound and limit positioning of the transducer to a few specific acoustic windows to image the heart. In the horse the apex of the heart sits on

the sternum, preventing a true apical image that is obtainable in humans and small animals.

A variety of different modalities of ultrasound can be applied, but 2D, 2D-guided M-mode measurements and colour flow Doppler mapping of abnormal blood flow are the most useful. The following section concentrates on their use.

Equipment

Many practices now have ultrasound machines and will be interested in using them to image the heart. While most machines will be useful in situations of gross pathology involving the myocardium or pericardium, these conditions are rare and specific equipment is required for a full echocardiographic examination. A 2.0–3.0 MHz sector probe with a depth display of 30 cm is ideal.

Technique

Although the novice will have little difficulty identifying anatomical landmarks, the subjective evaluation of cardiac function and the consistency of technique that is essential for accurate measurements to be made require a good deal of practice. Echocardiographic examination allows assessment of the structure of the heart, so that gross abnormalities such as ventricular septal defect (Fig. 9.5), or large bacterial vegetations (Fig. 9.6) can be detected. Assessment of the motion of the valves and walls of the ventricles can also give useful information about valvular and myocardial function (Fig. 9.7). In addition, an echocardiogram is of particular value for assessing the effects of disease by measuring changes in the chamber size compared with normal animals. Interpretation therefore relies upon accurate measurement and comparison of the results to a suitable normal range. Serial measurements over time in the same individual are useful for monitoring the progression of disease and guiding the prognosis. Valvular insufficiency is the most common cardiac abnormality, and will result in volume overload of appropriate chambers once it becomes haemodynamically significant (Figs 9.6, 9.7). For example, moderate to severe mitral regurgitation will result in volume overload of the left atrium and ventricle. The degree of volume overload

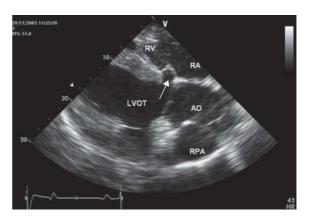


Figure 9.5 Inlet ventricular septal defect in a 3-year-old Thoroughbred colt. This is a right parasternal long axis view of the aorta. The septal defect is in the usual position at the junction of the interventricular septum and the aortic root (arrow). AO, aorta; LVOT, left ventricular outflow tract; RA, right atrium; RPA, right pulmonary artery; RV, right ventricle.

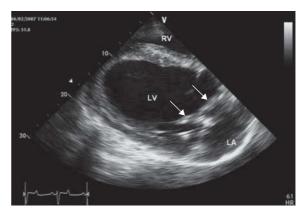


Figure 9.6 Large vegetations on the mitral valve and chordae tendineae (arrows) of a yearling filly with a 1-month history of pyrexia and shifting lameness. Over the filly's clinical history, she developed a grade 5/6 murmur over the left cardiac apex. The image is a right parasternal long axis view of the left and right ventricles. The apex of the left ventricular volume overload. LA: left atrium; LV, left ventricle; RV, right ventricle.

can be assessed by measuring the dimensions of these structures to gain an objective measure of the significance of the condition.

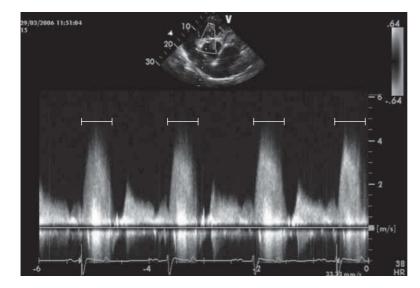
Doppler echocardiography is a technique in which the change in frequency of ultrasound caused

181

by reflection off moving structures is used to measure the direction and velocity of blood flow. It can be used to detect abnormal jets of blood within the heart and great arteries, such as those associated with valvular disease or congenital defects (Fig. 9.8 (Plate 13)). A semiquantitative assessment of the severity of valvular regurgitation can be made by measuring the size of the jet, and the haemodynamic effects of conditions such as a ventricular

septal defect can be measured objectively by calculating the velocity of blood flow through and pressure gradients across the defect.

A standardized imaging technique for guided M-mode echocardiography and publications describing the ranges of measurements found in clinically normal horses are listed in 'Further reading'. It should be emphasized that measurements made from poor images or using any method other than



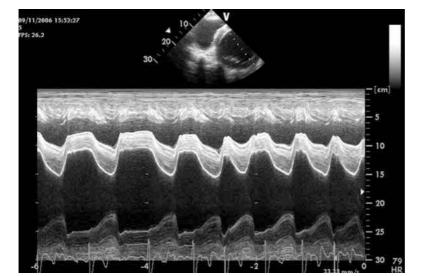


Figure 9.7 M-mode through the right and left ventricle of a 16-year-old Thoroughbred with an irregular heart rhythm, elevated resting heart rate and grade 6/6 murmur over the left heart base. The left ventricle is dilated and there is grossly abnormal septal motion. This arises because of diastolic volume overload subsequent to the ejection of a high proportion of the stroke volume into the relatively low-pressure left atrium through an incompetent mitral valve. The ECG confirms that atrial fibrillation is the cause of the horse's abnormal rhythm.

Figure 9.8 (Plate 13 in colour plate section) Continuous wave Doppler study of the abnormal flow through the ventricular septal defect of the horse in Figure 9.5. To perform this study, the transducer has been rotated from the position in Figure 9.5 to provide the best possible alignment with the abnormal flow through the defect. Blood flows at high velocity (4 m/s) through the defect from left to right ventricle (flow towards the transducer as shown by bars) during systole.

that described for the published range used for comparison are not only valueless but also positively misleading.

Although all too few clinicians will have the opportunity to develop skills in equine echocardiography, it is important that they appreciate the immense value of the technique in providing objective information. This can be of particular value in assessing the significance of mild or moderate heart disease in relation to athletic performance, or following the identification of a murmur at a prepurchase examination.

Radiography

The use of radiography in the horse for the investigation of cardiovascular disease is severely limited. High-output X-ray equipment capable of producing voltage of at least 125–150 kV and current in excess of 300 mA, is required for radiography of the adult equine chest. Portable machines are suitable only for examinations of young foals. In addition, radiography is a very insensitive method of detecting cardiomegaly in the horse. However, it can be useful in detecting gross pulmonary or pleural changes that may be of relevance where cardiac disease is suspected (see 'Thoracic radiography' in Ch. 12: 'Respiratory diseases').

Exercise tests

The examination of horses after exercise is part of the examination for purchase and it can be a useful part of the clinical evaluation of animals with suspected heart disease. Unfortunately, there are many factors other than heart disease that affect the cardiovascular system during exercise. The fitness and athletic ability of each individual horse will be different, and assessment of exercise performance is most valuable when compared with the individual's known exercise capacity. In addition, if the exercise test is limited by environment or lack of a suitable rider, then less information can be derived from it. As a general rule, horses should be examined when ridden and not on a lunge. Auscultation should be performed after submaximal exercise (a brief period of trot and canter), and then after maximal exercise.

The horse should be brought to a rapid standstill from fast work and auscultation should commence immediately and continue until the heart rate is no longer falling rapidly. The examination can then be repeated approximately 20 minutes after the animal has returned to rest.

Exercise and arrhythmias

The most common situation in which exercise is of use is to see whether arrhythmias detected at rest persist at higher heart rates. Vagally mediated arrhythmias such as second-degree atrioventricular block and sinus block are usually abolished by trotting the horse in hand. More vigorous exercise may also result in arrhythmias that were infrequent or absent at rest becoming more frequent, paroxysmal or sustained. It is important that these are detected and characterized as they are often associated with poor athletic performance. Occasionally, arrhythmias usually associated with pathological change, such as ventricular premature beats, are abolished at higher heart rates. While this makes them less likely to be a significant problem, it is important that they are recognized. The abolition of an arrhythmia by an increase in the heart rate is not in itself a diagnosis of a vagally mediated arrhythmia. In order to fully ascertain the effects of exercise on an arrhythmia the ECG recording should take place throughout exercise; it is not sufficient to record the ECG as the horse pulls up.

Exercise and murmurs

It is a commonly held belief that murmurs that are inapparent after exercise are insignificant. In my view this rule of thumb, although not wholly without foundation, is not entirely reliable. Numerous factors may affect the intensity of the murmurs, including stroke volume, blood pressure and blood viscosity. Functional murmurs associated with ejection of blood through the semilunar valves are of variable intensity at different heart rates. Sometimes these murmurs are less obvious or absent at higher heart rates. On other occasions they may become apparent in animals during excitement or exercise although they were absent at rest. Quiet holosystolic plateau-type murmurs associated with mitral insufficiency may be more difficult to hear at higher heart rates. While a quiet murmur of this type may not always be significant at the time of examination, it is important that it is detected and not attributed to normal flow.

Unfortunately, auscultation after exercise is often hampered by extraneous noise and respiratory sounds. Under these circumstances it is easy to miss murmurs of valve regurgitation even though they may be of significance to the horse.

Exercise and heart rate

The speed of recovery of the heart rate of animals with suspected heart disease is helpful in some cases. However, it is very difficult to know the normal rate of recovery, which depends on numerous variables such as the fitness of the horse, the state of the ground and presence of other abnormalities. For example, the heart rate of a lame horse may be higher than that of a sound animal at an equivalent level of exercise. As a rough guide, the heart rate should return to within 10% of the normal level within 15 minutes of the end of moderate exercise and within 30 minutes of more rigorous exercise.

Standardized exercise tests using a high-speed treadmill

The use of a high-speed treadmill allows graded exercise. Usually the heart rate (and often ECG) is monitored during the exercise so that the speed and incline of the treadmill can be altered depending upon the response of the horse. It is somewhat easier to know what constitutes the expected rate of heart rate slowing under these circumstances. The technique is often used for investigation of poor athletic performance in racehorses, where the level of fitness is more predictable and uniform than in pleasure horses.

II. MURMURS AND VALVULAR DISEASE

Murmurs

The haemodynamics of normal and abnormal blood flow result in audible vibrations that

cause functional or pathological heart murmurs. These can be identified by their timing, duration, intensity, character and PMI (see above under 'Auscultation'). Recognition of these characteristics often enables the clinician to pinpoint the cause of the murmur, an essential step in assessing its significance.

Alterations in blood flow due to valvular incompetence leading to regurgitation are relatively common. Abnormal flow is associated with congenital structural defects. But in horses, valvular narrowing (stenosis) of sufficient severity to obstruct blood flow is very rare.

Systolic murmurs

Functional systolic murmurs

The most common murmur heard in horses (approximately 50% of all horses) is an earlymid-systolic ejection-type murmur, usually of grade 1-3/6, with a PMI over the left heart base. These murmurs are often most striking in foals and in young, fit horses but can be present in all breeds. They are often variable at different heart rates and may become quieter or more intense after exercise. These murmurs are often called 'flow' or 'ejection' murmurs because they are associated with normal blood flow through the semilunar valves. They are of no clinical significance except that they must be distinguished from other systolic murmurs resulting from abnormal blood flow. The most distinctive features are that they end well before the end of systole and that they have a crescendo-decrescendo or decrescendo character.

Systolic murmurs due to valve incompetence

Systolic murmurs of clinical significance result from AV valve regurgitation or, less commonly, congenital heart defects.

The most common congenital defect in horses is a ventricular septal defect (VSD). This usually causes a grade 4-6/6 harsh, pansystolic, plateau-type murmur with a PMI just above the sternum on the right side of the chest (see below under 'Congenital lesions in the growing/adult animal').

Regurgitation through the AV valves results in a grade 2-6/6 plateau-type holo- or pansystolic murmur. Mitral regurgitation (left AV valve regurgitation) has a PMI over the left apical impulse area, while tricuspid regurgitation (right AV valve regurgitation) is best heard on the right side of the chest, often well underneath the triceps muscle. In some instances, the murmurs associated with these conditions are late systolic with a crescendo character. Mitral regurgitation can result in development of CHF and/or atrial fibrillation (see below) but, if neither occurs and the heart continues to compensate for the abnormal flow, the condition is well tolerated and often entirely asymptomatic. Rupture of the mitral valve chordae tendineae usually results in a loud harsh murmur with a precordial thrill. The prognosis for horses with mitral regurgitation following chordae tendineae rupture is poor, as volume overload and congestive heart usually progress rapidly. Tricuspid regurgitation may also be an incidental finding; it is particularly common in large, fit Thoroughbred racehorses but very rarely causes clinical problems.

Clinical guides to the severity of pathological systolic murmurs are the presence of signs indicating heart failure, including dependent oedema, jugular distension and tachycardia. Most often these signs are not present and judgement of their severity is then more difficult. The more widespread and louder the murmur, the more likely it is to be significant, because of a propensity to progress. Murmurs due to mitral regurgitation with a precordial thrill are usually sufficiently severe that progression to CHF is likely and the horse's athletic career is usually curtailed.

Further investigation can also be helpful in assessing the severity of the condition. ECGs are of little value except for identification of arrhythmias, particularly during exercise. By far the most useful technique is echocardiography, which allows an objective assessment of the severity of the disease. Haemodynamically significant valvular regurgitation usually results in volume overload, the extent of which can be estimated from the echocardiogram (Fig. 9.7). In addition, Doppler echocardiography can be used to estimate the size of the regurgitant jet.

184

Diastolic murmurs

Functional diastolic murmurs

Functional diastolic murmurs are also commonly found in athletic horses (up to 30% of Thoroughbred racehorses); however, they can be found in horses of all types and any age.

Early diastolic murmurs occur between S2 and S3, are high-pitched and musical in character (sometimes called a '2-year-old squeak') and are best heard over or just ventral to the heart base on the left or right side of the chest. Their intensity varies from grade 1 to 3/6, and can vary at different heart rates, often being greatest at slightly elevated rates of 40–60 bpm. There is no evidence that they are associated with valve pathology.

Diastolic murmurs due to valvular disease

Holodiastolic murmurs are relatively common in older horses and are nearly always associated with aortic valve regurgitation. Mild pulmonary regurgitation is fairly common but is not audible and seems to have no clinical significance. Murmurs of aortic valve regurgitation are decrescendo, with a variable pitch, and are often musical with a buzzing, cooing or rumbling character. These murmurs can be very loud (up to 6/6), even in horses without significant volume overload, and the intensity of the murmur is therefore a poor guide to significance. A useful clinical guide can be the arterial pulse quality, which may become short but strong (waterhammer) in horses with moderate or severe aortic regurgitation. The most objective means of assessment is echocardiography. Most horses with aortic regurgitation are retired from athletic use for reasons other than heart disease but in some cases poor exercise tolerance or even CHF can develop; particularly if mitral regurgitation also develops. Advanced aortic valve regurgitation results in left ventricular dilatation and increased cardiac work and afterload. thus directly increasing myocardial oxygen demand. Simultaneously, diastolic aortic pressure progressively decreases as valve dysfunction progresses, reducing coronary perfusion and myocardial oxygen delivery. Myocardial oxygen demand is yet further increased during exercise and, in advanced cases of

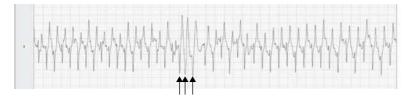


Figure 9.9 ECG taken during hack canter from a 14-year-old Thoroughbred with a grade 5/6 pandiastolic cardiac murmur. There is a triplet of ventricular premature beats (arrows) indicating myocardial ischaemia and hyperexcitability. As ventricular premature beats are a trigger for ventricular fibrillation, the horse was immediately retired from ridden work.



Figure 9.10 Second-degree atrioventricular block. An ECG recorded from a 4-year-old Thoroughbred with no clinical signs of heart disease but a periodically irregular cardiac rhythm. An isolated P wave without subsequent QRS complex is shown by an arrow. On auscultation an isolated S4 (A) heart sound could be heard during the pause between the ventricular contractions.

aortic valve regurgitation, ventricular ischaemia during exercise can lead to ectopic ventricular depolarization activity, increasing the risk of sudden death, or collapse. These changes are often present before the onset of clinical signs of heart failure and, as a result, regular exercising ECG is mandatory in this group of patients if they continue to be ridden (Fig. 9.9).

Presystolic murmurs

Presystolic murmurs (i.e. late diastolic) may be difficult to differentiate from S4 and are low-pitched and rumbling or grating in character. There is no evidence that they are of any clinical significance.

III. ARRHYTHMIAS

Supraventricular arrhythmias

Second-degree atrioventricular block

Second-degree AV block (2° AVB, a 'blocked', 'dropped' or 'missed' beat) is the most common arrhythmia in horses. Around 20% of horses have this arrhythmia at rest, which is caused by high vagal tone (Fig. 9.10). It is usually present at low heart rates and is abolished by increased sympathetic tone

and decreased parasympathetic tone (e.g. excitement or exercise). Occasionally, 2° AVB is found during heart rate slowing following exercise. There is no evidence that 2° AVB in resting horses has any pathological effect. It is of no clinical importance unless it is very frequent and persists when there is a demand for increased cardiac output.

Diagnosis

On auscultation the underlying rhythm is regular, but periodic pauses of twice the usual diastolic interval are present, resulting in a characteristic arrhythmia. During these long pauses, an atrial contraction sound (S4) is usually heard. The block often comes after a fixed number of sinus beats (often four or five), giving rise to the expression of a 'regularly irregular rhythm'.

ECG shows periodic P waves that are not followed by a QRS complex or T wave (Fig. 9.10). Excitement, or exercise, will abolish the block in normal horses, although it may return rapidly as heart rate slows.

Sinus arrhythmia

Sinus arrhythmia is a vagally mediated arrhythmia that may be present at low heart rates but is most commonly detected after submaximal exercise during heart rate slowing. If detected at rest, it is usually abolished by increased sympathetic tone (e.g. excitement or exercise). After exercise, sinus rhythm usually returns once the heart rate returns to normal. It is a normal physiological arrhythmia related to high vagal tone and there is no evidence that it is associated with significant cardiac disease.

Diagnosis

On auscultation the rhythm has phases during which the heart rate increases and slows, which may give the impression of irregularity, but there is usually an underlying cyclical regularity. The phases may be associated with respiration, particularly deep sighs. ECG shows a phasic variation in the R–R interval. Occasionally 2° AVB is also present.

Sinoatrial block/arrest

Sinoatrial block/arrest (sinus block) is a vagally mediated arrhythmia that is usually abolished by increased sympathetic tone. There is no evidence that it is correlated with significant cardiac disease.

Diagnosis

On auscultation there are long diastolic pauses during which the sinus node fails to discharge. Consequently, there is no atrial contraction and these pauses are silent (cf. 2° AVB: see above). The rhythm may be regular or irregular. An ECG reveals that the pauses are double or more than double the normal R–R interval.

Third-degree atrioventricular block

Third-degree AV block (3° AVB, complete heart block) is present when sinus impulses cannot pass through the AV node so that tissues distal to this point (junctional or ventricular) have to take up the role of pacemaker. The block is rare and always pathological.

Diagnosis

On auscultation there will be a slow, regular rhythm (a 'junctional' or 'ventricular escape' rhythm). Atrial contraction sounds (S4) may be heard, but these have no fixed relationship to S1 and S2 and they are usually rapid.

The ECG trace will show regular QRS complexes, which may be normal in configuration (junctional or supraventricular) or abnormal (ventricular) in configuration. P waves are also present but have no relationship to the QRS complexes. The P wave rate is usually very fast.

Atrial premature complexes (APCs)

APCs result from abnormal impulse formation in the atrial myocardium. Isolated APCs can be an incidental finding. However, frequent APCs can be a sign of myocardial disease, electrolyte imbalance, toxaemia, septicaemia, hypoxia or chronic AV valvular disease. APCs, or the disease underlying them, may result in poor racing performance. In some instances APCs are thought to be associated with previous episodes of respiratory disease. If APCs are detected, further investigation is indicated to establish the underlying cause.

Diagnosis

On auscultation, APCs can be recognized by the short diastolic interval, usually without a compensatory pause (cf. ventricular premature complexes). This means that there is an early beat followed by a normal diastolic interval prior to the subsequent S1. Depending on the degree of prematurity, S1 and S2 vary in intensity. Further diagnostic options include exercising ECG, 24-hour Holter recordings, echocardiography, haematology, a routine serum biochemistry profile, cardiac troponin 1 assay and viral serology.

As the name implies, APCs occur early causing a shortened P–P and R–R interval on ECG. They originate outside the SA node (they are ectopic), and may be of a different configuration from the normal P wave (Fig. 9.11). If APCs are sufficiently early, or at high heart rates, they may be lost in the preceding T wave or QRS complex. They usually reset the SA node so that the subsequent P wave follows after a normal P–P interval.



Figure 9.11 Atrial premature complexes (arrows) in a 16-year-old cob with a cardiac arrhythmia at rest. The premature beats interrupt the normal sinus rhythm, but each one is preceded by a P wave.



Figure 9.12 Atrial tachycardia (rate = 157/min) with variable second-degree AV block in a 6-year-old warmblood dressage horse. The AV block ensures that the resting rate is only slightly increased above normal.

APCs and tachycardia

Atrial tachycardias have a rapid rate and usually a regular rhythm due to multiple APCs. They may be paroxysmal (short-lasting) or sustained. The P waves are often buried in, or closely apposed to, the previous T wave and may be difficult to identify. The QRS complexes are normal. If 2° AVB is also present, the rate can be more normal but is still irregular (Fig. 9.12).

Atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia affecting performance in the horse. It is important for clinicians to recognize AF, in particular to distinguish it from 2° AVB, because long pauses can occur in both arrhythmias. Presenting signs include poor performance and occasionally epistaxis, ataxia and tachypnoea during or after exercise. In a significant number of cases, particularly in non-athletic horses, AF is an incidental finding.

AF is commonly found in horses without underlying cardiac disease because the large atria can support the persistence of the arrhythmia once it is established. AF also occurs in animals with dilatation of the atria secondary to valvular heart disease (especially mitral regurgitation), and those with frequent APCs. Very seldom is AF found in animals under 15 hands (150 cm) in height. Those horses without severe underlying heart disease can be successfully converted to sinus rhythm by the oral administration of quinidine sulphate. Once treated, these horses usually return to previous performance levels. However, animals with underlying heart disease are less likely to be successfully treated, and in those horses with an elevated heart rate (>60 bpm), or signs of CHF, treatment is both inappropriate and dangerous. Recently some referral centres are performing DC cardioversion to normal sinus rhythm with reasonable success.

Diagnosis

Auscultation reveals an irregular heart rhythm. The heart rate may be normal, slow or elevated (in contrast to the dog, where AF is almost always accompanied by a tachycardia). There may be long pauses of up to 8 seconds, sometimes followed by flurries of beats. Sometimes these flurries will come in a cyclical fashion. The S1 and S2 sounds will vary in intensity because of the variable position of the mitral valve at the beginning of systole. The characteristic finding is the absence of S4. When a long pause is heard, it is important to try to identify S4, because if S4 is present then the cause of the arrhythmia is not AF. In contrast, when 2° AVB is present, S4 is likely to be heard during the pause. In this instance the pauses are usually multiples of the normal R-R interval and the arrhythmia has a

Diagnostic techniques in equine medicine



Figure 9.13 Atrial fibrillation in an 11-year-old hunter. There are irregular baseline undulations ('f' waves) and no identifiable P waves. The underlying rhythm is completely irregular.

regularly irregular predictable nature. It may be helpful to use your foot as a metronome to get used to the underlying rhythm. *There is no underlying rhythm in AF: the rhythm is irregularly irregular.*

ECG will show that no P waves are present in any lead because of the absence of any coordinated atrial activity. Fibrillation (f) waves are usually seen in horses, except at high heart rates. The R–R interval is irregular (Fig. 9.13). The QRS complexes are normal in configuration. Grossly different QRS complexes are likely to be ventricular in origin and probably indicate more widespread cardiac disease.

Paroxysmal atrial fibrillation

In some animals AF occurs for a short time before sinus rhythm returns, without treatment. This usually occurs during exercise, and sinus rhythm is re-established within hours to days. The condition can result in a significant reduction in performance during exercise. It can be difficult to establish the diagnosis in these cases because the paroxysm has often ceased by the time that a veterinary examination is performed. Exercising ECG is a very useful diagnostic aid for these cases. Animals that experience repeated bouts of paroxysmal AF may have atrial disease, e.g. as a result of a previous viral infection. Electrolyte imbalance has also been implicated and the rhythm is not uncommonly encountered in endurance horses during or after competition.

Ventricular arrhythmias

Ventricular premature complexes (VPCs)

VPCs are caused by abnormal impulse formation in the ventricular myocardium. The presence of an

occasional, isolated VPC is not necessarily abnormal, particularly during recovery from maximal exercise. However, if the VPCs are frequent, occur during exercise or in runs, there are signs of CHF or a loud cardiac murmur(s) is present, their presence is significant and they should be taken seriously. VPCs are a potential trigger for ventricular fibrillation and affected horses should not be ridden until the abnormal rhythm has resolved and/or further investigations have established that the rhythm does not deteriorate during ridden exercise. VPCs also result from disease in other body systems and, as a result, do not necessarily reflect cardiac disease. Systemic disease must always be ruled out in affected horses.

Diagnosis

Auscultation will reveal an early beat followed by a longer than normal diastolic pause. The S1 intensity may be greater than normal and S2 may be relatively quiet depending on the duration of diastole. Echocardiography and clinical pathology (routine haematology and biochemistry, including electrolyte levels) are useful. Exercising ECG and 24-hour Holter ECG recordings are valuable to document the frequency of the arrhythmia and the effects of exercise.

On ECG, VPCs are early and therefore disturb the R–R interval, resulting in an irregular rhythm. They are ectopic and therefore do not follow the normal conduction pathways, resulting in a different QRS complex conformation from those of sinus origin (Fig. 9.14). However, in horses the duration of the QRS interval may or may not exceed the normal range (>0.14 s), so this is not a reliable means of identifying VPCs. If sinus beats are present it is pos-

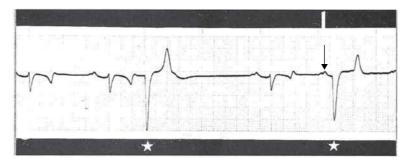


Figure 9.14 Ventricular premature complexes. Bizarre large QRS complexes are seen (stars), which are not preceded by conducted P waves. The first of these can be seen to be followed by a prolonged pause (compensatory pause). More of the strip needs to be seen to assess the frequency of the VPCs and the underlying rhythm, but in this example a P wave may be buried in the T wave following the first VPC, and the P wave preceding the second VPC is too close to it to have been conducted (arrow). On auscultation there was a premature beat with a loud S1 and rather quiet S2, followed by a pause longer than the usual S1–S1 interval.

sible to recognize a VPC from its different configuration and amplitude. However, it is more difficult to recognize some VPCs if there is a persistent ventricular tachycardia, in which case there are no sinus beats for comparison. If different QRS forms are present the condition is described as multiform in origin. Usually this indicates more widespread myocardial disease and a less favourable prognosis. The T wave is also widened and of opposite polarity to the QRS complex. The ectopic beat is almost always followed by a full compensatory pause, but it can be found between two normal QRS complexes not disrupting the R–R interval, in which case it is called an interpolated beat.

Ventricular tachycardia

'V tach' is defined as four or more VPCs in succession. It may be paroxysmal or sustained. There is nearly always serious underlying cardiac or systemic disease present.

Diagnosis

Auscultation will demonstrate a rapid rhythm which is regular during the periods of ventricular tachycardia, but which may appear irregular if there are short paroxysms interspersed with normal sinus rhythm. P waves may be seen on ECG or hidden by the abnormal QRS complexes. Fusion beats or capture beats may be seen. There is always atrioventricular dissociation.

Ventricular fibrillation

Ventricular fibrillation (VF) is usually a terminal event in which there are no organized ventricular depolarizations or contraction. The horse collapses and no pulse is palpable. VF is associated with increased myocardial irritability that is usually caused by severe systemic or cardiac disease.

Diagnosis

No clear heart sounds are detected on auscultation and the ECG shows irregular baseline undulations with no QRS complexes, P or T waves.

IV. OTHER HEART DISEASES

Congestive heart failure

Congestive heart failure is relatively uncommon in horses. The most common underlying cause is valvular disease, particularly mitral or aortic valve regurgitation. CHF is seldom reversible, except in some cases of myocardial or pericardial disease. Occasionally, towards the end of pregnancy, mares will show signs of CHF that resolves after the birth of the foal.

Most horses with CHF show exercise intolerance. Signs can be divided into those resulting from leftsided or right-sided failure. However, this is a gross over-simplification. In addition, most horses with left-sided disease eventually develop right-sided CHF.

In acute left-sided CHF, the predominant clinical signs are due to the development of pulmonary oedema. Hyperpnoea, tachypnoea and dyspnoea may be observed. In right-sided CHF, the first signs to develop are distension of the jugular veins and dependent oedema, which usually forms a plaque along the ventral abdomen. Filling of the sheath and distal limbs may be seen. Diarrhoea (due to intestinal oedema) and weight loss may be observed in severe cases.

Auscultatory findings should enable the underlying cause of the CHF to be identified, and arrhythmias that complicate the condition may also be detected. Electrocardiography and echocardiography are valuable to define the cause further, assess its severity and guide prognosis and treatment where appropriate.

Horses with CHF have a poor prognosis unless the underlying cause can be reversed, but this is seldom possible. Horses with mild CHF may be stabilized on treatment in the short term so that they can be used as breeding animals or kept as pets, but they are unsuitable for riding purposes. Sudden death may occur due to the development of malignant ventricular rhythms or following pulmonary arterial or left atrial rupture.

Congenital lesions in the growing/adult animal

Congenital heart defects are relatively uncommon in horses compared with other domestic species. Although they may result in fetal or neonatal death, most are not identified until the animal fails to thrive as a youngster, has poor athletic performance when first put into work, or when a murmur is detected at a routine examination. Not infrequently, the congenital lesion is not identified until well into adult life.

Ventricular septal defect

By far the most common congenital lesion in horses, particularly in growing and adult horses, is a ventricular septal defect (VSD). Other defects usually present earlier in life, while VSDs vary in severity and can even be asymptomatic. VSDs result in loud systolic murmurs and usually there is a precordial thrill. Clinical signs associated with a VSD depend upon its size: small defects allow only a limited amount of blood to shunt from the left to right ventricle. Performance can be normal, particularly in non-athletic horses. Other horses may exhibit reduced performance, tachycardia or signs of CHF. Some horses do well until, later in life, they develop aortic regurgitation due to deformation of the aortic ring, which is close to the site of most VSDs.

Diagnosis

Definitive diagnosis requires the use of echocardiography, angiography or catheterization. Echocardiography is the most accurate of these, provides the best guide to severity and is non-invasive. Because VSDs can have limited effects on exercise tolerance, animals in which this lesion is suspected should not be condemned unless clinical signs are severe or echocardiography demonstrates significant haemodynamic abnormalities.

Using two-dimensional echocardiography, the location of the VSD can be identified and its size measured (Fig. 9.5). Doppler echocardiography can be used to measure the velocity of blood flow through the defect and from this information the pressure gradient across the defect.

Myocardial disease

Myocardial disease is poorly defined in the horse. Clinical signs vary from poor athletic performance to sudden death, but severe myocardial disease is much less common in horses than in small animals. However, arrhythmias are an important cause of poor athletic performance and may be related to low-grade myocardial disease.

Myocarditis is an inflammatory process that is thought to occur in some horses in association with respiratory viral infection. However, this association is anecdotal. Horses with poor athletic performance following a respiratory infection benefit from cardiac investigation, but it must be considered that the reduced exercise tolerance may be due to other postviral effects. Exercising and 24-hour Holter monitor ECG recordings may show evidence of intermittent arrhythmias such as supraventricular premature beats or tachycardia.

Where signs of CHF develop in horses without loud cardiac murmurs, myocardial or pericardial disease must be suspected. Heart sounds may be quiet and the apical impulse and pulse quality may be weak. A variable murmur may be detected and arrhythmias, especially VPCs, are common.

Severe myocardial disease can be toxic or idiopathic in origin. Monensin toxicity is the most common cause of severe myocardial disease such that investigation of dietary history is helpful.

Clinical pathology may be of value in some cases. Serum cardiac muscle isoenzymes of lactate dehydrogenase (LDH) have been reported to be an indicator of myocardial injury; however, there is little evidence for this at present. Animals with severe disease such as monensin toxicity may appear to have high cardiac isoenzymes, but the true origin may be from skeletal muscle damage. However, the fact that the serum muscle enzyme creatine phosphokinase (CPK) is elevated in the acute stages of monensin toxicity is diagnostically useful. Haematology and viral serology may provide evidence of viral infection, but clinical signs of myocarditis may develop some weeks after infection when these indicators are even less reliable than in the acute phase. Cardiac troponin I is a protein that is released specifically from damaged myocytes and its levels in serum are occasionally elevated in horses with suspected myocardial disease. However, although more specific than LDH isoenzymes and creatine phosphokinase for detection of cardiac disease, the sensitivity of cardiac troponin I appears to be low in this context.

Bacterial endocarditis

Bacterial endocarditis is a very uncommon condition that carries a grave prognosis if it is not diagnosed early and treated aggressively. It is slightly more common in foals and aged horses but is sufficiently rare that it is difficult to give a predilection group. The aortic and mitral valves are the most frequently affected. When the right-sided valves are affected prognosis seems to be more favourable.

The predominant clinical signs are usually malaise and weight loss. Fever is often identified. A murmur typical of incompetence of the affected valve may be heard and in a significant proportion of cases ventricular premature beats are present. The source of the infection is seldom identified.

Diagnosis

Clinical pathology is usually extremely helpful, revealing evidence of an acute inflammatory process. A neutrophilia may or may not be present but hyperfibrinogenaemia is usually very marked, with values in the region of 8–12 g/l being not uncommon. Blood culture can be helpful: several samples should be taken into large quantities of blood for aerobic and anaerobic culture (see 'Blood culture' in Ch. 8: 'Blood disorders'). Unfortunately, cultures are often negative, but antimicrobial sensitivity should be performed if cultures are obtained because a wide variety of organisms may be involved and treatment needs to be specific and long-term. Even with a successful bacteriological cure, clinical signs of valvular disease may persist.

When clinicopathological tests are suggestive of a severe inflammatory process, echocardiography is indicated in order to pinpoint the location of the infection. An echocardiogram in horses with endocarditis usually shows one or more large, vegetative, echogenic lesion on the affected valve (Fig. 9.6). The chordae tendineae may also be affected and may be uniformly thickened in the early stages. Large degenerative nodules are not always present in horses so that any suspicious lesions on valves or chordae detected in a horse with the appropriate clinical history should be considered to be positive. If there is any uncertainty, it is worth repeating the echocardiogram in a few days. Echocardiography is also useful for assessing the severity of valvular regurgitation. If volume overload is severe, treatment may not be warranted.

Pericarditis

Pericardial disease is very uncommon in horses but may be recognized rather more frequently in future now that the use of echocardiography is more widespread.

The development of clinical signs depends on whether diastolic filling of the heart is normal. Filling can be limited by the pericardial sac if it becomes fibrosed (constrictive pericarditis), or if it fills with a significant effusion. If the effusion forms rapidly or is substantial, it may compress the right atrium and limit venous return (tamponade). Presenting signs may be malaise, poor athletic performance or CHF. If the pericardium becomes associated with a pleuropneumonia, respiratory signs will also be present.

Auscultation may reveal muffled heart sounds. If there is a small amount of fluid present, a pericardial 'friction rub' may be detected. This may have one to three components and has a quality similar to a creaking door. Unlike a pleural rub it is synchronous with the cardiac cycle.

Diagnosis

Identification of a pericardial effusion is best made using echocardiography, when an anechoic space is seen between the echogenic pericardium and the myocardium. Layers or fronds of fibrin on the pericardium or epicardium or echogenic specks in the effusion may be seen if there is a bacterial aetiology. If tamponade is present, the right atrium will have a concave outer surface. However, echocardiographic diagnosis of constrictive pericarditis is difficult. The pericardium may appear thickened and the pattern of filling is altered, with a step in the M-mode contour of the ventricular wall and valve motion at mid-diastole.

Appropriate clinicopathological tests are haematology with plasma fibrinogen estimation to identify an inflammatory process. Cytology and culture of the effusion can be helpful in identifying the aetiology of the disease.

Pericardiocentesis

Pericardiocentesis is required to relieve tamponade and may be helpful in obtaining pericardial fluid for cytology and culture. The procedure is not without risk because of the sensitivity of the epicardium, which can result in arrhythmias when stimulated. Monitoring the ECG and placing an intravenous catheter prior to pericardiocentesis is therefore advisable.

Blind insertion of a catheter is possible in the 5th or 6th intercostal space on the left or right side of the chest, just dorsal to the midway point between the elbow and the shoulder level. A long 10–14G catheter, through which a fine polythene tube such as a dog urinary catheter can be threaded, is inserted after infiltration of the skin and intercostal muscle with local anaesthetic. The procedure is best performed under ultrasound guidance.

V. VASCULAR DISEASES

The association of vasculitis with systemic disease is considered under 'Vascular disorders' in Chapter 8: 'Blood disorders'. The diagnosis and evaluation of much larger vascular lesions is possible by the use of ultrasonography and is considered here.

Ultrasonography of peripheral vasculature

Ultrasonography is a useful technique for evaluation of vascular diseases. Two-dimensional imaging using a linear array or a sector scanner can be used to identify gross lesions. Doppler equipment can be used to investigate blood flow within a vessel, but this facility is generally restricted to specialist centres. These techniques can be useful in investigating a number of clinical situations:

- Aortic or iliac thromboembolism
- Arteriovenous fistulae
- Venous thrombosis
- Identification of moving fluid
- Major vessel rupture.

Aortic or iliac thromboembolism

Thromboembolism of the terminal aorta or iliac arteries is an uncommon cause of hindlimb pain

and weakness that is exacerbated by exercise. The ficult thrombus may be palpable on rectal examination, thorac

thrombus may be palpable on rectal examination, although on occasion thrombosis has been diagnosed using ultrasound where no abnormalities were detected by palpation.

A 5 or 7.5 MHz transducer scanner is suitable. For most examinations performed per rectum a linear array transducer is used, but some sector scanners are designed to be suitable for rectal use. Many practices will have equipment designed for examination of the reproductive tract that is equally suitable for this investigation. The thrombus is of mixed echogenicity, with areas of marked hyperechogenicity, particularly in chronic cases in which fibrous tissue has been laid down. Up to 80% of the lumen of the aorta has been shown to be occluded in some cases. The examination should also include the internal and external iliac arteries. The prognosis for athletic use in animals with this condition is poor.

Arteriovenous fistulae

Peripheral arteriovenous fistulae are rare but have been identified by ultrasonography in some animals. The clinical significance of these lesions depends on their size and location. They may be congenital, acquired or iatrogenic. An example of the latter is fistula formation between the jugular vein and carotid artery as a result of poor catheterization technique. Doppler ultrasonography can be used to measure blood flow through the fistula.

Venous thrombosis

Ultrasound is a useful technique to detect the buildup of a thrombus within a vein. A good example is identifying thrombus formation around a jugular catheter, which may prompt the removal of the catheter before complete occlusion of the vessel occurs. Ultrasound can also be used to investigate swelling around veins and identify thrombophlebitis.

For the investigation of superficial vessels, a highfrequency transducer such as a 7.5–10 MHz probe is useful and a stand-off may be required. Both sector scanners and linear array transducers can be used but linear array transducers may be more difficult to manipulate in confined areas such as the thoracic inlet.

Identification of moving fluid

In some investigations of soft tissue structures, areas will be identified that are filled with material of a homogenous density, usually hypoechoic in nature, and the observer will be uncertain whether this structure is a vessel or some other fluid-filled structure. In these situations, pulsation of the structure may lead to its identification as an artery. In some instances echogenic particles will be seen to be moving within the vessel lumen.

Once again, the most useful technique for investigating flow in a vessel is Doppler ultrasound. Not only can the contents of a vessel be confirmed to be moving, but also measurements of the velocity of flow and the diameter of the vessel will allow calculation of the volume of blood that is flowing through the vessel.

Major vessel rupture

One of the most common causes of sudden death in horses is rupture of a major artery. By definition, it is therefore difficult for any investigative technique other than post-mortem to be useful in these cases. However, in some horses death is not immediate and investigation may identify the source of haemorrhage in collapsed animals.

The pulmonary vessels are commonly involved. This usually manifests as a profuse bilateral nasal haemorrhage. Occasionally, a vessel ruptures into the pleural space and the presence of a bloody effusion may be identified by thoracic radiography, ultrasonography and thoracocentesis. However, ultrasonography is the most useful of these techniques and thoracocentesis should be avoided if possible. Ultrasonography has the advantage that it can provide information on the type of fluid present, its extent and the underlying pulmonary disease.

The major pulmonary vessel is the pulmonary artery. This may rupture as a sequel to CHF. Echocardiography may show hypoechoic regions around the base of the artery, a hyperdynamic heart and abnormal motion of the pulmonary valve in those cases that do not die immediately.

Aortic vessel rupture is a potential cause of sudden death and is not diagnosed ante-mortem. Occasionally the aorta ruptures at its root into the interventricular septum or right ventricle. This results either in sudden death caused by a malignant arrhythmia due to massive disruption of the cardiac conduction tissue or in right-sided congestive failure. In the latter cases a machinery murmur, present in systole and diastole, is detected from the right hemithorax. A definitive diagnosis can be made with echocardiography. Rupture of a mesenteric artery may be associated with acute colic. In such cases abdominocentesis may reveal frank blood. Fractures of long bones are sometimes associated with haemorrhage from major arteries and in the case of pelvic fractures the blood loss may result in death. In these instances ultrasonography may show the fracture and an associated haematoma. A 3.5-5 MHz linear array or sector-scanning transducer is ideal and can be used transcutaneously or per rectum.

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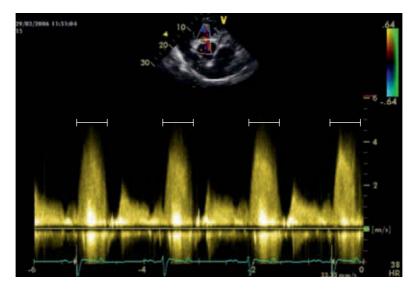


Plate 13 (Fig. 9.8) Continuous wave Doppler study of the abnormal flow through the ventricular septal defect of the horse in Figure 9.5. To perform this study, the transducer has been rotated from the position in Figure 9.5 to provide the best possible alignment with the abnormal flow through the defect. Blood flows at high velocity (4 m/s) through the defect from left to right ventricle (flow towards the transducer as shown by bars) during systole.



Plate 14 (Fig. 15.8) Closer naked eye examination of the eye and adnexa should note external details shown here such as the angle of the cilia on the upper eyelid, the dorsal and ventral orbital sulci (which divide the eyelids into tarsal and orbital portions), the position of the third eyelid and caruncle, and the amount of pigmentation present on the eyelids and conjunctiva. The limbus should be clearly defined; note the pigmented rim in this animal. The cornea should be transparent, allowing the fine structure of the iris to be clearly visualized. In this horse the grey line which marks the insertion of the pectinate ligament into Descemet's membrane and the cornea is very obvious laterally, less so medially. The pupil should be an almost symmetrical horizontal ellipse and granula iridica are usually obvious on the dorsal pupillary border, less so on the ventral pupillary border. In order to appreciate internal details beyond the pupil, examination must be continued in the dark.

CHAPTER 10

Lymphatic diseases

Chapter contents

I. Practical techniques 195 Lymphadenopathy 195 Lymph node biopsy 195

II. Investigating specific diseases 197

Lymphangitis 197 Sporadic lymphangitis 197 Infectious causes of lymphangitis 198 Lymphosarcoma in horses 198 Abdominal lymphosarcoma 199 Thoracic lymphosarcoma 200 Multicentric lymphosarcoma 200 Cutaneous lymphosarcoma 201 The clinical pathology of lymphosarcoma 201

Further reading 202

I. PRACTICAL TECHNIQUES

Lymphadenopathy

Lymph node enlargement is the result of reactive hyperplasia, infection or neoplastic invasion and is usually incidental to other clinical signs.

In healthy horses, the palpable superficial lymph nodes are usually small and include the submandibular and precrural nodes. Deeper lymph nodes that become palpable as a result of enlargement include retropharyngeal, prescapular, thoracic inlet, supramammary and inguinal nodes.

Lymph node swellings may obstruct dependent lymphatic drainage and cause oedema, as in sporadic lymphangitis, 'strangles' (*Streptococcus equi* infection) and tumours of the lymphosarcoma complex. As space-occupying lesions, swollen lymph nodes may obstruct the pharynx, oesophagus, trachea, bronchi or intestinal tract, depending upon their location.

Although neoplastic infiltration may be the result of metastasis from tumours in adjacent tissues, the most common tumour causing lymphadenopathy in horses is lymphoid in origin, i.e. lymphosarcoma (lymphoma).

If the associated clinical signs are inconclusive, biopsy is required to differentiate between inflammatory enlargement (lymphadenitis) and neoplastic infiltration.

Lymph node biopsy

A reasonable-sized, representative sample of the node is essential for adequate histopathological examination.

Lymph node excision is ideal for diagnostic pathology, but impractical in the horse.

Fine-needle aspiration and smear preparation occasionally provides diagnostic information. However, the sample is small and unlikely to be representative of the lesion, thus producing a 'false negative'. An exception is lymph node abscessation.

The best option is incisional biopsy, in which a wedge section is excised under local anaesthesia, coupled, if necessary, with sedation. This relatively large sample retains better morphology than a needle core biopsy and improves the chance of a definitive diagnosis.

Wedge section biopsy technique

A wedge section biopsy is most easily collected from a superficial lymph node that can be adequately immobilized against the overlying skin. The local anatomy of the area of lymph drainage should be considered carefully to avoid inadvertent damage to adjacent structures.

- Depending on the site, an area some 10–15 cm square is clipped and surgically prepared over the node. Local anaesthesia is produced by subcutaneous injection of lidocaine along a line following the proposed site of the skin incision. The remainder of the technique must be performed under strict surgical asepsis.
- Ideally the lymph node is immobilized between the fingers and thumb of one hand, leaving the other to make the skin incision. Once the initial incision has been made, the lymph node is maintained in a fixed position against the skin opening. A deep elliptical incision is then made through the capsule of the node and this is continued in a convergent direction into the body of the node. This is most easily performed using a No. 15 scalpel. The wedge section is grasped with tissue forceps and gently withdrawn, while scissors are used to cut any remaining tissue connections.
- In most instances the section is then divided into halves. One half is submitted for bacterial culture and the other is put into formalin for histopathology.
- Prior to closure the source of any significant haemorrhage should be identified and ligated. The cut edges of the node are then apposed

with a horizontal mattress suture using an absorbable material. Similar material can be used to close the subcutaneous tissues in a simple continuous pattern. The skin incision is then closed with non-absorbable material in a simple interrupted pattern. Local swelling is usually minimal following the procedure and resolves in 5–7 days.

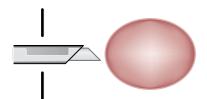
Comments

- Complications of wedge section biopsy are infrequent, but more common than with the needle technique (see below). The most common complications are local haemorrhage and/or a failure of first-intention healing. These risks are reduced by careful attention to good surgical technique during the course of the procedure.
- Poor healing is likely following the incision of a neoplastic mass.
- As with all invasive techniques, the patient's tetanus status should be ascertained.

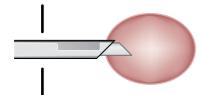
Needle core biopsy technique

Because of the potential problems associated with healing, a pragmatic alternative to wedge section is needle core biopsy. A core biopsy may be obtained using the Tru-Cut needle (Baxter Healthcare Corporation, California). The Tru-Cut is a 14G punch needle designed for sampling superficial and subcutaneous tissue masses. Its design enables a tissue plug to be trapped and excised within the outer casing of the needle (Fig. 10.1). The technique is as follows:

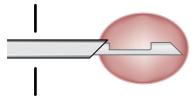
 A representative lymph node is chosen, ideally one that is superficial and can be held immobile against the overlying skin. An area of skin over the node is clipped and prepared as for the wedge biopsy. A small volume (1–2 ml) of local anaesthetic is infiltrated subcutaneously at the site of needle entry. This allows a small stab incision to be made through the skin with a scalpel. Core biopsy needles are not designed to penetrate the skin and a prior stab incision is always necessary.



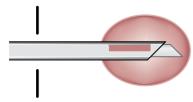
The closed needle is inserted through a skin incision



The tip is positioned within the edge of the node



The obturator is advanced



The cannula is advanced to cut the specimen

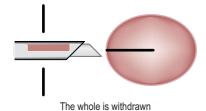


Figure 10.1 Technique for needle core biopsy of a lymph node using the Tru-Cut instrument.

- The needle, in the closed position, is then inserted through the skin incision and positioned so that the tip is just entering the lymph node (Fig. 10.1). Where possible, the entry of the needle should be along the direction of the long axis of the node.
- The obturator alone is then advanced into the lymph node, allowing tissue to insinuate into its specimen notch. The cannula is advanced in a secondary action to cut the tissue core. Both obturator and cannula are then withdrawn in the closed position. It is not usually necessary to suture the stab incision.
- The sample is retrieved from the needle by advancing the obturator to reveal the specimen notch. It can then be teased into formalin using a sterile disposable needle. Providing the biopsy needle is kept sterile, several samples may be collected. However, it is usually preferable to collect a second sample from a different site within the node.

Spring-loaded automated biopsy instruments are also available (see under 'Liver biopsy' in Ch. 4: 'Liver diseases').

II. INVESTIGATING SPECIFIC DISEASES

Lymphangitis

Sporadic lymphangitis

Sporadic lymphangitis is a common lymphatic obstruction of uncertain aetiology that usually affects one or other of the hindlimbs and is traditionally blamed on a protein-rich diet fed during a rest period. However, an infectious aetiology cannot be discounted and culture of skin biopsy specimens should be considered. Inflammation of the lymphatic tracts and associated regional lymph nodes leads to lymph stasis and, at the extreme, a thickening of the whole limb.

Diagnosis is by recognition of the clinical signs. The earliest change is an acute lameness, but swelling of the limb quickly follows and may be sufficiently severe to cause serum ooze over taut skin surfaces. Secondary infection with the development of cellulitis is possible. Early recognition and treatment of the acute case to resolve oedema formation is extremely important. If oedema persists for more than 7 days, severe fibrosis can develop in the interstitial spaces, resulting in permanent swelling and reduced function. This chronic disfigurement is usually refractory to all treatments. There is a tendency for the condition to recur.

Infectious causes of lymphangitis

Inflammation of lymphatic vessels and regional lymph nodes in association with local infection is common. Infections that cause primary lymphangitis are relatively uncommon and usually involve the limbs, producing local swelling and oedema. In contrast to sporadic lymphangitis, these conditions are contagious and involve identifiable bacterial or fungal agents.

Ulcerative lymphangitis is the most common of these conditions and may be associated with wound infection in conditions of poor hygiene. Transmission is by direct contact (grooming kit) but biting flies are possible vectors. The result is multiple foci of nodular abscessation along lymphatic tracts that erupt to discharge a greenish exudate. A number of bacterial causes are implicated including Corynebacterium pseudotuberculosis, Pseudomonas aeruginosa, and staphylococcal and streptococcal species. Diagnosis is based on culture. Skin biopsy may also be performed and typically reveals superficial and deep perivascular dermatitis that may be pyogranulomatous or suppurative. Percutaneous ultrasound of the affected limb may assist in identifying and draining abscesses underneath the skin.

The clinician should be aware of two other forms of infectious lymphangitis that, although very rare, are still notifiable in the UK and other parts of the world. *Cutaneous glanders* or 'farcy' is the cutaneous form of a debilitating pneumonic disease caused by *Pseudomonas mallei*. It has been eradicated from most of western Europe and is now confined to parts of Asia. Lymphatic infection is associated with the development of nodules that discharge a 'honeylike' purulent exudate. Diagnosis is based on culture and serum antibody assay. In the UK an intradermal hypersensitivity test would be undertaken by Animal Health using an antigen extract of the bacterium (the Mallein test). *Epizootic lymphangitis* is caused by a yeast-like fungus, *Histoplasma farciminosum*, which gains access to superficial wounds to produce skin nodules from which a thick creamy exudate erupts. The disease is now of limited occurrence worldwide, being found in parts of the African and Asian continents. The clinical presentation is similar to the cutaneous form of glanders, from which it can be distinguished by smear cytology, serology, and/or Mallein test.

Lymphosarcoma in horses

Lymphosarcoma is the most common tumour of the equine haematopoietic system and is probably the most common internal tumour of horses. It tends to occur in middle-aged and older horses, but cases are recorded in yearlings and young horses. There is no predisposition of breed or sex.

The site of tumour development and the associated range of presenting signs can usually be grouped conveniently into one of four categories:

- Abdominal lymphosarcoma probably the most common form
- Thoracic lymphosarcoma
- Multicentric lymphosarcoma
- Cutaneous lymphosarcoma probably the least common form.

However, individuals often present tumour foci and clinical signs that overlap these divisions, resulting in a wide variety of clinical presentations. In most cases there is weight loss and a common nonspecific finding is intermittent fever that is probably associated with tumour necrosis.

A definitive ante-mortem diagnosis of lymphosarcoma is obtained by demonstrating neoplastic lymphocytes in the peripheral blood, bone marrow, pleural or peritoneal fluids, or in a biopsy sample of a lymph node or tumour mass. Typically a diagnosis is obtained from the histology of a tumour mass. Otherwise, the clinical pathology of lymphosarcoma tends to be non-specific.

The techniques employed to narrow down the differential diagnoses of the presenting clinical signs

are outlined below. They are described in detail elsewhere in this book under the appropriate organ systems.

Abdominal lymphosarcoma

Abdominal lymphosarcoma (alimentary form) is characterized by the development of discrete (focal) or diffuse lesions. Occasionally, both conditions occur together.

Discrete lesions

Sites of lymphocyte infiltration include the intestinal wall, abdominal lymph nodes, mesentery, liver, omentum and spleen. Such lesions can achieve considerable size before causing clinical signs. In general, clinical signs are associated with external pressure on the gastrointestinal tract (causing acute colic), or focal invasion of the intestinal wall itself (causing recurrent colics). Ventral oedema may occur.

Diagnosis

- A palpable, solid mass (or masses) at rectal examination is suspicious.
- Abdominocentesis may provide diagnostic exfoliative cytology, but this is rarely the case with lymphosarcoma.
- Clinical pathology is likely to provide non-specific information (see later).
- Percutaneous ultrasound examination may demonstrate masses in the liver (Fig. 10.2), kidneys or spleen (Fig. 10.3) that may be accessible by needle core biopsy.
- Laparotomy and occasionally laparoscopy demonstrate the mass(es) and the extent of gross infiltration.

Diffuse lesions

Extensive infiltration of the intestinal mucosa/submucosa with lymphocytes causes destruction of the villous architecture. Local lymph nodes are also likely to be involved. The result is malabsorption and the presenting signs depend upon which part of the intestine is involved.

Infiltration of the small intestine is associated with a loss of weight despite an adequate food



Figure 10.2 Hypoechoic lesions in the liver identified by percutaneous ultrasound in a horse with lymphoma. (Image copyright © University of Georgia/Dr A. M. House.)

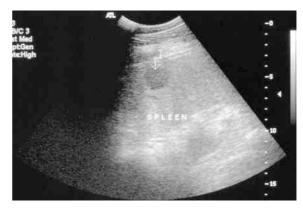


Figure 10.3 Percutaneous ultrasound examination of a horse with metastatic tumours in the spleen. (Image copyright © University of Georgia/Dr A. M. House.)

intake. However, the appetite is often variable. Ventral oedema may be present and occasionally there are signs of chronic low-grade pain such as teeth grinding or repetitive yawning. Faecal consistency is usually normal.

Infiltration of the large intestine (or large intestine plus small intestine) produces the same signs as above, but with chronic diarrhea.

Diagnosis

- In a serum biochemistry profile,
 - hypoalbuminaemia is common (protein-losing enteropathy) and in these circumstances an oral

glucose absorption test may be performed (see 'Tests of intestinal malabsorption' in Ch. 2: 'Alimentary diseases'). The serum alkaline phosphatase may be raised (see below).

- The oral glucose absorption test will usually indicate malabsorption if the small intestine is involved.
- In cases of diarrhoea, a rectal biopsy may demonstrate lymphocyte infiltration of the large intestine.
- Abdominocentesis is indicated, but rarely provides diagnostic exfoliative cytology.
- Abdominal ultrasound examination will sometimes reveal increased wall thickness (>5 mm) of the small or large intestine.
- Intestinal biopsies obtained by laparotomy, laparoscopy and occasionally transendoscopically from the duodenum may provide diagnostic histopathology.

Comment

 At laparotomy or post-mortem examination, diffuse lymphocytic infiltration of the intestinal tract is usually not appreciable. There is often no palpable thickening of tissues and a definitive diagnosis always requires histopathology. However, both diffuse and discrete lesions can occur together.

Thoracic lymphosarcoma

Thoracic lymphosarcoma (mediastinal form) is characterized by space-occupying lesions within the thorax owing to the development of thymic/mediastinal tumours with associated pleural effusion. There may be oedema at the thoracic inlet (which gravitates ventrally), together with jugular vein distension, dyspnoea and weight loss. Invasion of lung parenchyma is possible.

Diagnosis

- Thoracic radiography will reveal pleural effusion and possibly abnormal mass(es).
- Percutaneous ultrasonography will demonstrate pleural effusion and the presence of any



Figure 10.4 Thoracic ultrasound examination demonstrating nodular infiltrates at the lung periphery. (Image copyright © University of Georgia/Dr A. M. House.)

abnormal thoracic mass. Pulmonary nodules can sometimes be identified (Fig. 10.4). The technique can be used to indicate a suitable site for thoracocentesis/needle biopsy.

- Thoracocentesis may demonstrate numerous lymphoblasts in the effusion (this is in contrast to abdominocentesis, which rarely reveals evidence of abdominal lymphosarcoma).
- Other clinical pathology is likely to provide non-specific information (see later).

Multicentric lymphosarcoma

Multicentric lymphosarcoma (generalized form) is characterized by the widespread infiltration of lymph nodes and other organs. There is a generalized lymphadenopathy of superficial (Fig. 10.5) and internal lymph nodes. Metastases can occur to the bone marrow, liver, spleen, intestines, kidneys, lung and elsewhere. Ventral oedema may occur.

In some patients the generalized form may be associated with leukaemia. However, unlike other species, it is less common to identify leukaemia in horses with lymphosarcoma.

Diagnosis

- Biopsy of an enlarged lymph node (see above) demonstrates a neoplastic infiltrate.
- Thoracic radiography may reveal evidence of metastases (Fig. 10.6).

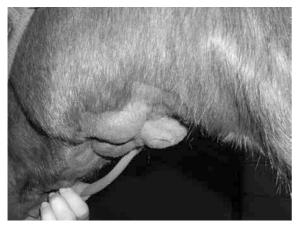


Figure 10.5 Enlarged submandibular lymph nodes from a pony with lymphoma (multicentric form). (Image copyright © University of Georgia/Dr A. M. House.)

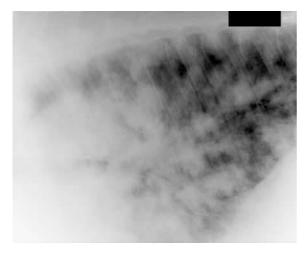


Figure 10.6 Thoracic radiographs of a pony with multicentric lymphosarcoma demonstrating a diffuse nodular pattern. (Image copyright © University of Georgia/Dr A. M. House.)

- Haematology may reveal leukaemic forms, in which case bone marrow aspiration/biopsy is indicated.
- Other clinical pathology is likely to provide non-specific information (see below).

Cutaneous lymphosarcoma

Cutaneous lymphosarcoma is characterized by the presence of single or multiple non-painful subcuta-

neous swellings. These are of variable diameter, up to 10 cm, and are randomly distributed over the body. Sometimes there is infiltration into muscle tissue. Eventually there is weight loss but as long as there is no metastasis to internal organs the patient may survive for several years.

Diagnosis

• Biopsy of a subcutaneous mass, as described above for lymph node biopsy.

The clinical pathology of lymphosarcoma

In all cases of wasting disease, clinical pathology should include haematology and serum biochemistry profiles. Haematology provides information with respect to anaemia and may reflect inflammatory processes. A serum biochemistry profile should be selected that will reveal abnormalities consistent with hepatic, renal or alimentary disease. However, in patients with lymphosarcoma the clinical pathology tends to be non-specific as indicated below.

Haematology

Haematology usually demonstrates anaemia, which is probably the result of chronic inflammatory suppression of erythropoiesis. On rare occasions, immune-mediated haemolytic anaemia is identified in cases of equine lymphosarcoma (see Ch. 8: 'Blood disorders').

Leukocytes usually show non-specific changes. A neutrophilia is possible, but horses with lymphosarcoma rarely show leukaemia. Those that do show a marked lymphocytosis, which usually comprises atypical or immature lymphocytes, or a mixture of both. In such cases, bone marrow aspiration/biopsy is likely to reveal infiltration by immature lymphocytes.

Plasma fibrinogen concentration

Hyperfibrinogenaemia is usual though not invariable. It may reflect the proinflammatory response to tumour necrosis.

Serum biochemistry

Patients often present with hyperproteinaemia, which is usually due to hyperglobulinaemia. However, in the alimentary form albumin concentrations may be depressed as a result of a protein-losing enteropathy.

Serum protein electrophoresis in cases of hyperglobulinaemia shows a polyclonal increase in beta and gamma globulins, reflecting a non-specific inflammatory response. On very rare occasions a *monoclonal gammopathy* is identified as a dense narrow band of gamma protein. Such a finding is indicative of either a lymphoproliferative or myeloproliferative neoplasm and should prompt the search for a neoplastic process if one has not been determined already (see also 'Plasma cell myeloma' in Ch. 8: 'Blood disorders'). Serum alkaline phosphatase is often raised and presumably reflects damage to the architecture of the intestinal epithelium. In the presence of hypoalbuminaemia it is consistent with an enteropathy, but it is not pathognomonic for lymphosarcoma.

FURTHER READING

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CHAPTER

Fluid, electrolyte and acid-base balance

Chapter contents

Fluid balance 204

Assessment of fluid balance 205

Plasma protein status 208

Assessment of the plasma protein requirement 209

Electrolyte balance 210

Sodium 210 Potassium 211 Chloride and bicarbonate 211 Calcium and magnesium 212 Calculation of fluid and electrolyte losses 212 Clinical example 212 Acid-base balance 213 Assessment of acid-base balance 214 Common conditions affecting fluid and electrolyte balance 214 Reduced water intake 214 Colic 215 Diarrhoea 215 Exertional dehydration 216 Further reading 216

The purpose of this chapter is to address the diagnostic assessment of fluid, electrolyte and acid-base balance in various disease states of the adult horse.

In the text that follows it is necessary to consider fluid, electrolyte and acid–base balance separately, but the clinician must never lose sight of their mutual interdependence. There is a dynamic relationship between these parameters; a change in one will induce changes in the others. Because of this the assessment of changes that have been caused by disease must be monitored throughout the period of corrective treatment; a treatment aimed at correcting one parameter will certainly have an effect on the others. Although it is outside the scope of this book to describe treatments in detail, the required action will be indicated by the interpretation of clinical findings. The first section of this chapter deals with fluid balance and its assessment. However, quite apart from the volume of fluid that is needed to correct an imbalance, the required composition of that fluid must be determined by assessment of the plasma protein status, blood electrolyte concentrations, and acid-base considerations. These parameters are considered individually in the subsequent sections and their application to common conditions is summarized in the final section on 'Common conditions affecting fluid and electrolyte balance'.

Fluid balance

An understanding of physiology and pathophysiology of fluid balance is essential for optimizing

Diagnostic techniques in equine medicine

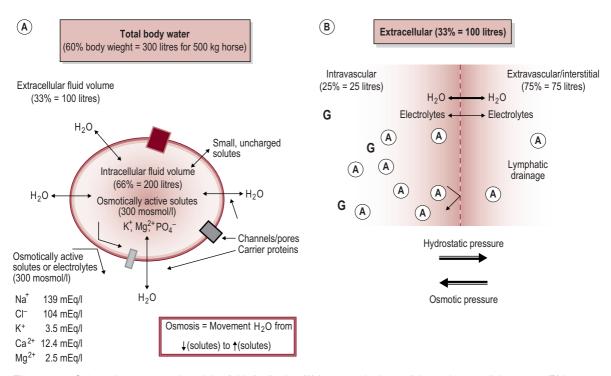


Figure 11.1 Schematic representation of the fluid distribution (A) between the intracellular and extracellular space; (B) between the intravascular and extravascular space. (A, albumin; G, globulin).

patient care. Critically ill patients should not be over- or under-hydrated and careful monitoring is essential to accomplish this goal. Figure 11.1 illustrates the distribution of water between the intracellular and extracellular space and between the intra- and extravascular spaces that comprise the extracellular space. Disturbances in the water balance result from either inadequate intake or excessive loss. The most common reasons for inadequate intake in horses include water deprivation (e.g. recumbency, lameness, frozen water source, mismanagement), an inability to drink (e.g. dysphagia, oesophageal obstruction, oral lesions) or systemic illness causing inappetence and reluctance to consume water (e.g. neurological disease, colic, pleuropneumonia). Excessive water is most commonly lost into the gastrointestinal tract (e.g. colitis, enteritis) or is associated with haemorrhage, excessive sweating, hypersalivation (e.g. choke, dysphagia) or polyuric renal failure. Electrolyte abnormalities usually accompany these alterations in fluid balance.

Dehydration refers to total body water loss and hypovolaemia is used to describe an inadequate intravascular water volume. Dehydration and hypovolaemia do not occur independently; however, recognition of each is important to direct fluid therapy. Differentiating dehydration from hypovolaemia is important when selecting the type of fluid therapy because crystalloid fluids are ideal for correcting dehydration because they are distributed throughout the extracellular (and intracellular) fluid space, and colloids are most effective for correcting hypovolaemia because they are retained within the vascular space and increase the intravascular volume.
 Table 11.1
 Nomenclature for systemic conditions affecting horses with an acute abdomen, based on information from human and veterinary critical care

Nomenclature (acronym)	Definition			
Endotoxaemia	Endotoxin (lipopolysaccharide from Gram-negative bacteria cell wall) circulating in the blood. Endotoxin can stimulate a systemic inflammatory response (SIRS)			
Systemic inflammatory response syndrome (SIRS)	Systemic inflammatory response to severe clinical disease with two or more of the following: 1) fever or hypothermia, 2) tachycardia, 3) tachypnoea or hypocapnia, and 4) leukopenia, leukocytosis or a high number of circulating immature (band) neutrophils			
Multiple organ dysfunction syndrome (MODS)	Functional abnormality of more than one vital organ system including lungs, kidneys, cardiovascular, central and peripheral nervous systems, coagulation, gastrointestinal tract, liver, adrenal glands and skeletal muscle			
Sepsis	SIRS plus infection			
Severe sepsis	Sepsis plus MODS, hypoperfusion or hypotension			
Septic shock	Sepsis-induced hypotension despite adequate fluid resuscitation plus perfusion abnormalities (lactic acidosis, oliguria, altered mentation)			
Hyperdynamic shock	Tachycardia, tachypnoea, hyperaemic mucous membranes, rapid capillary refill time, decreased borborygmi compared to normal, muscle fasciculations and dullness. Hyperdynamic shock is characterized by a high cardiac outpu and low peripheral vascular resistance			
Hypodynamic shock	Tachycardia, tachypnoea (rapid, shallow respiration), prolonged capillary and jugular refill times, dry and purple to pale mucous membranes, weak peripheral pulses, cool extremities and hypothermia. Hypodynamic shock is characterized by low cardiac output, high peripheral vascular resistance and systemic hypotension. MODS often follows signs of hypodynamic shock			
Disseminated intravascular coagulopathy (DIC)	Abnormality in three out of five of the following categories: 1) thrombocytopenia, 2) hypofibrinogenaemia, 3) prolonged clotting time tests (prothrombin time (PT), partial thromboplastin time (PTT), activated clotting time (ACT)), 4) decreased antithrombin III (ATIII) activity compared to normal, 5) high fibrin (fibrinogen) degradation products (FDP)			

Shock is, by definition, an imbalance between oxygen delivery and oxygen consumption that results in inadequate adenosine triphosphate (ATP) production and, if not corrected, cell death. Common causes of shock in horses include hypovolaemia, endotoxaemia, haemorrhage, sepsis and hypoxia. Endotoxaemia is a common cause of shock in horses and results in a systemic inflammatory response syndrome (SIRS), which, if not corrected, can lead to hyperdynamic and then hypodynamic shock and ultimately multiple organ dysfunction syndrome (MODS) and death (Table 11.1).

Assessment of fluid balance

An acute loss of fluid from the ECF will increase its osmolality and water is transferred from the ICF. When the loss exceeds 5% of total body water, the result becomes clinically detectable as dehydration.

Clinical signs

The collective clinical signs can provide a diagnostic guide to the extent of fluid loss and its effect upon the circulation. Key features are as follows:

- High heart and pulse rates (tachycardia) are indicative of circulatory compromise (the most common being hypovolaemia) or pain. The patient's history, physical examination and laboratory data can help determine the predominant reason for tachycardia
- Changes in pulse pressure reflect the integrity of the peripheral circulation. If the pulse is weak or absent and the capillary refill time prolonged, the patient is hypovolaemic
- Changes in the capillary refill time (CRT) reflect the integrity of the peripheral circulation. Refill times in excess of 2 seconds indicate hypovolaemia with poor peripheral perfusion and circulatory compromise
- Dryness of the oral mucous membranes indicates dehydration
- Poor jugular distensibility when the vein is raised indicates a fall in venous pressure and hypovolaemia
- Poor skin elasticity is consistent with dehydration. However, this is a very subjective test in horses. A fold pulled up at the point of

the shoulder is probably more reliable than the skin of the neck region

- Cool extremities and a low rectal temperature indicate shock; generally the extremities will be cool well before hypothermia is evident on rectal temperature
- Inadequate urine production accompanies renal hypoperfusion and should be monitored closely. Normal urine output in an adult horse is 15–30 ml/kg/d (7.5–15 litres/500 kg/d) and is assessed subjectively in adult horses.

The collective clinical information will not provide an accurate measure of the per cent dehydration of body weight, but it allows a subjective assessment of mild, moderate or severe dehydration. All signs are accentuated in acute dehydration and at their extreme indicate the development of hypovolaemic shock (Table 11.2).

Clinical pathology

In addition to clinical signs, simple blood parameters can be used to indicate the severity of dehydration. However, where facilities are available they are best used in a serial manner to follow the course of dehydration over a critical period.

Packed cell volume (PCV). A blood sample taken into anticoagulant (EDTA or heparin) is suitable for

Dehydration (%)	Heart rate (bpm)	CRT (s)	PCV/TPP (%/g/dl)	Cr (mg/dl)	Additional clinical signs
<5%	30–40	<2	WNL	WNL	Not detectable
6	41–60	2	40/7	1.5–2	
8	61–80	3	45/7.5	2–3	Possible dry mucous membranes; eyes possibly sunken in orbits
10	81–100	4	50/8	3–4	Definite dry mucous membranes; eyes sunken in orbits; possibly signs of shock (cool extremities, rapid and weak pulse)
12	>100	>4	>50/>8	>4	Definite signs of shock; imminent death

Table 11.2 Clinical signs in patients that can be used to estimate percentage of dehydration

Cr, serum or plasma creatinine concentration; CRT, capillary refill time; PCV, packed cell volume; TPP, total plasma protein; WNL, within normal limits for the laboratory. Source: adapted from Hardy 2004 and DiBartola S P 2000 Fluid therapy in small animal practice. Saunders, St Louis.

PCV estimation but the technique has potential drawbacks. Excitement of the patient at collection may introduce error as a result of splenic contraction. Alternatively, an anaemic animal that has become dehydrated may show a PCV that is within a normal range. However, in all cases serial measurements should reveal any progressive dehydration. In general terms a PCV of more than 45% indicates a reduction in the ECF volume.

Total plasma protein (TPP) estimation. A heparinized blood sample is suitable for total plasma protein estimation, which may be undertaken in the field using a refractometer. However, concurrent protein loss can produce a spuriously low result. Thus a patient suffering a protein-losing enteropathy as well as dehydration may show a total plasma protein that is within the normal range. In addition, chronic infection can raise the plasma fibrinogen and globulin concentrations so that the total plasma protein will be high, even in the absence of dehydration. However, as with PCV, serial measurement should reveal any progressive dehydration.

Urea and creatinine concentrations. Most serum or plasma biochemistry parameters, including urea and creatinine, are high in patients with acute dehydration. However, increases in urea and/or creatinine also reflect prerenal failure associated with hypovolaemia (i.e. renal hypoperfusion). This condition is usually reversible if fluid therapy is not delayed. Renal failure should be suspected and urinalysis performed on any patient that has a persistently high creatinine concentration despite apparently adequate hydration.

Lactate concentration. Lactate is the end product of anaerobic glycolysis and is used as a marker of peripheral perfusion and oxygen delivery. Lactate concentration can be increased for many reasons; however, hyperlactataemia is most often attributed to tissue hypoxia as a result of hypovolaemia, hypoxaemia, hypotension or, less commonly, a hypermetabolic state. Lactate concentration can be used to monitor the adequacy of fluid therapy during patient resuscitation. Normal lactate concentration is less than 2 mmol/l and a horse on intravenous fluids should have a lactate concentration below 1 mmol/l. Venous oxygen saturation $(S_vO_2)/venous$ oxygen partial pressure (P_vO_2) . A low S_vO_2 or P_vO_2 indicates that the tissues are extracting more oxygen than that delivered to them and in combination with hyperlactataemia is an indication of tissue hypoxia as a result of hypovolaemia, hypoxaemia, hypotension or, less commonly, a hypermetabolic state. While the jugular vein is most accessible for obtaining a venous sample, mixed venous (pulmonary artery) or central venous (cranial vena cava) blood is more representative of the entire body. A central venous blood sample can be obtained following placement of a central venous catheter (see below). Normal jugular S_vO_2 is reported to be 65–75% and P_vO_2 is 45.6 ± 4.7 mmHg in adult horses.

Urine specific gravity (USG). The renal tubules concentrate and dilute urine based on water requirements. Horses that need to lose total body water (i.e. are over-hydrated) have a USG below 1.008 and dehydrated horses have concentrated urine with a USG as high as 1.060. Urine specific gravity can be used to assess and guide fluid therapy. For example, fluid administration rate should be slowed in a horse with a USG of 1.003 and may be increased in a horse with a USG of 1.030 depending on other measurements such as TPP, creatinine and lactate concentrations, and central venous pressure (CVP). Urinalysis and assessment of urine function should be performed on any horse that is persistently isosthenuric (USG 1.008-1.012) after discontinuing fluid therapy.

Cardiovascular system

Arterial blood pressure. While we are unable to actually measure blood flow, mean arterial pressure provides the best available estimate. Arterial blood pressure can be measured directly with an arterial catheter in the facial or transverse facial artery (adults or foals) or the great metatarsal artery (foals) or indirectly using a tail (adults and foals) or limb cuff (foals). Arterial blood pressure in adult horses is most easily measured using the oscillometric method with a cuff over the coccygeal artery (tail cuff). The length of the internal inflatable bladder should be 80% and width 20–25% of the tail circumference. Measurements should be taken with

the horse standing quietly and the head in a normal resting position; three consecutive measurements should be taken and the consistency between measurements observed; and the heart rate measured should be the same as the heart rate obtained on physical examination. Normal indirect blood pressure in adult horses is: systolic 110–130 mmHg, diastolic 55–80 mmHg and mean 80–100 mmHg. Mean arterial blood pressure should be maintained at more than 60 mmHg in most cases; this can usually be accomplished with crystalloid and colloid fluid therapy; inotropes and vasopressors are rarely necessary.

Central venous pressure. Central venous pressure is the intraluminal pressure within the intrathoracic cranial vena cava and can be measured using a water manometer via a catheter placed in the jugular vein and terminating in the intrathoracic cranial vena cava. Central venous pressure provides an estimate of preload and right ventricular filling pressure and can be used to guide fluid therapy in hypovolaemic patients. Normal CVP in adult horses is reported to range from 8 to 12 cmH₂O. In cases with a low CVP, intravenous crystalloid and colloid fluid therapy should be used to get the CVP within the reference range. A high CVP can indicate the need to decrease the rate of intravenous fluid administration or in rare cases heart failure.

Comment

• It is important to remember that the key in monitoring fluid balance in patients is to use multiple methods of assessment and take serial measurements to monitor the response to fluid therapy.

Plasma protein status

Albumin, globulins and fibrinogen are the major plasma proteins. Colloid osmotic (oncotic) pressure (COP) is maintained by the plasma proteins, principally by albumin, and is necessary to maintain intravascular volume. Normal COP in adult horses is 15–22 mmHg.

Some 60% of an adult horse's body weight consists of water, which is distributed between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments. The ICF is approximately 66% and the ECF 33% of the total body water (Fig. 11.1A). Water moves freely across the cell membrane between these compartments by osmosis to maintain the osmotic equilibrium (300 mosmol/l). A decrease in the water volume of either compartment changes its osmotic forces, which results in a redistribution of water between the two sides until the osmotic equilibrium is resumed. The osmolality of the ICF is maintained for the most part by potassium and phosphates whereas ECF osmolality is primarily a function of sodium and chloride ions. Osmotically active solutes are not able to freely cross the cell membrane and move between compartments through specific channels or pores.

The extracellular fluid (ECF) space is divided by the capillary wall into intravascular and extravascular spaces. The extravascular space comprises approximately 75% and the intravascular space 25% of the ECF (Fig. 11.1B). Water and electrolytes move freely across the capillary wall between the intra- and extravascular space. Hydrostatic pressure tends to push water from the intravascular space and the COP is responsible for maintaining the water within the intravascular space. Albumin is responsible for most (75%) of the intravascular COP. There tends to be a net movement of fluid from the intravascular to the extravascular space, which is returned to the intravascular space via the lymphatic system.

Hypoproteinaemia is most often associated with hypoalbuminaemia and indicates protein/albumin loss during a disease process. In horses this is commonly associated with loss of albumin through the vascular wall because of endothelial changes associated with the systemic inflammatory response syndrome (SIRS), or actual vascular injury and/or a protein-losing enteropathy with loss into the intestinal lumen because of mucosal damage and inflammation (e.g. enterocolitis, right dorsal colitis, strangulating intestinal lesions and infiltrative bowel disease). Horses with extensive burns or other wounds and acute haemorrhage are also hypoproteinaemic/hypoalbuminaemic. In critically ill patients, inadequate protein intake and high metabolic demands associated with fever, trauma, infection and surgery should also be considered as reasons for hypoproteinaemia/hypoalbuminaemia. Protein-losing nephropathy, chronic hepatic fibrosis and parasitism are less common causes of hypoproteinaemia in horses.

Hyperproteinaemia is most often associated with hypovolaemia and dehydration (see above under 'Fluid Balance'). Gastrointestinal disease with loss of water into the intestinal lumen or wall (e.g. enterocolitis) and inadequate intake because of illness or dysphagia (e.g. botulism, choke) is the most common clinical reason. Hyperproteinaemia associated with hyperglobulinaemia can be associated with chronic infections, immune-mediated disease or neoplasia. Hyperfibrinogenaemia is unlikely to cause hyperproteinaemia because of its relatively low concentration, i.e. a marked hyperfibrinogenaemia of 1000 mg/dl will increase the TPP by 1 g/dl. Hyperfibrinogenaemia is associated with generalized or localized inflammation, including infection, and follows accidental or surgical trauma.

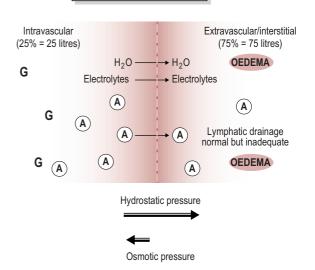
Hypofibrinogenaemia (absolute or relative) can result from disseminated intravascular coagulopathy or liver disease and a coagulation and liver profile should be performed in these cases (see under 'Coagulation disorders' in Ch. 8: 'Blood disorders').

Assessment of the plasma protein requirement

Total plasma protein (total solids) is most often measured in practice using a refractometer. Albumin and globulins are measured in the laboratory using protein electrophoresis, and fibrinogen can be measured using several different techniques. Colloid oncotic pressure can be measured directly using a colloid osmometer or indirectly using various equations such as the Landis–Pappenheimer equation:

 $COP = 2.1TPP + (0.16TPP^2) + (0.0009TPP^3).$

Direct measurement of COP should be measured in critically ill patients. Oedema formation occurs with TPP below 40 g/l or albumin concentrations above 15 g/l (Fig. 11.2). It is critical to avoid over-hydration



Extracellular (33% = 100 litres)

Figure 11.2 Loss of intravascular albumin causing oedema formation.

in hypoproteinaemic/hypoalbuminaemic patients; however, fluid therapy should not be restricted to maintain adequate TPP and albumin concentrations. In these cases, administration of either blood products (plasma, whole blood) or a synthetic colloid (e.g. hetastarch) is necessary.

Fresh frozen plasma should be given to any horse where continued protein losses are occurring, or where continued fluid therapy has resulted in the dilution of plasma proteins to a concentration below 40 g/l. It is important to remember that plasma has benefits (e.g. coagulation factors, antiendotoxin antibodies) in addition to its colloid properties. The principal colloid in plasma is albumin. Excluding ongoing losses, 1 litre of plasma should increase the TPP by 0.05–0.1 g/dl; therefore, to increase the TPP by 1 g/dl in a 500 kg horse 10–20 litres of plasma is necessary. The volume of plasma necessary can be more precisely calculated as follows.

The plasma volume of a horse is approximately 5% of its body weight and the normal range of TPP is approximately 60–70 g/l. Assuming a mean TPP of 65 g/l, a horse with a plasma concentration reduced to 40 g/l has a deficit of some 25 g/l. From these suppositions, a 500 kg horse would have a

11

plasma volume of 25 litres (5% of 500) and its total protein deficit would be 25 g/l \times 25 = 625 g. If a suitable donor has a TPP of 70 g/l, then the volume of plasma required to replace 625 g protein is 625/70 = 8.9 litres.

Because of the expense of plasma, the large volumes necessary and the ongoing loss of albumin, plasma is not necessarily the best colloid to maintain COP. Hetastarch 10 ml/kg/d (5 l/500 kg horse) can be used as a colloid. The main complication with the use of hetastarch is coagulopathy associated with a decrease in von Willebrand's factor/factor VIII; however, at a dose of 10 ml/kg/d this complication is not a problem in normal horses. In horses with disseminated intravascular coagulopathy, the effect of hetastarch on coagulation is unknown and it should be used with caution in these patients. Pentastarch is thought to have fewer side effects, but is more expensive.

Electrolyte balance

The problem in assessing electrolyte balance is that only the concentration of electrolytes in the plasma portion of the ECF can be determined with ease. ICF electrolyte concentrations can be determined, but the techniques are not usually available to the practitioner. However, knowing the distribution and function of the various electrolytes enables an empirical interpretation of their status from the results of a blood sample. Those of clinical importance in fluid and acid-base balance are sodium. potassium, chloride and bicarbonate. The first three can be measured in either serum or plasma, but bicarbonate estimation can only be undertaken on a blood sample collected into lithium heparin (see later). Whole blood samples must be separated soon after collection, since any tendency to haemolysis will alter electrolyte concentrations in serum or plasma. Typical blood electrolyte ranges for the adult horse are given in Appendix 1.1 of Chapter 1. It is important to remember that in patients with normal renal function, the kidneys can correct the majority of electrolyte and acid-base abnormalities following resuscitation, providing the patient receives adequate maintenance intravenous fluids and electrolytes.

Sodium

Sodium is the major cation within the ECF and is largely responsible for maintaining the compartment's osmolality and thereby its fluid volume. The ECF osmolality can be calculated from the following formula:

```
ECF osmolality (mosmol/kg) = 2(Na^+ + K^+) + glucose/18 + urea/2.8.
```

Because cell membranes are permeable to potassium and urea, they are ineffective osmoles. The effective ECF osmolality (mosmol/kg) = $2 \times Na^+ +$ glucose/18 and because glucose contributes little to the osmolality, the effective ECF osmolality can be estimated by doubling the ECF sodium concentration.

The laboratory estimation of serum or plasma (ECF) sodium concentration cannot be interpreted in absolute terms as a blood deficit or excess. This is because its concentration at any one time depends upon fluctuations within the total 'exchangeable' body stores of water, sodium and potassium, which can be transferred between compartments. The relationship of these factors has been defined by the following equation:

From this equation it follows that decreases in serum or plasma sodium concentration below the normal range (hyponatraemia) may be due to an excess of total body water, a loss of sodium or potassium, or a combination of these factors. However, increases in serum or plasma sodium concentration above the normal range (hypernatraemia) may be due to a loss of total body water, an excess of sodium or potassium, or a combination of these factors.

Hyponatraemic states (<135 mmol/l) usually occur in diarrhoeic diseases where massive losses of fluid and electrolyte are followed by oral intake of water and partial replacement of the lost fluid. Hypernatraemic states (>145 mmol/l) are rare but can follow acute dehydration or excessive sodium replacement in fluid therapy.

Because sodium is critical in maintaining osmolality any correction of sodium imbalance should be made slowly, particularly in more chronic states (>48 h) of hyper- or hyponatraemia. In states of hyper- or hyponatraemia (hyper- or hypoosmolality), the brain cells either accumulate idiogenic osmoles (hypernatraemia) or lose potassium and organic osmolytes (hyponatraemia) to maintain isotonicity between the ICF and ECF. With correction of the ECF sodium or osmolality, the idiogenic osmoles need to be eliminated from, or potassium and organic osmolytes restored to, the brain cells to prevent a discrepancy between the osmolality of the ICF and ECF and subsequent cerebral oedema (too rapid correction of hypernatraemia) or osmotic demyelination syndrome (too rapid correction of hyponatraemia). In chronic cases of hypernatraemia it is recommended to correct the serum or plasma sodium concentration at a rate of less than 0.5 mEq/l/h and in chronic cases of hyponatraemia, sodium should not be increased by more than 12 mEg/l/24 h or more than 18 mEg/l/48 h. Resuscitation with a balanced polyionic isotonic fluid is acceptable in most adult horses and the sodium concentration should be monitored during the resuscitation period.

Potassium

Potassium is the major cation of the ICF; only 2% of its total body store is present in the ECF. Consequently, serum or plasma potassium concentrations are of very limited value in estimating total body potassium. Even when the blood concentration is normal or raised, total body potassium stores could be depleted.

It is commonly observed that the serum or plasma concentration of potassium increases during acidosis and decreases in alkalosis. Cells tend to take up hydrogen and release potassium in states of acidosis and the reverse occurs in alkalosis. Knowledge of blood potassium concentration, together with a clinical assessment of the circulatory status, may therefore be used to infer extremes of acid-base balance. However, the situation may be complicated in conditions where there is a net loss of potassium from the circulation.

Decreases in serum or plasma potassium concentration below the normal range (hypokalaemia: <3.3 mmol/l) are often associated with diarrhoea or, importantly, a decreased food intake. Large amounts of potassium are excreted by the normal equine kidney, so that deficits soon occur when a horse's feed intake is reduced. Deficits are readily replaced by the intake of sufficient quantities of hay. Marked hypokalaemia is usually indicative of a severe acid-base imbalance (alkalosis). If the blood potassium concentration is less than 3.3 mmol/l, or the horse is unable to eat, then supplementation must be considered. Potassium should be supplemented in any horse that is inappetent. Routinely available polyionic isotonic fluids in 5 litre bags are replacement fluids because of the high sodium and low potassium concentration. In most adult horses, the kidneys can excrete the high sodium load; however, supplementation with potassium is necessary. Potassium chloride should be added to intravenous fluids used for maintenance at a concentration of 20 mEq/l. During resuscitation, potassium should not be administered at a rate above 0.5 mEq/kg/h and potassium is therefore not usually added to the resuscitation fluids.

Increases above the normal range (hyperkalaemia: >5 mmol/l) are unusual in the horse unless associated with severe acidosis, haemolysis or, rarely, impaired renal function (e.g. renal failure and ruptured bladder). Spurious hyperkalaemia in blood samples may follow spoilage associated with haemolysis or leakage of potassium out of red cells. It is for this reason that prompt separation of serum or plasma from blood cells is important when sampling. If possible, hyperkalaemia should be confirmed by a second sample. For obvious reasons, whole blood samples are unsatisfactory if laboratory processing is delayed.

Chloride and bicarbonate

Chloride and bicarbonate are the major anions of the ECF and demonstrate an inverse relationship. Since chloride is largely located in the ECF, changes in its serum or plasma concentration tend to reflect changes in its whole-body status. A decrease in the

serum or plasma concentration of chloride below normal range (hypochloraemia: <93 mmol/l) is usually the result of an increased loss to the gastrointestinal tract (diarrhoea or high obstruction), impaired renal function or, alternatively, a massive loss in sweating.

The role of bicarbonate is to act as a buffering system and its plasma concentration therefore reflects the horse's acid-base status. Marked decreases in bicarbonate accompany moderate to severe acidotic states. In a state of metabolic acidosis there is a decrease in plasma bicarbonate and an increase in serum or plasma chloride concentration. In metabolic alkalosis the reverse is true. See below for further discussion on acid-base balance.

Calcium and magnesium

Calcium and magnesium are critical electrolytes. Hypocalcaemia and hypomagnesaemia are common in critically ill horses, particularly horses with gastrointestinal disease and endotoxaemia. While the actual deficit is not possible to calculate, calcium and magnesium can and should be supplemented in these cases. It is important to remember that approximately half of calcium is protein-bound and hypoalbuminaemia will result in low total calcium concentrations; therefore, measurement of ionized calcium is ideal in these cases. Supplementation of calcium in cases with ischaemia-reperfusion injury is somewhat controversial because there is not a total body calcium deficit and intracellular accumulation of calcium can potentially exacerbate cell death in these cases. The current recommendation is to supplement calcium following resuscitation.

Calculation of fluid and electrolyte losses

In many instances a polyionic fluid such as lactated Ringer's solution is given empirically to correct fluid loss and the effect is subsequently monitored using clinical and clinicopathological parameters. However, as the following example shows, it is possible to make a crude but revealing calculation of fluid and electrolyte deficits based upon clinical examination and simple clinicopathological data.

Clinical example

The example is a 500 kg horse with severe diarrhoea. Clinical examination reveals a raised heart rate (60–80 bpm), a thready pulse, a prolonged CRT (3–4 s) and reduced skin elasticity. These findings suggest modest dehydration (8–10%). Clinical pathology reveals a PCV in excess of 45% and low plasma concentrations of sodium (130 mmol/l) and potassium (3.0 mmol/l). From this assessment the animal is clearly dehydrated and there is a loss of electrolytes to the bowel. Above all, the sodium deficit needs to be corrected, which in the first instance should stabilize the ECF volume. *The correction of sodium and volume deficits are the major concerns when considering fluid and electrolyte therapy*.

Once the percentage clinical dehydration has been assessed, the magnitude of the required fluid volume for replacement can be estimated as follows:

```
Approximate fluid deficit (litres) =
Supposed clinical dehydration (%) Body weight (kg).
```

In this example the magnitude of fluid requirement is therefore:

8-10% of 500 litres = 40-50 litres.

Using these figures and the given clinicopathological data, it is then possible to estimate the sodium and potassium deficits. For this we make two assumptions: 1) that the horse's plasma sodium concentration in health is the mean of the laboratory's normal range (135-145 = 140 mmol/l) and 2) that the horse's total body water prior to dehydration was 60% of its body weight, i.e. 300 litres.

Since the plasma concentration of sodium has the following relationships (see above under 'Electrolyte balance'):

 $Serum Na^{+} = \frac{Exchangeable Na^{+} + Exchangeable K^{+}}{Total body water}$

then *before* dehydration the sum of exchangeable Na^+ and K^+ is derived from:

This, by substitution of the given figures becomes:

But *after* dehydration the sum of the exchangeable Na^+ and K^+ is reduced because the plasma Na^+ concentration is low (130 mmol/l) and there is a fluid deficit of 40–50 litres as follows:

 $130\times(300-50)$ to $130\times(300-40)$ = 32 500 to 33 800 mmol.

The deficit of $Na^+ + K^+$ therefore becomes:

[42 000 – 33 800 mmol] to [42 000 – 32 500 mmol] = 8200 to 9500 mmol.

In diarrhoea, some 70% of the $Na^+ + K^+$ loss is sodium. In consequence:

The Na $^{+}$ deficit = [8200 0.7] to [9500 0.7] = 5740 to 6650 mmol,

and by subtraction:

The K^+ deficit = 2460 to 2850 mmol.

In summary, this crude assessment of the patient's requirement indicates:

- A total body water deficit of 40–50 litres
- A Na⁺ deficit of 5740–6650 mmol
- A K⁺ deficit of 2460–2850 mmol.

By selecting 40–50 litres of a polyionic solution the sodium deficit would almost be corrected. Polyionic solutions approximate in ionic composition and concentration to plasma and in such solutions the sodium content is usually of the order of 130–140 mmol/l. However, the potassium deficit would remain far short of replacement. This is not a problem if the horse is eating or oral supplementation with potassium chloride is permissible. Nevertheless, if fluid therapy is repeated over several days in the absence of oral supplementation, substantial potassium deficits can result.

Comment

• This calculation provides a rough idea of the immediate replacement volume. However, after

replacement is achieved there will be a continuing maintenance requirement of 2 ml/ kg/h (1 l/500 kg/h) *plus* estimated losses from diarrhoea or gastric reflux (often 2–4 l/500 kg/h), which the patient may or may not be able to sustain for itself. While the maintenance fluid rate selected in many cases is somewhat empirical, continued monitoring of physical examination, laboratory and cardiovascular measurements of fluid balance, with appropriate adjustment of the fluid rate to maintain hydration and euvolaemia, is most important.

Acid-base balance

Disturbances of acid-base balance may be due to an increased production, or a decreased excretion, of acids or bases. A number of mechanisms can cause these disturbances. The accumulations of organic or inorganic acids and bases in the body produce states of metabolic acidosis or metabolic alkalosis, respectively. These acid-base imbalances often accompany conditions for which fluid therapy is indicated. Corrective fluid therapy will usually redress the acidbase balance by diluting out acid or base excesses and improving tissue perfusion and renal function. In consequence, specific correction of acid-base imbalance is often unnecessary and on occasion can even be harmful. However, any alteration in acid-base disturbance should be monitored and the underlying cause identified and treated.

Metabolic acidosis is the most common acidbase disorder in horses. It occurs most frequently in association with obstructive gastrointestinal disease and diarrhoea. Much more rarely, it is associated with renal failure. The underlying causes of acidosis in these situations are either increased base loss and/or reduced peripheral perfusion causing a switch from aerobic to predominantly anaerobic metabolism in tissues, with a consequent build up of lactate. The physiological response is an increase in the respiratory rate (to blow off CO₂), which may be seen among the clinical signs. Mild metabolic acidosis usually causes few adverse effects.

Metabolic alkalosis is uncommon in horses but is usually associated with a depletion of serum or plasma chloride concentration. Alkalosis may occur transiently with hypochloraemia in the early stages of diarrhoea or small intestinal obstruction.

Changes in blood pH can also follow changes in respiratory ventilation. Hypoventilation produces respiratory acidosis due to an increasing partial pressure of CO_2 (P_aCO_2) in the blood. Respiratory acidosis occurs during general anaesthesia in the horse in association with a central depression of respiration and an increase in the partial pressure of CO_2 in the blood. With due care, it is of little consequence. In contrast, hyperventilation produces respiratory alkalosis due to a decreasing P_aCO_2 . Respiratory alkalosis may follow hyperventilation associated with exercise, pain or hypoxaemia.

Assessment of acid-base balance

Although arterial blood gas and pH measurements provide the only accurate guide to acid-base status, plasma bicarbonate estimations are acceptable for most clinical situations. Plasma bicarbonate concentrations are most commonly estimated from the total CO₂ in plasma, of which 95% is considered to be bicarbonate. However, delays in determining total CO₂ concentration can cause spuriously low values, and to avoid this, venous blood samples must be collected anaerobically into heparinized syringes and processed as soon as possible. Unfortunately, the close proximity of sophisticated equipment is required for these analyses and this is not usually the case in practice. In practical terms, however, the need to correct a metabolic acidosis by specific bicarbonate therapy is rare - unless the plasma concentration falls below 15 mmol/l, or in cases with marked ongoing losses such as horses and foals with prolonged diarrhoea.

The decision to administer sodium bicarbonate solution requires caution since it may cause persistent metabolic alkalosis with respiratory depression, hypernatraemia (by supplementing excess sodium), hypokalaemia and a net hyperosmolality. However, in the exceptional cases where plasma bicarbonate falls below 15 mmol/l, supplementary bicarbonate should be given because many of the body's metabolic processes cannot function below a pH of 7. At specialist centres, estimations of the quantity of bicarbonate required to restore a deficit are usually related to replacement of the deficit in extracellular fluid. Since the lower range of normal plasma bicarbonate concentration is usually some 25 mmol/l, a *base deficit* (i.e. bicarbonate deficit) is calculated by subtracting the patient's plasma bicarbonate concentration (mmol/l) from 25 mmol/l and substituting in the following equation to derive the bicarbonate requirement:

```
\label{eq:hco3} \begin{array}{l} \text{HCO}_3^- \text{ requirement } (\text{hmol/l}) = \\ 0.3 \times \text{Body weight } (\text{kg}) \times \text{Base deficit } (\text{hmol/l}). \end{array}
```

This equation allows calculation of the bicarbonate deficit in extracellular fluid, which is presumed to be some 30% (0.3) of body weight. To continue the earlier example of the 500 kg horse with severe diarrhoea (see above), if its plasma bicarbonate concentration was found to be very low, say 12 mmol/l, then its base deficit would be 25 - 12 = 13 mmol/l. By substituting in the above equation, its bicarbonate requirement would be $0.3 \times 500 \times 13 = 1950 \text{ mmol}$.

Since 1 g of NaHCO₃ yields 12 mmol HCO₃, the horse's requirement is 1950/12 = 163 g, which can be given intravenously as a 5% solution over 30–45 minutes. It is standard practice to replace half the calculated deficit so that overcorrection is avoided. Further blood samples should be taken during treatment to monitor bicarbonate concentrations.

Common conditions affecting fluid and electrolyte balance

Examples of the common conditions of the adult horse that require investigation and treatment for fluid and electrolyte imbalance are given below.

Reduced water intake

Any disease process associated with reduced water and food intake will inevitably produce signs of progressive dehydration. In these circumstances the loss of Na⁺ from the ECF is low, but K⁺ deficits soon develop if the food intake is reduced. *Clinical assessment:* clinical signs of dehydration will not develop until after 2–3 days of deprivation.

Clinical pathology: in the early stages clinical pathology will be unremarkable, but after 2–3 days there will be modest increases in PCV, TPP and serum or plasma Na⁺, K^+ and Cl⁻.

Requirement: within 1–2 days of the onset of deprivation, oral fluids are sufficient to meet the daily requirement. After 3 days the patient will have developed more severe dehydration, which must be treated initially with an intravenous polyionic replacement fluid, followed by oral maintenance fluid (50–100 ml/kg/d).

Colic

Fluid, electrolyte and acid–base disturbances are associated with those acute colic cases in which fluid is sequestered in the gut lumen and/or there is associated strangulation. Examples include horses with small-intestinal strangulating lesions and largecolon volvulus.

Clinical assessment: the clinical signs of colic will be coupled with signs of hypovolaemia and shock.

Clinical pathology: the PCV and TPP are high, indicating haemoconcentration, hypovolaemia and dehydration, but serum or plasma Na⁺, K⁺ and Cl⁻ may be within normal limits. Hypochloraemia is common in horses with a small-intestinal obstruction. Blood lactate and serum or plasma creatinine concentrations are often high in these patients, indicating poor tissue perfusion. If plasma bicarbonate estimations are possible, these may be low in cases of severe circulatory disturbance (metabolic acidosis).

Requirements: Fluid requirements are minimal for horses with simple colic. Following correction of the obstruction, oral fluids and electrolytes or a shortduration of maintenance intravenous polyionic fluids are necessary. In critically ill colic patients, intravenous polyionic replacement fluids (20– 60 ml/kg) are necessary for resuscitation. In severe cases, hypertonic saline (4 ml/kg or 2 l/500 kg) may be used as a resuscitation fluid to rapidly expand the plasma volume; however, the effects of hypertonic saline are short-acting because it is a crystalloid fluid and is distributed across the entire ECF space. Hypertonic saline should always be followed with 10 litres of isotonic polyionic fluids for every 1 litre of hypertonic saline administered. Critically ill colic patients often need treatment with plasma (2–10 litres or more for a 500 kg horse) and/ or a synthetic colloid such as hetastarch. Maintenance fluid rates can be high in these patients and should be based on physical, laboratory and cardiovascular monitoring. Isotonic sodium bicarbonate is rarely necessary and is usually contraindicated in horses with colic unless it is complicated by colitis and diarrhoea.

Diarrhoea

The extent of fluid and electrolyte losses and the development of acidosis depend upon the severity and duration of the enteric lesion and whether or not the patient continues to drink during the illness.

Clinical signs: clinical signs of dehydration will be minimal in mild cases, but signs of toxaemia and dehydration will accompany severe diarrhoea.

Clinical pathology: mild cases in which the patient is not systemically ill and continues to drink will hardly affect clinicopathological parameters. In severe cases the PCV will be high as a result of splenic contraction, haemoconcentration and dehydration, and the serum or plasma electrolytes will be low because of enteric loss. There is usually an associated loss of albumin to the gut lumen, so that the TPP may not markedly increase with dehydration and these patients are usually hypoproteinaemic following resuscitation. If plasma bicarbonate estimations are possible, these will be low in severe or prolonged cases.

Requirements: in mild cases, oral fluids may be sufficient. At most, intravenous polyionic fluids would be required for replacement, followed by oral fluids for maintenance. Severe cases require intravenous polyionic infusions for replacement and maintenance and in most cases plasma and a synthetic colloid (e.g. hetastarch) to maintain colloid oncotic

pressure and the intravascular volume. In severe cases, hypertonic saline (4 ml/kg or 2 l/500 kg) may be used as a resuscitation fluid to rapidly expand the plasma volume; however, caution should be exercised with the use of hypertonic saline in patients with chronic hyponatraemia (see previously). If plasma bicarbonate estimations are possible, isotonic NaHCO₃ may be indicated if the acidaemia does not improve with replacement fluid therapy.

Exertional dehydration

Endurance exertion over relatively short distances is unlikely to be associated with clinical signs of dehydration. Exertion over long distances in which there is also profuse sweating may produce clinicopathological evidence of dehydration with falls in Na⁺ and especially K^+ and Cl^- .

Requirements: mild cases simply require oral fluids. More extreme cases require intravenous polyionic replacement fluid.

Comment

 On occasion, hypocalcaemia is also associated with long-distance exertion and results in an increase in neuromuscular irritability (see 'Disorders of calcium metabolism' in Ch. 5: 'Endocrine diseases').

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CHAPTER 12

Respiratory diseases

Chapter contents

I. Practical techniques: upper respiratory tract 218 Endoscopy 218 Technique 218 Nasal chambers 218 Nasopharynx 219 Guttural pouches 219 Palatal arch 220 Epiglottis 221 Larvnx 221 Trachea and bronchi 221 Treadmill endoscopy 222 Examination of the paranasal sinuses 222 Percussion 222 Endoscopy 223 Sinus centesis 223 Radiography 225 Computed tomography/magnetic resonance imaging 226 Direct sinus endoscopy (sinoscopy) 226 Trephination 227 Nuclear scintigraphy 228 Examination of the guttural pouches 228 Endoscopy 228 Catheterization 229 Radiography 229 Examination of the larynx 229 Palpation 229 Arytenoid depression test 229 Slap test 230

Endoscopy 230 Radiography 231 Ultrasonography 231

II. Practical techniques: lower respiratory tract 231

Auscultation 231 Abnormalities of normal airflow sounds 232 Adventitious sounds 233 Percussion of the chest 233 Collection of samples from the lower airways 233 Aspiration of tracheal fluid 233 Transtracheal aspiration 233 Complications of transtracheal aspiration 235 Endoscopic tracheal aspiration 235 Bronchoalveolar lavage 236 Endoscopic technique 236 Blind technique 236 Thoracic radiography 237 Lung biopsy 237 Percutaneous technique 237 Transbronchial technique 238 Ultrasonography of the chest 238 Pleuroscopy (thoracoscopy) 239 Thoracocentesis 240 Arterial blood collection 241 Carotid artery 241 Transverse facial artery 241 Facial artery 242

Nasal and nasopharyngeal swabbing 242 Sampling from the guttural pouch 242

III. Clinical pathology 243

Cytology of tracheal aspirates and bronchoalveolar lavage fluid 243 Preparation 243 Interpretation 243 Pleural fluid analysis 243 Normal fluid 243 Pleural effusions 244 Biochemical evaluation of pleural fluid 244 Arterial blood gas analysis 244 Viral serology 244 Sinus aspirates 244 Haematology 244 Microbial culture 245

Chapter appendices 245

Further reading 245

APPENDIX 12.1 246

Some applications of diagnostic techniques for the investigation of nasal discharge

APPENDIX 12.2 248

Some applications of diagnostic techniques for the investigation of coughing

I. PRACTICAL TECHNIQUES: UPPER RESPIRATORY TRACT

Diseases of the upper and lower respiratory tracts are common in horses. This chapter describes practical techniques for the diagnosis of these conditions using equipment that is available to the practising veterinary surgeon.

Endoscopy

Endoscopy allows direct visualization of many parts of the respiratory tract, and the use of flexible fibreoptic or videoendoscopic equipment has become an essential component in the evaluation of respiratory tract diseases. The parts of the tract that are accessible to examination are determined by the equipment available. Although a narrow 1 or 1.2 m endoscope will permit examination of most parts of the upper respiratory tract in adult horses, it is unlikely to have sufficient length to allow examination of the bronchial tree – an instrument of 2 m or longer is needed for bronchoscopy. Fine paediatric endoscopes may be required for foals. Adequate disinfection of the instrument between horses is essential to prevent cross-infection by pathogens.

Technique

The horse is restrained as necessary. A nose twitch is usually sufficient, and this helps to stabilize the head. Chemical sedation is sometimes required, but this should be avoided where functional assessment of the larynx or palatal arch is needed. It is helpful to have three people to perform the examination: one holds the horse and twitch, one stabilizes the endoscope at the nostril, and one has charge of the controls. For routine examinations, the endoscope is introduced through one nostril and passed along the ventral nasal meatus.

The regions of the tract that can be examined can be conveniently divided into seven areas:

- Nasal chambers
- Nasopharynx
- Auditory tube diverticula (guttural pouches)
- Palatal arch
- Epiglottis
- Larynx
- Trachea and bronchi.

Nasal chambers

The ventral nasal meatus and ventral concha (Fig. 12.1) are examined as the endoscope is advanced to

Respiratory diseases



Figure 12.1 Endoscopic view of the ventral nasal meatus and ventral concha.

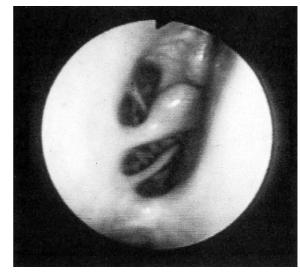


Figure 12.2 Endoscopic view of the ethmoidal labyrinth and great ethmoturbinate.

the nasopharynx. However, detailed examination of these areas is more easily performed as the instrument is slowly withdrawn towards the nose. As the endoscope is retracted within the nasopharynx to the choana, dorsal deviation of the tip will allow examination of the ethmoidal labyrinth and great ethmoturbinate (Fig. 12.2). The nasomaxillary opening of the paranasal sinuses opens into the lateral wall of the middle meatus in this region. Although this opening cannot be examined directly, discharges originating within the sinuses may be seen streaming into the nasal cavity here. Progressive ethmoidal haematomas commonly occur in the region of the ethmoidal labyrinth and appear as a grey/ green mass protruding rostrally into the middle nasal meatus.

The middle nasal meatus can also be examined from the nares but it is narrower than the ventral meatus and is easily traumatized if the endoscope is forced along it. The conchal surfaces should be examined for fungal plaques, ulcers, masses, etc. Paranasal sinus disease may result in distension/ swelling of the conchae and narrowing of the meati. Nasal septal disease (deviation, cysts, thickening, etc.) may be observed.

Nasopharynx

The walls of the nasopharynx and pharyngeal recess commonly show *hyperplastic lymphoid nodules* in young horses up to 5 years of age (Fig. 12.3). This is considered to be a normal finding but it can result in adventitious respiratory noises if excessive.

Pharyngeal paralysis is an important cause of dysphagia and nasal return of food. Endoscopically, this appears as sagging of the pharyngeal walls, persistent dorsal displacement of the soft palate and dissemination of food debris/saliva within the nasopharynx.

Guttural pouches

Examination of the guttural pouches is possible by introducing the endoscope from the ventral nasal meatus into the nasopharynx and passing it in a dorsal direction under the cartilaginous flap of the auditory tube (Fig. 12.4). A flexible wire leader, passed down the biopsy channel of the instrument, is passed under the flap first and used to elevate the flap by rotation of the endoscope, thus allowing entry of the endoscope itself into the auditory tube. A rotating movement as the endoscope is advanced

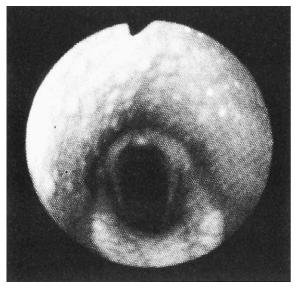


Figure 12.3 Endoscopic view of the nasopharyngeal wall of a young adult horse showing hyperplasia of the lymphoid follicles.

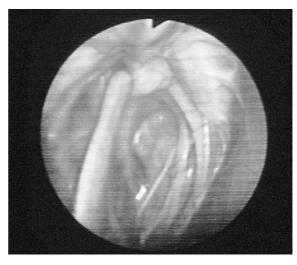


Figure 12.5 Endoscopic view of the medial compartment of the guttural pouch.

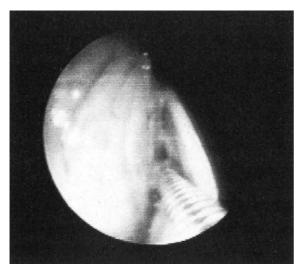


Figure 12.4 Endoscopy of the guttural pouch. A lead wire is passed underneath the cartilaginous flap of the auditory tube to aid subsequent passage of the endoscope into the tube.

facilitates this entry. The endoscope is then advanced into the pouch. Each pouch is divided into medial and lateral compartments by the stylohyoid bone. The medial pouch (Fig. 12.5) is larger than the lateral pouch, and coursing along its caudolateral wall are several important structures including the internal carotid artery, the cranial cervical ganglion and the vagus, glossopharyngeal, hypoglossal, spinal accessory and sympathetic nerves.

Palatal arch

The soft palate is a continuous sheet that forms the floor of the nasopharynx. Its free (caudal) border normally lies beneath the epiglottis (Fig. 12.6) and is therefore obscured from view unless it becomes displaced dorsally during swallowing (Fig. 12.7). Congenital lesions of the soft palate are a cause of nasal reflux of milk in foals. They are readily diagnosed endoscopically – the defect allows direct visualization of the oral cavity from the nasopharynx.

Laryngopalatal dislocation (dorsal displacement of the soft palate) involves movement of the free caudal edge of the palate dorsal to the epiglottis. In performance horses this condition results in exercise intolerance associated with an adventitious respiratory noise (gurgling). The condition is usually intermittent and cannot be diagnosed at rest. At a specialist centre, endoscopy during exercise on a high-speed treadmill may allow an accurate diagnosis. Horses twitched for endoscopy often displace the soft palate initially, but it usually returns to its

Respiratory diseases

Pharyngeal cysts are most commonly located in the subepiglottic tissue and are usually visible by endoscopy, but occasionally cysts may be obscured by the caudal margin of the soft palate.

Epiglottis

The epiglottis is a leaf-like structure that projects rostrodorsally from the base of the larynx. The edge is serrated, and the dorsal surface possesses fine arcuate blood vessels (Fig. 12.6). Epiglottic entrapment involves the dorsal reflection of subepiglottic tissue and arytenoepiglottic folds over the epiglottis, thus obscuring its apex, lateral margins and dorsal surface. Endoscopically, the outline of the epiglottis is visible, but the serrated edges and dorsal vessels are obscured by the entrapping tissue. Hypoplasia of the epiglottis resulting in reduced length, width and thickness of the epiglottis can be assessed subjectively by endoscopy but requires radiography to confirm the diagnosis. The condition predisposes epiglottic entrapment and laryngopalatal to subluxation

Larynx

The larynx is viewed from the nasopharynx but the eccentric position of the endoscope invariably produces a degree of perceived asymmetry of the rima glottidis. If uncertainty about the significance of slight asymmetry exists, the procedure should be performed via each nostril in turn.

In most normal horses, an endoscope can be introduced into the larynx and trachea without inducing a severe cough response. However, in horses affected by lower airway disease, the cough reflex may be very sensitive and paroxysmal coughing occurs. In these cases, the topical application of diluted lidocaine solution (50:50 sprayed over the larynx via a catheter passed down the biopsy channel) will abolish this response.

Trachea and bronchi

The trachea (Fig. 12.8) can be examined in its entirety if a sufficiently long endoscope is used (>1.8 m). Strictures of the tracheal lumen

Figure 12.6 Endoscopic view of the soft palate. The caudal border lies beneath and is obscured by the epiglottis. The epiglottis has a serrated edge and fine vessels are visible on its dorsal surface.

Figure 12.7 Endoscopic view of the soft palate in its dorsally displaced position. The caudal border is visible, since it has become displaced above the epiglottis.

normal position after swallowing. Frequent displacement of the palate or many unsuccessful swallowing attempts to correct it at rest are suspicious of laryngopalatal dislocation, but not diagnostic. Similarly, billowing and displacement of the soft palate during nostril occlusion, with difficulty of replacement, are also suspicious.



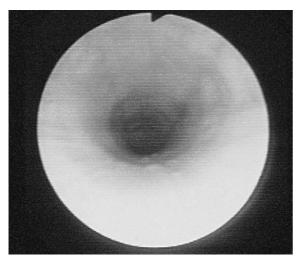


Figure 12.8 Endoscopic view of the trachea.

(congenital, iatrogenic or traumatic) can be observed, although radiography may yield more useful information. Discharges often accumulate at the thoracic inlet, and their nature (mucus, purulent material, blood) can be assessed and samples obtained by aspiration (see below). A haemorrhagic discharge observed after exercise is suggestive of *exercise-induced pulmonary haemorrhage*. Foreign bodies such as brambles may lodge in the distal trachea/main bronchi and are an uncommon cause of chronic coughing – they can usually be identified and retrieved by endoscopy.

The bronchial tree can be examined as far as the length and diameter of the endoscope permits. The carina (Fig. 12.9) normally presents as a sharp angle at the junction of the right and left principal bronchi. Thickening of the angle (due to mucosal oedema and inflammation) and hyperaemia may be seen in chronic lower airway diseases. A unilateral purulent discharge draining from only one principal bronchus indicates a focal lung lesion on that side (e.g. focal pneumonia, pulmonary abscess or foreign body). Passage of the endoscope down the bronchial tree may induce significant coughing, which renders the examination difficult. This reaction can be reduced by repeated infusions of small volumes of dilute lidocaine solution as the endoscope is advanced. The bronchial walls can be assessed for

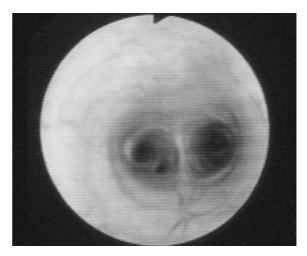


Figure 12.9 Endoscopic view of the carina and tracheal bifurcation.

thickening, inflammation and collapse. Intraluminal masses are rarely diagnosed in the horse.

Treadmill endoscopy

Endoscopic examination of the upper respiratory tract during high-speed treadmill exercise enables the clinician to identify dynamic causes of upper respiratory obstruction that are not present at rest. For racehorses, the treadmill must be capable of speeds up to 14 m/s and an incline of up to 10° uphill. Horses should be fit, and they should be acclimatized to the treadmill before the examination is performed. Videoendoscopy with either video or digital recording is essential. Conditions that may be diagnosed in this way include intermittent dorsal displacement of the soft palate, vocal fold or arytenoid cartilage collapse, nasopharyngeal collapse, intermittent epiglottal entrapment, axial deviation of the aryepiglottic folds and epiglottic retroversion

Examination of the paranasal sinuses

Percussion

The horse has five paired paranasal sinuses: frontal, sphenopalatine, ethmoidal, superior maxillary and inferior maxillary. Of these, the frontal and maxillary

Respiratory diseases



Figure 12.10 Percussion of the paranasal sinuses. The fingers of one hand are tapped sharply on the bone overlying the sinus.

sinuses are the most important sites of disease. Percussion can be helpful in detecting fluid or spaceoccupying lesions, or in demonstrating pain in the region of the sinus. The fingers of one hand are tapped sharply over the bone overlying the sinus (Fig. 12.10). Changes in resonance will be more easily appreciated if the mouth is opened at the same time (e.g. by placing a finger in the interdental space). Comparisons of resonance or pain reaction are made on both sides of the face. The topographical anatomy and areas of percussion of the frontal and maxillary sinuses are shown in Figures 12.11 and 12.12.

Endoscopy

The paranasal sinuses are interconnected and open into the middle meatus via the nasomaxillary ostium. This opening cannot be visualized directly but drainage of discharges from the opening into the nasal cavity may be observed by examining the caudal aspect of the middle nasal meatus (see endoscopy section above under 'Nasal chambers'). Deformation of the nasal meati may occur in chronic sinus disease as a result of expansion of the sinus cavities.

Sinus centesis

This allows the collection of fluid samples for cytology and culture. The precise site for centesis may be determined by clinical and radiographic features,

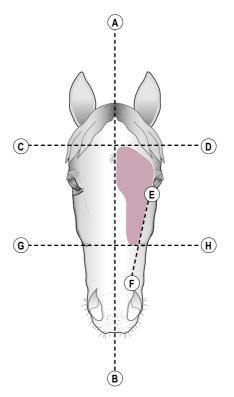


Figure 12.11 Topographical anatomy of the left frontal sinus. The left and right sinuses are separated by a bony septum in the midline of the head (A–B). The caudal margin of the frontal sinus is rostral to the temporomandibular joint at a line drawn through the middle of the zygomatic arches (C–D). The lateral margin is a line drawn from the medial canthus of the eye to the nasomaxillary notch (E–F). The rostral extent of the sinus lies on a line drawn between the midpoints of the medial canthus to the nasomaxillary notch on each side (G–H).

but in cases of generalized sinus disease the sites shown in Figure 12.13 are satisfactory. The procedure is performed in the standing, sedated horse as follows:

- The site is clipped and prepared for aseptic surgery and the skin is infiltrated with local anaesthetic. A 0.5–1.0 cm incision is made through the skin and subcutaneous tissues.
- A 2 mm Steinmann pin attached to a Jacob's chuck is used to drill a hole through the bone (Fig. 12.14). It may be necessary to displace the levator labii superioris muscle dorsally from the

Diagnostic techniques in equine medicine

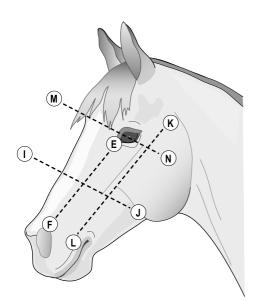


Figure 12.12 Topographical anatomy of the maxillary sinuses. The dorsal margin is a line drawn from the medial canthus of the eye to the nasomaxillary notch (E–F). The rostral limit is at a line drawn at right angles to the dorsal margin to meet the rostral end of the facial crest (I–J). The ventral margin runs parallel to the dorsal margin along the facial crest (K–L). The caudal limit is a line drawn from the middle of the orbit to the facial crest (M–N).

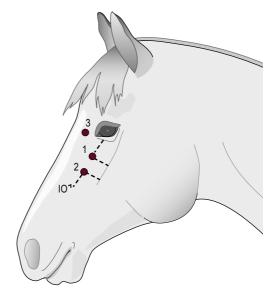


Figure 12.13 Sites for routine sinus trephination. (1) Caudal maxillary sinus: 2.5–3 cm rostral to the medial canthus. (2) Rostral maxillary sinus: 2.5–3 cm dorsal to the facial crest and 2.5–3 cm caudal to the infraorbital foramen (IO). (3) Frontal sinus: midway between the medial canthus of the eye and the dorsal midline of the face.

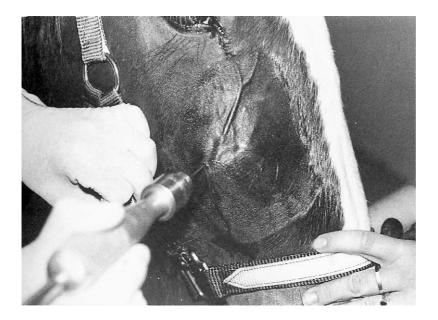


Figure 12.14 Sinus centesis (rostral maxillary sinus). A Steinmann pin is used to drill a hole through the bone into the sinus cavity.

Respiratory diseases

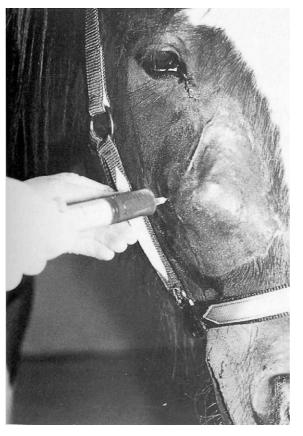


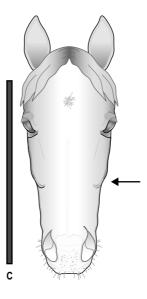
Figure 12.15 Sinus centesis. Fluid is aspirated from the sinus via a needle inserted through the pinhole.

rostral maxillary site. In many horses with chronic paranasal sinus disease the overlying bone is sufficiently thinned to allow access into the sinus with a 14 or 16G needle and there is no need for a prior drill hole.

- Fluid can be collected via a needle or polythene tubing (Fig. 12.15). Lavage with sterile saline may be helpful if the fluid content of the sinus is inaccessible or highly viscous.
- The centesis site is left to heal by second intention.

Radiography

Radiography is invaluable in the assessment of paranasal sinus disease. Lateral radiographs in the standing horse (usually sedated), with the affected side

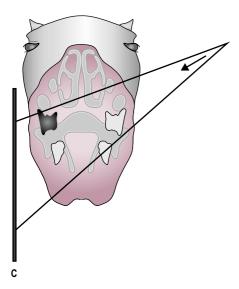


C = cassette

Figure 12.16 Lateral radiography of the paranasal sinuses and nasal cavity. The cassette (C) is placed alongside the head and the horizontal X-ray beam is centred at the rostral limit of the facial crest.

nearest the film, are most helpful. A drip stand can be employed as a cassette holder by placing the cassette in a bag suspended from the stand. The horizontal X-ray beam is centred at the rostral end of the facial crest or the specific area of interest (Fig. 12.16). A rope or cloth halter is used to hold the horse. The use of large cassettes $(35 \times 43 \text{ cm})$, especially cassettes with rare-earth intensifying screens, is helpful. The use of a grid is unnecessary since high-quality radiographs are not required and they involve an increased risk of radiation exposure. Thirty degree oblique views are useful to profile the affected side away from the non-affected side and to assess the cheek teeth roots (Fig. 12.17). Dorsoventral views can be obtained by positioning the cassette underneath and parallel to the hemimandibles with the X-ray beam directed perpendicular to the plate and centred on the midline at the level of the rostral end of the facial crest. Fluid lines and soft tissue masses can be identified by these techniques. Oblique views taken under general anaesthesia may be necessary if head movement is a problem.

Diagnostic techniques in equine medicine



C = cassette

Figure 12.17 Thirty degree oblique radiography to demonstrate the roots of the maxillary cheek teeth. Cross-section through the head at the level of the rostral end of the facial crest. The cassette (C) is placed on the affected side, and the X-ray beam is angled down from the contralateral side. The maxillary cheek teeth and roots of the affected side (shaded) will be highlighted on the film away from the other teeth.

Computed tomography/magnetic resonance imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are techniques that are becoming increasingly applied at specialist centres to the evaluation of some causes of paranasal sinus disease in the horse. Both techniques are usually performed under general anaesthesia. They are most useful for evaluating the presence and position of masses within the paranasal sinuses (e.g. progressive ethmoidal haematoma, sinus cysts and neoplasia) and can be helpful for surgical planning.

Direct sinus endoscopy (sinoscopy)

The sinuses may be examined in the standing, sedated horse using a flexible fibreoptic endoscope or a rigid arthroscope, introduced through a tre-

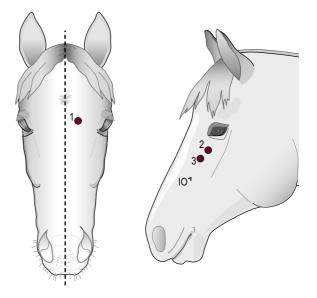


Figure 12.18 Recommended portal locations for endoscopic examination of the paranasal sinuses. (1) Frontal sinus: 1.5 cm lateral to the midline between a line joining the caudal margins of the eyes and a line joining the rostral canthi. (2) Caudal maxillary sinus: 1.5 cm ventral to the most ventral point of the ventral orbital rim. (3) Rostral maxillary sinus: 2 cm dorsal to the rostral aspect of the facial crest. Rostral maxillary sinoscopy is only possible in older (>10 years) horses.

phine hole. The landmarks for the trephine holes are as follows:

- *Frontal sinus*: 1.5 cm lateral to the midline between a line joining the caudal margins of the eyes and a line joining the rostral canthi
- *Caudal maxillary sinus*: 1.5 cm ventral to the most ventral point of the ventral orbital rim
- *Rostral maxillary sinus*: 2 cm dorsal to the rostral aspect of the facial crest. Rostral maxillary sinoscopy is only possible in older (>10 years) horses.

The sites for endoscopic examination are shown in Figure 12.18. Procedure as follows:

- The site is prepared aseptically and the skin is infiltrated with local anaesthetic. A 1.5 cm incision is made through the skin, subcutaneous tissue and periosteum
- When an arthroscope or narrow flexible endoscope is being used, a portal can be drilled

Respiratory diseases



Figure 12.19 Direct sinus endoscopy. An endoscope has been introduced into the frontal sinus via a small trephine hole.

through the bone using a 5 mm Steinmann pin in a chuck or a modified drill bit. If a largerdiameter endoscope is being used, the hole may need to be made with a surgical trephine (see below)

• Once the sinus cavity is entered, the arthroscope/endoscope is introduced and the cavity examined (Fig. 12.19).

For general examination of the frontal and caudal maxillary sinuses, the frontal sinus portal is preferred. Examination of the dorsal conchal sinus is also performed via the frontal sinus. The sphenopalatine sinus is examined via the caudal maxillary sinus portal.

Trephination

The creation of trephine holes into the sinus cavities allows biopsy and aspiration in the standing horse. The sites for trephination are shown in Figure 12.20. Procedure as follows:

- The site is prepared aseptically and the skin is infiltrated with local anaesthetic
- A circle of skin is marked with the trephine (Fig. 12.21) and excised with a scalpel, along with the subcutaneous tissue and fascia (Fig. 12.22)
- The trochar point is used to make a small hole in the bone, which stabilizes the trephine as it is rotated to cut through the bone. The plug of

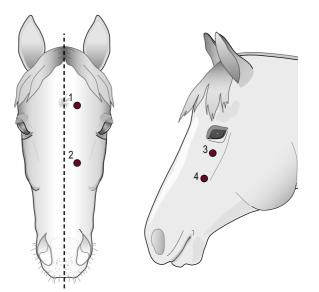


Figure 12.20 Routine sites for sinus trephination. (1) Frontal sinus (frontal portion): 4 cm from the midline on a line joining the supraorbital processes. (2) Frontal sinus (turbinate portion): 5 cm from the midline at a level 3–4 cm caudal to the rostral end of the facial crest. (3) Caudal maxillary sinus: 2.5–3 cm dorsal to the facial crest, and 2.5–3 cm rostral to the medial canthus; as far into the angle formed between the rim of the bony orbit and the facial crest as possible. (4) Rostral maxillary sinus: 2.5 cm caudal and 3.5 cm dorsal to a point at the rostral limit of the facial crest. NB: In young horses this opening will be adjacent to the cheek teeth and there will be minimal access to the sinus.

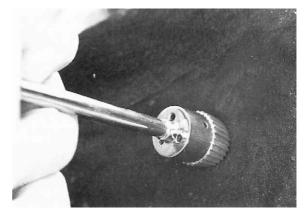


Figure 12.21 Trephination (caudal maxillary sinus). The trephine is pressed against the skin to mark the site of the skin incision.

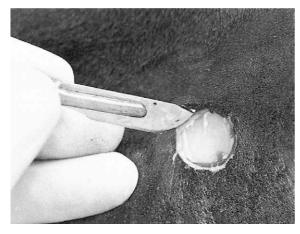


Figure 12.22 Trephination. The skin, subcutaneous tissue and fascia are excised to reveal the surface of the bone.

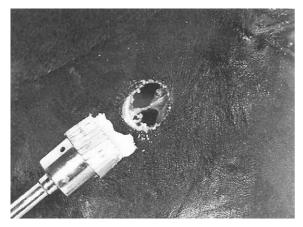


Figure 12.23 Trephination. The trephine is used to create a window in the bone, allowing entry into the sinus cavity.

bone is removed, thereby allowing direct access to the sinus cavity (Fig. 12.23).

Comments

- The trephine hole will heal rapidly by granulation and second intention healing, and requires minimal aftercare other than daily cleaning with dilute antiseptic solution.
- If the trephine hole is to be used for daily medication or flushing, the opening should be plugged with a rolled plug of sterile gauze to delay healing (Fig. 12.24).



Figure 12.24 Trephine hole into the rostral maxillary sinus plugged with sterile cotton gauze to prevent too rapid healing. A Foley catheter is secured in the frontal sinus via another trephine hole to permit medication/flushing.

Nuclear scintigraphy

Nuclear scintigraphy can sometimes be helpful in the evaluation of horses with paranasal sinus disease. In particular it can be helpful for the identification of periapical tooth root infections. Most examinations are performed using the bone marker ^{99m}Tc-MDP at 2–4 hours post-injection. The use of ^{99m}Tc-labelled leukocytes has also been described.

Examination of the guttural pouches

Endoscopy

Evidence of possible guttural pouch disease may be apparent by routine endoscopic examination of the pharynx (see above under 'Endoscopy'). The following observations may be suggestive of guttural pouch disease:

- Blood or pus draining from the opening of one or both auditory tubes into the pharynx
- Pharyngeal paralysis

- Laryngeal hemiplegia
- Collapse of the pharyngeal roof.

NB: It is possible for blood or discharges to be aspirated from the nasopharynx into the auditory tube, thereby giving the false impression during endoscopic examination of a discharge draining from the auditory tube.

Guttural pouch mycosis may be confirmed by the identification of a mycotic plaque within the pouch. In most cases, the mycosis affects the dorsomedial wall of the pouch over the internal carotid artery. Extreme care must be exercised when examining horses with potential guttural pouch mycosis, especially those with a history or evidence of haemorrhage. The presence of free blood/haematoma within the pouch hinders the examination, and care must be taken to ensure that the endoscope does not dislodge a blood clot over the artery that could initiate further haemorrhage. The degree of neurological dysfunction (pharyngeal paralysis; laryngeal hemiplegia) should be assessed prior to undertaking treatment of mycotic disease.

Endoscopy can provide limited information about conditions such as tympany, empyema and chondroids. Although these diseases may be confirmed by endoscopy, other techniques, especially radiography, will supply more useful information. *Diverticulitis* is recognized as a generalized inflammation of the walls of the pouches and may be associated with neuropathies such as pharyngeal and laryngeal paralysis.

Catheterization

A catheter can be passed into the guttural pouch either blindly or under endoscopic guidance to allow the collection of samples for cytology and culture.

If a catheter is to be inserted blindly, the distance from the nostril to the opening of the auditory tube (which corresponds to the level of the lateral canthus of the eye) should be marked on the catheter. The catheter should be stiffened by threading it over a wire guide that has a 30-degree curve at the distal 2 cm level. The catheter is passed along the ventral nasal meatus until the mark reaches the nose. The curved end is turned laterally and the catheter advanced under the flap-like opening of the auditory tube. This is facilitated if the catheter is advanced as the horse swallows. Entry into the auditory tube is recognized by a lack of resistance as the catheter is advanced into the pouch.

Radiography

Air within the guttural pouches provides a good, natural radiographic contrast agent, which makes radiography a useful clinical technique in the assessment of guttural pouch disease. Standing lateral views are taken in the same manner as for the paranasal sinuses (see above). The medial and lateral compartments of the pouches can be identified. Fluid lines may be seen in cases of empyema or haemorrhage. Chondroids can also be identified. Compression by retropharyngeal masses causes distortion of the outlines of the pouches. Abnormally large air-filled pouches are seen in cases of guttural pouch tympany. To separate the left and right sides, the affected side can be radiographed next to the cassette, angled at 10-15° in a rostrocaudal direction.

Examination of the larynx

Palpation

The index fingers of each hand are pushed under the tendon of the sternocephalicus muscle on either side of the neck, and the cranial dorsal larynx in the region of the muscular process is palpated. In advanced cases of idiopathic laryngeal hemiplegia, atrophy of the dorsal cricoarytenoid muscle on the left side makes the muscular process more prominent.

Arytenoid depression test

The first and second fingers of both hands are located over the muscular processes of the arytenoids, which are then depressed. In horses with laryngeal hemiplegia, this results in a stridorous respiratory noise. The test is best performed shortly after exercise. Vibration of the left arytenoid cartilage

may also be detected at this time in horses with left laryngeal hemiplegia.

Slap test

This test is used to assess the adductor function of the arytenoids. The response can be evaluated either by palpating the muscular process or by visualizing the larynx by endoscopy. An assistant gently slaps the horse on one side of the thorax; this results in a reflex movement or flicking of the contralateral muscular process, resulting in adduction of the corniculate process and vocal cord. In a horse with left laryngeal hemiplegia, the flicking of the left muscular process in response to slapping the right thorax is diminished or absent. In normal horses, the response of both arytenoids is symmetrical. The test should be performed while the horse is breathing quietly and during expiration.

Endoscopy

Endoscopy is used to view the larynx and to evaluate the range of movement of the arytenoid cartilages. The procedure may need to be performed at rest and after exercise. Examination during exercise on a treadmill may be particularly helpful in subtle cases of laryngeal hemiplegia.

The larynx is viewed by passing the endoscope along the ventral meatus in the usual way. The visible parts of the larynx are assessed for gross abnormalities of structure (e.g. arytenoid chondritis) and evidence of previous surgical interference (e.g. absence of one or both laryngeal ventricles). The paralaryngeal structures (epiglottis, palatine arch and pharyngeal walls) are also evaluated.

The symmetry of the glottis and positions of the corniculate processes and vocal cords are examined at rest. In cases of laryngeal hemiplegia where the paralysis is complete, the affected arytenoid cartilage shows little or no movement and is displaced (along with the vocal cord) to the midline (Figs 12.25, 12.26). The opening to the laryngeal ventricle on the affected side is more obvious.

The depth of respiration and degree of arytenoid abduction during expiration can be increased by temporary occlusion of the nostrils or the injection



Figure 12.25 Endoscopic view of the normal resting larynx.

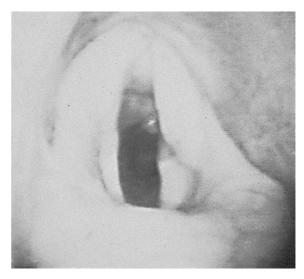


Figure 12.26 Endoscopic view of larynx with left laryngeal hemiplegia. The left arytenoid cartilage and vocal cord are displaced towards the midline.

of a respiratory stimulant (e.g. doxapram hydrochloride). Observation of arytenoid adduction following stimulation of swallowing (e.g. by flushing water through the endoscope) is also useful. Asynchronous abduction of the arytenoids is not in its own right regarded as abnormal, provided that both arytenoid cartilages can abduct maximally. However, a deficient, exaggerated or biphasic arytenoid abduction is abnormal.

Comment

• In horses with mild degrees of laryngeal hemiplegia, abnormalities of arytenoid abduction may be difficult to detect at rest. These horses should be reassessed immediately after strenuous exercise or, at specialist centres, during exercise on a high-speed treadmill. During or immediately after exercise a normal horse will have a symmetrically dilated larynx with both arytenoids at maximal abduction. In horses with laryngeal hemiplegia, the larynx will appear asymmetrical, with incomplete abduction on the affected side.

Radiography

Standing lateral radiographs of the pharynx and larynx can provide useful additional information about the condition of the larynx and surrounding structures. The absence of one or both laryngeal ventricles indicates previous surgery (laryngeal ventriculectomy). Radiographic examination of the larynx prior to laryngeal prosthesis surgery may be helpful in identifying osseous metaplasia of the cartilages that might complicate the surgery. Persistent dorsal displacement of the soft palate, subepiglottic cysts, epiglottic entrapment, chronic arytenoid chondritis and fourth brachial arch defect syndrome may all be diagnosed radiographically. In addition, epiglottic size and conformation can be assessed.

Ultrasonography

Transcutaneous ultrasonography allows identification of portions of the hyoid apparatus, laryngeal cartilages, associated soft tissues, and intrinsic and extrinsic musculature. A 7.5–10 MHz linear array or curvilinear probe may be used.

II. PRACTICAL TECHNIQUES: LOWER RESPIRATORY TRACT

Auscultation

Auscultation of the trachea and chest should be performed in a quiet environment. Air movement sounds in the trachea are clear and well defined with inspiratory sounds being similar to expiratory sounds. Referred sounds from both the upper and lower airways may be heard at the trachea. Gurgling/ bubbling sounds can be heard over the distal cervical trachea of horses that have large amounts of lower airway secretions. The secretions tend to pool in the trachea in this region.

Lung sounds audible over the chest will vary depending upon the bodily condition of the horse and the depth of breathing. Lung sounds may be difficult to appreciate in fat horses, whereas in thin horses many more sounds can be heard. Respiratory sounds can be accentuated by increasing the depth of breathing using a rebreathing bag (Fig. 12.27). A large reservoir bag, such as a plastic bin liner, is placed over the nostrils and the resulting accumulation of carbon dioxide in the air stimulates the depth of breathing. The bag can be left in place for



Figure 12.27 Use of a rebreathing bag. A reservoir bag is placed over the horse's nostrils and the horse is allowed to breathe several times into and out of the bag until the depth of respiration is sufficiently increased.

Diagnostic techniques in equine medicine

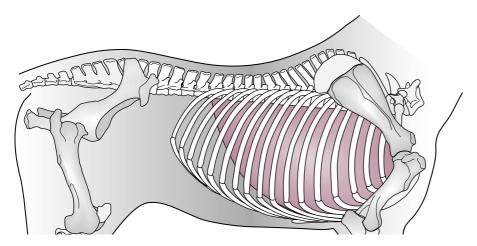


Figure 12.28 Topographical anatomy showing the relationship between the caudal border of the lung and the intercostal spaces.

a variable period depending on the response of the horse. Horses with recurrent airway obstruction (RAO), formerly termed 'chronic obstructive pulmonary disease' (COPD), or pneumonia may cough paroxysmally when the bag is used, which limits its usefulness. Care should be taken when using the technique in horses with painful pleural conditions.

The normal caudal lung border runs in a gently curving line from the 18th rib, with the following landmarks (Fig. 12.28).

- 17th intercostal space border level with the tuber coxae
- 13th intercostal space border level with the mid-thorax
- 11th intercostal space border level with the point of shoulder; then curving down to the elbow.

Airflow sounds in the chest are normally slightly louder on the right side than the left and inspiratory sounds are slightly louder than expiratory sounds. The sounds are most readily heard over the area of the carina where the large airways divide, but air sounds at the lung periphery may be difficult to detect in normal horses. Comparisons should always be made between the left and right sides.

Intestinal sounds are commonly heard on thoracic auscultation that may mimic abnormal

respiratory sounds, such as pleural rubbing sounds. True respiratory sounds will occur at the same phase of respiration with every breath, whereas intestinal sounds are random. It is therefore essential to watch the respiratory rhythm as the lungs are auscultated.

Abnormalities of normal airflow sounds

These include the following:

- A generalized increase in the intensity of sounds, e.g. mild RAO
- The expiratory sounds are louder than inspiratory sounds, e.g. consolidation or pleural effusion (owing to increased transmission of large airway sounds)
- A localized or unilateral absence or decrease in the intensity of sounds, e.g. pulmonary or pleural abscess, pleural effusion
- An abrupt change from soft to harsh sounds, e.g. effusion or consolidation
- A bilateral absence of sounds in the ventral chest, e.g. bilateral pleural effusion (often accompanied by the radiation of heart sounds over a larger than normal area)
- The complete absence of sounds in the dorsal chest (uni- or bilateral), e.g. pneumothorax.

Adventitious sounds

Adventitious airflow sounds are abnormal lung sounds, which include crackles ('rales') and wheezes ('rhonchi').

Fine crackles are described as 'Velcro-like' and tend to occur mainly during late inspiration – they can be heard in RAO and pulmonary oedema/congestive heart failure. Coarse crackles are popping sounds that occur during inspiration or expiration, and are commonly heard in RAO.

Wheezes are musical notes of varying pitch and duration. They occur in obstructive airway diseases, including RAO and bronchopneumonia, and may arise during inspiration or expiration.

Pleural friction (rubbing) sounds may be heard in pleuritis cases, although the sounds are lost when a significant effusion is present. These are usually fine crackling, crunching or creaking sounds that are heard mainly at end inspiration/early expiration.

Percussion of the chest

Percussion of the chest is particularly useful for the detection of pleural pain and effusions. The technique may be performed using a plexor and pleximeter, or with the fingers. When using the fingers, the first two fingers of one hand serve as a pleximeter (placed in an intercostal space) and the first two fingers of the other hand serve as a plexor to sharply tap the fingers of the first hand (Fig. 12.29). The area of percussion is similar to the area of auscultation, remembering that there is an area of cardiac dullness (larger on the left than the right) in the ventral thorax. The entire chest on both sides should be percussed working in parallel lines from dorsal to ventral and anterior to posterior. Normal lung tissue will sound resonant and hollow, whereas solid tissue or fluid will sound dull and flat.

Comment

• Pain on percussion is common in pleuritis cases, especially before large quantities of effusion have accumulated.

Figure 12.29 Chest percussion using the fingers of one hand to sharply tap the fingers of the other hand pressed against the chest wall.

Collection of samples from the lower airways

Aspiration of tracheal fluid collects secretions from the larger airways (trachea and bronchi) and also from the more distal airways via mucociliary clearance. In contrast, bronchoalveolar lavage harvests secretions specifically from the smaller, distal airways and alveoli. The choice of which technique to use is determined by the suspected underlying disease process and the reason for obtaining the sample (e.g. cytology or culture). In some cases, both tracheal aspiration and bronchoalveolar lavage will be required from the same horse. Tracheal aspirates collected 30-60 minutes after exercise may yield samples of greater diagnostic value than resting samples because they will contain more secretions originating from all areas of the lower respiratory tract.

Aspiration of tracheal fluid

Transtracheal aspiration

This technique allows the aseptic collection of samples from the lower respiratory tract that are suitable for cytology and bacterial culture. This technique is generally recommended when samples for





Figure 12.30 Transtracheal aspiration. A small stab incision is made through the skin in the midline over a bleb of local anaesthetic.

microbiological culture are required (e.g. in suspected pneumonia) since it avoids contamination by flora of the upper airway (which is likely in samples obtained through an endoscope). However, for isolation of specific organisms (e.g. Streptococcus pneumoniae, Actinobacillus equuli or Mycoplasma spp.), culture of tracheal aspirates obtained endoscopically is suitable. The procedure is performed in the standing horse using restraint (sedation of the horse is often helpful). The procedure must be performed under aseptic conditions and the clinician should wear sterile gloves. A small area over the middle cervical trachea is clipped and prepared aseptically. A variety of needle/catheter combinations may be used. Commercially available kits are convenient, and usually include a catheterover-needle and flushing/aspiration catheter combination. Alternatively, a 12 gauge needle, 7.5 cm over-the-needle cannula and a 5 French gauge canine urinary catheter may be used. The technique is then as follows:

• A bleb of local anaesthetic (e.g. 0.5 ml 2% lidocaine) is injected under the skin in the midline, and a small stab incision is made with a scalpel blade (Fig. 12.30)



Figure 12.31 Transtracheal aspiration. A 12G over-theneedle catheter is pushed between two tracheal rings into the tracheal lumen.

- The trachea is grasped by one hand to stabilize it, and the tracheal lumen is entered by pushing the needle or cannula (directed downwards) through the skin incision and between two tracheal rings (Fig. 12.31). NB: Care should be taken to avoid penetrating a cartilage ring or damaging the opposite wall of the trachea, and the bevel of the catheter/needle should face downwards. Once the tracheal lumen is entered, air may be heard passing in and out of the catheter/needle as the horse breathes
- The needle (+ stylet) is removed, and the flushing catheter is then passed through the cannula and threaded down the trachea until the end is at the level of the thoracic inlet (Figs 12.32, 12.33)
- 20–30 ml of sterile saline (without any bacteriostatic agents) is injected through the catheter and immediately aspirated. Only a small proportion of the delivered fluid is likely to be retrieved. The position of the urinary catheter may need to be adjusted several times, or the flushing/aspiration repeated several times until a satisfactory volume of fluid is recovered.

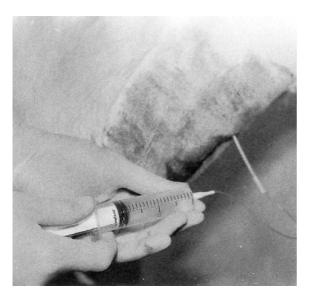


Figure 12.32 Transtracheal aspiration. A sterile dog urinary catheter is threaded into the tracheal lumen and lavage/ aspiration is performed with sterile saline.

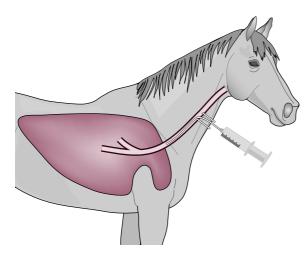


Figure 12.33 Diagram illustrating transtracheal aspiration. The catheter is passed until the distal tip is at the level of the thoracic inlet.

Gently lowering the horse's head or slow retraction of the catheter while applying suction may facilitate collection of a suitable sample.

On completion of aspiration, the urinary catheter is removed, followed by the introducer catheter.

However, if a needle is used as an introducer, it should be removed with the urinary catheter to prevent it from cutting off the catheter tip within the trachea.

Complications of transtracheal aspiration

The clinician should be aware of the potential complications of this technique:

- Damage to the tracheal cartilage, with resultant chronic infection
- Breaking of the catheter within the trachea. Most horses will cough up the damaged catheter within 30 minutes without long-term sequelae
- Breaking of the catheter in a subcutaneous site as a result of initial misplacement of the cannula/needle. The catheter must be removed surgically in these cases
- Local infection/cellulitis at the site of tracheal puncture due to contamination of the site by bacteria from the lower airways. In this instance hot fomentations, surgical drainage and appropriate antibiotic therapy will be necessary. Routine prophylactic antibiotics given systemically or injected locally may prevent this complication, and should always be used if malodorous or frankly purulent material is aspirated.

Endoscopic tracheal aspiration

This procedure allows the collection of tracheal aspirate samples suitable for cytology and limited microbiological culture. It relies on the availability of a sufficiently long endoscope (usually 2 m) to reach the distal cervical trachea. The procedure is well tolerated by most horses and has largely replaced transtracheal aspiration (apart from pneumonia cases) because it is much less invasive and has fewer complications. Endoscopy allows visualization of the lower airways at the same time and permits targeted aspiration from specific sites.

A flexible endoscope is passed in the usual way via the nares into the trachea. When the tip has reached the distal trachea, a catheter is passed down

the biopsy channel until it protrudes from the end. Sterile saline (10–15 ml) is injected through the catheter, which forms a pool at the thoracic inlet. The catheter tip is positioned into this pool and the sample is aspirated. Only a small proportion of the delivered fluid is likely to be retrieved.

Comment

 Samples collected in this way are suitable for cytology, but bacteriological culture must be interpreted with caution since the endoscope is unavoidably contaminated by bacteria residing in the upper respiratory tract. Guarded aspiration catheters are available that will reduce the risk of contamination of samples obtained in this way. The endoscope must be thoroughly disinfected if samples are being obtained for microbiological culture (and to prevent cross-contamination between horses). Contamination of flexible endoscopes by Pseudomonas spp. is common. If the horse coughs frequently during endoscopic tracheal aspiration, there will be an increased risk of contamination by oropharyngeal organisms. Likewise, if the aspiration procedure is slow, there will be an increased risk of contamination by oropharyngeal flora due to aspiration of saliva/upper respiratory secretions along the outside of the endoscope.

Bronchoalveolar lavage (BAL)

This technique samples fluid and cells from the alveoli and distal airways. BAL is undertaken in the standing, sedated horse and may be performed using an endoscope or a 'blind' technique.

Cytology of BAL fluid provides a more accurate reflection of the state of the small airways and alveoli than that of tracheal aspirates. BAL is ideally suited to the assessment of diffuse lower airway and alveolar diseases. When performed blindly, the segment of lung being lavaged is unknown, and the technique should therefore not be used in focal lung diseases. Endoscopy permits more accurate placement of the lavage tube but the precise location can still be difficult to assess.

Endoscopic technique

A 120 cm (or longer) endoscope is passed in the usual way to the carina and then into a mainstem bronchus. It is advanced down the bronchial tree until it wedges in an airway (usually about a fourth to sixth generation bronchus if an endoscope with an external diameter of more than 8–10 mm is being used). Lavage is performed via a catheter passed down the biopsy channel. NB: Coughing can be severe as the endoscope passes down the bronchial tree. In most horses this abates after 10–20 seconds, but in horses with airway inflammation coughing can be persistent, in which case this can be reduced by infusing a small volume of dilute lidocaine solution (0.4%) at each bronchial division.

The volume and type of fluid used for BAL is a matter of personal preference. Most clinicians use sterile pre-warmed saline, infused in 50 ml aliquots to a total volume of about 300 ml. The retrieval rate for this fluid varies, but is usually in the region of 50–80%. Normal BAL samples will appear foamy because of the presence of surfactant, and this indicates a good sample. The samples should be pooled together prior to evaluation.

Blind technique

Commercially available nasobronchial BAL tubes should be used when the technique is performed blind (Fig. 12.34). The tubes usually have an external diameter of 11 mm and have an inflatable cuff at the distal end. With the horse's head extended, the tube is passed in the same way as the endoscope through the nasopharynx into the trachea until a cough reflex is elicited. Infusion of dilute lidocaine solution (as for the endoscopic technique) will alleviate the coughing and permit the tube to be advanced down the bronchial tree (the tube most commonly enters the right dorsocaudal lung). When the tube becomes lodged and will not advance any further, the cuff is gently inflated with 5-10 ml of air, and the lavage is performed as for the endoscopic technique (Fig. 12.35).

Complications of BAL are minimal. A mild neutrophilic inflammatory response in the lavaged lung segment is expected. Occasionally, mild

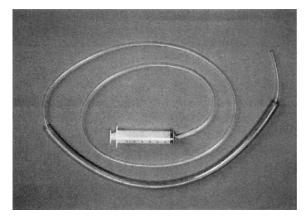


Figure 12.34 BAL tube consisting of a stomach tube (external diameter 12 mm) through which a smaller tube (external diameter 5 mm) is threaded. The small tube is lodged in a small bronchus and the lavage/aspiration is performed.

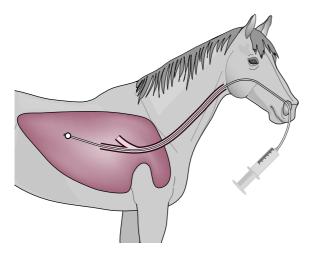


Figure 12.35 Diagram illustrating BAL performed with a balloon-tipped catheter. The catheter is passed via the nose, larynx and trachea into the bronchial tree. It is lodged in a small bronchus, and the balloon is inflated prior to performing lavage.

pyrexia is detected for about 24 hours after the procedure.

Thoracic radiography

Thoracic radiographs can be helpful in the diagnosis and monitoring of chest diseases in foals (especially pneumonia), and some lung diseases (especially interstitial disease, pneumonia, neoplasia, etc.) and pleural diseases (effusions) in the adult. Rare earth intensifying screens and high-speed radiographic film are necessary. The cassette should not be handheld and can be suspended in a bag hanging from a drip stand. An air gap of approximately 25 cm between the patient and the film eliminates the need to use a grid. The horse must be standing perfectly still when the radiograph is taken, and sedation should be used if necessary.

In the foal, both lateral and ventrodorsal or dorsoventral projections can be used, and a single 35×43 cm film may cover the entire chest. In the adult horse only lateral views are possible, and four overlapping views are often necessary to cover the entire thorax. A focus-film distance of 2 m is ideal, but if low power mobile or portable generators are used the distance may need to be reduced to 1 m in order to keep the exposure times short enough to avoid motion artefacts.

Good quality radiographs of a field that encompasses the caudodorsal lung area are possible using low-output generators. This view enables detailed evaluation of peripheral lung tissue and thirdgeneration pulmonary vessels. Radiographic examination of other lung fields requires the use of powerful generators at specialist centres. See 'Further reading'.

Lung biopsy

Lung biopsy is indicated in diffuse pulmonary diseases of unknown aetiology such as disseminated nodular or widespread interstitial diseases. Solitary lung masses identified by radiography, ultrasonography or endoscopy can also be investigated by biopsy.

Parenchymal lung biopsies may be obtained either by a percutaneous route using a biopsy needle, or by a transbronchial technique via endoscopy using a grasping biopsy wire. Both techniques are performed in the standing horse. In addition, biopsies may be obtained thoracoscopically.

Percutaneous technique

A Tru-Cut biopsy needle (Baxter Healthcare Corporation, California) is suitable for the percutaneous



Figure 12.36 Percutaneous lung biopsy using a Tru-Cut biopsy needle.

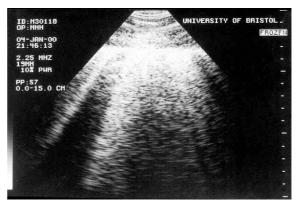


Figure 12.37 Ultrasonographic appearance of normal lung.

technique. The selected site on the chest wall is clipped and prepared aseptically. In diffuse lung diseases the 7th or 8th intercostal space (left or right), approximately 8 cm above the level of the elbow joint, is recommended. The technique is as follows:

- The skin, intercostal muscles and pleura are infiltrated with local anaesthetic, and a 5 mm stab incision is made through the skin.
- The biopsy instrument is introduced through the intercostal space, avoiding the vessels and nerves that run along the caudal margins of the ribs (Fig. 12.36). The needle can often be felt to 'pop' through the pleura
- The needle is then inserted about 2 cm into the lung tissue, and the biopsy taken. Two or three biopsies are usually taken
- The skin is sutured as necessary.

Comment

• Complications are rare with this technique, but could include haemorrhage and pneumothorax. Transient epistaxis or haemoptysis is sometimes observed following biopsy.

Transbronchial technique

An endoscope is passed in the same way as for BAL (above) until it lodges in a bronchus. The biopsy wire (jaws closed) is pushed down a small airway

until resistance is met. The jaws are opened and the wire is advanced as far as possible prior to closing the jaws and retrieving the sample. Four to six repeat samples are usually taken. Biopsies of bronchial wall or endobronchial masses can also be obtained by this method.

Comment

• Samples obtained by the transbronchial route tend to have crushing artefacts that may render histological interpretation difficult.

Ultrasonography of the chest

Ultrasonography is ideally suited to the investigation of pleural diseases, especially pleural effusions, where the technique can provide some clues as to the nature of the fluid (i.e. transudate or exudate) as well as the presence of adhesions and loculations. Consolidated lung, pulmonary abscesses and pulmonary masses may also be imaged if these lesions are adjacent to the pleural surface. Ultrasound waves are not transmitted through aerated lung (Fig. 12.37), so the technique is of little value in airway disease or focal lung diseases where the diseased area lies deep to the lung surface. The ribs can also be imaged by ultrasound.

Either linear array, curvilinear or sector scanners can be used. Superficial structures may be examined using a high frequency probe (7.5–10 MHz), but for





Figure 12.38 Ultrasonographic examination of the thorax using a 5 MHz linear array transducer.

deeper structures (>15 cm) a lower frequency probe should be used. A 5 MHz transducer is generally satisfactory – this produces an optimal image quality in the 5-10 cm depth range. In horses with large pleural effusions, a 2.5–3.5 MHz or lower-frequency transducer may be necessary. The area to be scanned should be clipped, cleansed and washed in spirit to degrease the surface. An acoustic coupling gel is then applied and massaged into the skin. The intrathoracic structures are imaged by placing the probe in the intercostal spaces and scanning both sides of the chest in a systematic fashion (Fig. 12.38). The normal anatomical boundaries of the lung (as described earlier in the section on 'Auscultation') are important landmarks. Imaging along the intercostal space from dorsal to ventral, progressing from caudal to cranial across the thorax, is recommended. At each intercostal space, structures should ideally be imaged in both transverse and longitudinal planes. Movement of the transducer should be slow to permit evaluation of each area during both inspiration and expiration.

The normal inflated lung has a highly echogenic surface. The reverberation artefact caused by the echogenic interface of pleura and aerated lung obscures the visualization of deeper structures. The pleural surfaces glide smoothly against each other as the lungs inflate and deflate. The two pleural surfaces normally appear as one echogenic line. Ribs are echogenic at their surface, smoothly marginated, convexly curved and cast a shadow. In the caudal thorax, the diaphragm may be visualized as a thin, smooth, echogenic line between the aerated lung and the cranial border of the liver.

Commonly identified abnormalities include:

- Pleural effusion separation of the parietal and visceral pleura by a band of hypoechoic material (fluid) deep to the intercostal muscles and superficial to the lungs. The echogenicity of the fluid varies depending on its nature (anechoic fluid represents fluid of low cellularity/protein content, whereas echogenic fluid indicates fluid with high cellularity/protein concentration. In the standing horse, free fluid will gravitate to the ventral thorax
- Pleuritis a roughened texture of the pleural surface causes narrow streaks of reverberation artefacts, commonly referred to as 'comet tails'. Comet tails can sometimes be identified in normal lungs, especially ventrally at the end of expiration. In chronic pleuritis, adhesions between the visceral and parietal pleura may restrict the normal gliding motion
- Pleural effusion with fibrin/fibrin tags linear echogenic structures that extend from the pleural surface into a region of effusion. These bands may float or wave within the pleural fluid
- Atelectasis and consolidation a consolidated lung appears similar to the liver ('hepatized') with tubular, hypoechoic branching vascular structures
- Pulmonary mass if a mass contacts the pleura it will be visible ultrasonographically, but not if it lies deep to aerated lung
- Pneumothorax this will cause a reverberation artefact without the normal gliding appearance of the pleura.

Pleuroscopy (thoracoscopy)

Direct endoscopic examination of the pleural cavity is occasionally helpful in the evaluation of selected cases of pleuropneumonia, thoracic abscesses, pericarditis and thoracic neoplasia. It may also be helpful in aiding biopsy of pulmonary and pleural masses. Either rigid endoscopes (such as arthroscopes or laparoscopes) or flexible endoscopes may be used. The technique is as follows:

- The horse should be sedated as necessary
- An area over the dorsal lung at the 8th to 10th intercostal space is clipped and prepared aseptically. Local anaesthetic is infiltrated in the skin and down through muscle to the level of the parietal pleura
- A stab incision is made through the skin and a purse-string suture is placed around it. The intercostal tissues are bluntly dissected (avoiding the blood vessels and nerves that run along the caudal margins of the ribs) down to and including the parietal pleura. As the pleural cavity is entered, air will be heard to rush in through the wound this creates a pneumothorax, which allows space for examination with the endoscope (Fig. 12.39)
- The endoscope is introduced and the pleural cavity is examined. The dorsal and middle parts of the thorax can be visualized in this way. Approximately two-thirds of the thoracic cavity, including the major portions of the lungs, mediastinum, thoracic aorta, oesophagus, diaphragm and sometimes the base of the heart/pericardium, can be visualized
- On completion of the examination, the endoscope is withdrawn and the purse-string suture is tightened to seal the chest wall defect

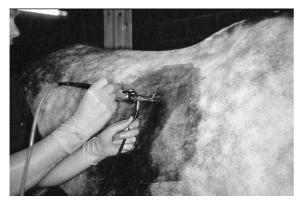


Figure 12.39 Pleuroscopy using a rigid arthroscope. A unilateral pneumothorax is created as soon as the chest cavity is entered, which provides space to allow the intrathoracic structures to be examined.

• The pneumothorax may be drained by inserting a sterile cannula with an attached three-way tap into the cavity dorsal to the endoscopy portal. Air is repeatedly aspirated through the cannula using a 50 ml syringe.

Comment

• Prophylactic broad-spectrum antibiotic therapy should be administered before and for several days after the procedure.

Thoracocentesis

Thoracocentesis allows sampling of pleural fluid for cytology and culture. Repeated thoracocentesis is also used in the treatment of pleuritis to provide drainage for accumulated effusions. Although horses usually have a fenestrated mediastinum, in disease states these fenestrations can become obstructed by fibrin, so that the two sides of the thorax become separate cavities. Consequently, it may be necessary to sample/drain each side of the thorax separately.

Thoracocentesis is undertaken in the standing horse and is generally performed in the ventral third of the chest, taking care to avoid damage to the heart. The precise site will vary depending on the distribution and quantity of fluid in each individual horse. When ultrasonography is available, it should be used to select the best site. The technique is as follows:

- The usual site is the 6th–7th intercostal spaces on the right or the 8th–9th intercostal spaces on the left
- A large area should be clipped and prepared aseptically. *Care should be taken to avoid the lateral thoracic vein that runs subcutaneously over the ventral part of the chest wall*
- Local anaesthetic solution (e.g. 10–20 ml of 2% lidocaine) is infiltrated subcutaneously and into the intercostal muscles down to the level of the parietal pleura
- A variety of needles/cannulas can be used. A 14G, 13.3 cm Teflon over-the-needle catheter is suitable. Alternatively, a blunt-ended teat cannula or metal bitch urinary catheter can be used for sampling small volumes of fluid. This

is introduced through a stab incision in the skin and pushed down through the intercostal muscles (avoiding the vessels and nerves which run along the caudal margin of the ribs) until it enters the pleural cavity. This can be appreciated by a palpable decrease in resistance ('pop')

- If fluid is not present in the cavity, air will be drawn into the chest once the pleura is penetrated, so a device such as a three-way tap is essential to seal the end of the cannula
- If excessive fluid is present, it will usually drain from the cannula under gravity, and samples can be collected for bacteriology (into aerobic and anaerobic blood culture bottles) and cytology (into EDTA). In normal horses, only a small volume (<8 ml) of fluid will be obtained
- Following completion of the procedure, the cannula is withdrawn; there is usually no need to suture the skin.

Complications (pneumothorax, lung laceration, haemothorax, cardiac arrhythmias, cardiac puncture, etc.) are very rare if the correct procedure is used.

Comment

• If large volumes of fluid are to be drained in addition to sampling, wider-bore sterile cannulas can be used (such as metal bitch urinary catheters or human thoracic drainage cannulas: Fig. 12.40). It is usually necessary to suture the skin after the use of these larger cannulas.

Arterial blood collection

In the adult horse, arterial blood samples for blood gas analysis are usually collected from either the common carotid artery, the transverse facial artery or the facial artery.

Carotid artery

The carotid artery is punctured in the region of the lower third of the neck as follows:

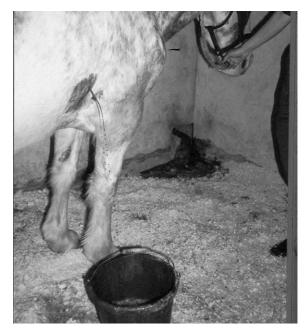
• The artery is palpated as a cord-like structure deep to the jugular vein

Figure 12.40 Thoracocentesis. Drainage of a large volume of pleural fluid using a metal bitch urinary catheter.

- The area is prepared aseptically, and a 2-inch × 19–21G (51 × 1.0 mm) needle is pushed into the vessel, which is stabilized with the fingers of one hand. When the artery is penetrated, blood will squirt out under pressure. Several attempts may be required to penetrate the artery. Alternatively, placement of the needle using ultrasound guidance may help
- Blood is collected into a 2 or 5 ml syringe that has been previously filled with a small volume of sodium heparin solution (1000 IU/ml) to eliminate any air space. All air bubbles are expelled from the syringe, which is then sealed with a cap
- Following removal of the needle from the artery, firm finger pressure is applied for several minutes to prevent haematoma formation.

Transverse facial artery

The transverse facial artery runs rostrally on the surface of the masseter muscle about 1.5 cm ventral



to the zygomatic arch. It is superficial and readily punctured if a needle is advanced at a shallow angle parallel to the axis of the palpebral fissure. Prior application of local anaesthetic ointment such as EMLA cream facilitates the procedure.

Facial artery

The facial artery is more easily sampled in the anaesthetized horse. It rounds the ventral border of the horizontal mandible and ascends along the rostral border of the masseter muscle. It is rostral to the vein. The facial artery can be sampled at a site just lateral to the lateral canthus of the eye. The area is prepared aseptically, and the artery is stabilized with the fingers of one hand. The vessel is punctured with a 1 inch \times 23G (25 \times 0.65 mm) needle and the sample is collected as described above.

Comment

• Arterial samples should be analysed immediately, or alternatively they can be stored in ice for up to 6 hours.

Nasal and nasopharyngeal swabbing

Nasal and pharyngeal swabs can be used for the isolation of respiratory viruses and bacteria. The viruses of clinical importance are: equine herpesvirus types 1 and 4 (EHV-1 and EHV-4); equine influenza virus and equine viral arteritis (EVA) virus. Lesser clinical significance is attached to equine adenovirus and equine rhinovirus.

Successful isolation of these viruses depends upon sample collection at the correct time (early in the course of infection), the use of suitable viral transport medium and rapid transport to the laboratory. Large gauze swabs are the most suitable (Fig. 12.41). Alternatively, a guarded mare uterine swab may be used. The swab is inserted through the nares and passed along the ventral nasal meatus to the nasopharynx (about 30 cm from the nares in a 500 kg horse). The swab should be rotated and left in the nasopharynx for several minutes before it is withdrawn and then placed immediately into viral



Figure 12.41 Gauze nasopharyngeal swabs suitable for collection of samples for virus isolation.

transport medium containing antibiotics. The swab is detached using wire cutters. Some respiratory viruses (e.g. EHV-1) may also be isolated from citrated or heparinized blood. Samples should be sent to the laboratory on ice or in a cool transport container as soon as possible.

Bacteriological examination of nasal or nasopharyngeal swabs is rarely helpful because of the large normal bacterial flora. However, in certain specific diseases, culture for a specific pathogen (e.g. *Streptococcus equi* subsp. *equi* in suspected strangles cases) may be helpful. Nasal washes may be more effective than swabs when small numbers of organisms are likely because a greater surface area can be sampled. Fifty millilitres of warm normal saline can be infused via a 15 cm long sterile, soft rubber tube inserted into the nasal cavity, and the washings collected into a sterile container for subsequent centrifugation and culture.

Sampling from the guttural pouch

Samples obtained from the guttural pouch may be helpful for both cytological examination and microbiological culture. Samples may be obtained by 'blind' catheterization or via a flexible endoscope (see 'Examination of the guttural pouches' above). Fluid samples for cytological, microbiological or molecular examinations (e.g. polymerase chain reaction (PCR)), can be collected by direct aspiration or lavage with 20–30 ml of sterile saline. If chondroids are present in the guttural pouch, one may be retrieved using endoscopic basket forceps and submitted for bacteriological culture.

III. CLINICAL PATHOLOGY

Cytology of tracheal aspirates and bronchoalveolar lavage fluid

Preparation

Tracheal aspirate samples may be sufficiently cellular to allow a smear to be made immediately after collection on to a glass slide, which is fixed or airdried and stained.

BAL fluid and less cellular tracheal aspirate samples should be centrifuged in conical tubes ($350 \times g$ for 10 min), and the sediment used to prepare a smear. Alternatively, a smear may be prepared using a cytocentrifuge. Smears should be made as rapidly as possible, since cellular changes occur within hours after collection. Generally, samples may be stored for up to 8 hours at room temperature or 24 hours at 4°C. If immediate processing is not possible, the cells can be fixed by adding an equal volume of fixative (e.g. 40–50% ethanol). Rapid bacterial overgrowth occurs in samples kept at room temperature.

Suitable stains for cytology include Diff-Quik, Wright's stain, Wright's-Giemsa stain, May-Grünwald-Giemsa stain, Sano Trichome and Papanicolaou stains. Gram stain can be used to identify bacteria. Perl's Prussian Blue stain can be used to identify haemosiderin. A Ziehl-Neelsen stain may be helpful to indicate mycobacterial infection.

Total nucleated cell counts can be useful in tracheal aspirates and BAL samples and are performed using a Neubauer haemocytometer. Cell counts obtained with Coulter counters are unreliable, and samples must be filtered to remove excess mucus and other debris before analysis. It must be recognized that the total nucleated cell counts will vary depending on the degree of dilution of the sample and this is not possible to quantify.

Interpretation

Tracheal aspirates

Tracheal aspirates from normal horses contain mainly ciliated columnar epithelial cells and alveo-

lar macrophages. Macrophages may be seen to contain phagocytosed material such as fungal spores and degenerate cells. Haemosiderin granules are commonly seen in the macrophages of Thoroughbred horses in race training – this is a normal finding and may not indicate clinically significant exercise-induced pulmonary haemorrhage. Varying numbers of neutrophils are found in normal horses, which may account for up to 20–40% of the total cells present. Other cells that may be found include goblet cells, squamous epithelial cells, eosinophils (normally <1%), lymphocytes (normally <1%), basophils and mast cells (normally <1%).

Inflammatory diseases such as RAO and bronchopneumonia usually result in large numbers of neutrophils (which may comprise 95–98% of the total cells) and increased amounts of mucus in tracheal aspirates. Curschmann's spirals (casts of inspissated mucous plugs from small airways) are also seen in chronic lower airway disease.

High numbers of eosinophils are seen in horses with lungworm (*Dictyocaulus arnfieldi*) infection. Larvae may also be recovered in tracheal washes in these cases.

BAL samples

BAL samples from normal horses contain mainly macrophages and lymphocytes (approximately 40–50% of each). Neutrophils normally account for less than 5%. Other cells include mast cells (normally <2%), epithelial cells and eosinophils (normally <0.5%). Horses with RAO tend to show an increase in neutrophils. Animals with pneumonia show large numbers of neutrophils with toxic changes (provided that a diseased segment of lung has been sampled).

Pleural fluid analysis

Normal fluid

Normal horses have a small volume (up to 8 ml) of pleural fluid that can be retrieved by thoracocentesis. The fluid is clear, watery and pale straw-coloured. The total nucleated cell count is normally less than 4×10^9 /l, the total protein less than 3 g/dl and the specific gravity about 1.015. Neutrophils predominate (approximately 70%) with smaller numbers of large mononuclear cells (20%), lymphocytes and eosinophils.

Pleural effusions

Pleural effusions may be classified as transudates, modified transudates, exudates or chylous effusions.

Transudates

Transudates have normal cell counts and protein levels. They may be identified in early neoplastic diseases, congestive heart failure and hypoproteinaemia.

Modified transudates

Modified transudates are transudates with added cells (mesothelial cells, macrophages, neutrophils or neoplastic cells) and/or protein. They tend to appear pink and slightly cloudy. In the horse they are most commonly associated with neoplasia.

Exudates

Exudates have high cell counts (more than 10×10^9 /l), high protein content (more than 3.0 g/dl) and high specific gravity (1.018). They are usually thick and cloudy, and tend to clot spontaneously. The majority of cells are neutrophils. Exudates are seen in pleuritis (pleuropneumonia) and some neoplastic conditions.

Chylous effusions

Chylous effusions appear milky white; the colour clears when the sample is shaken with ether. They possess high triglyceride concentrations, and a large proportion of the cells are lymphocytes.

Biochemical evaluation of pleural fluid

Total protein concentration is usually measured using a refractometer on samples collected into EDTA. The total protein concentration of normal pleural fluid is less than 25 g/l.

Glucose and lactate concentrations and pH: These measurements can be helpful to differentiate

septic from non-septic effusions. Samples should be collected into fluoride-oxalate. Low pleural fluid glucose concentration (<0.4 g/l), increased lactate concentration (> blood concentration), and decreased pH (<7.0) are suggestive of sepsis.

Arterial blood gas analysis

Normal values of partial pressures of O_2 (P_aO_2) and CO_2 (P_aCO_2) are 85–100 mmHg and 35–45 mmHg respectively.

Hypoxaemia ($P_aO_2 < 80$ mmHg) occurs in diseases resulting in mismatching of ventilation and perfusion, such as pulmonary oedema, pneumonia and RAO.

Hypoxaemia with hypercapnea ($P_{a}CO_{2} > 45 \text{ mmHg}$) indicates hypoventilation, e.g. severe RAO with airway obstruction.

Viral serology

Serology is the most convenient way of diagnosing viral infections but should be seen as complementary to nasopharyngeal swabbing rather than as an alternative technique. In most cases, acute and convalescent serum samples (taken early in the infection and 2–3 weeks later) are required. A fourfold rise in antibody titre is regarded as diagnostic of infection.

Sinus aspirates

Cytology and culture of fluid obtained by centesis can be helpful in differentiating primary sinusitis and sinusitis that is secondary to dental disease. The presence of food material indicates dental disease. Bacterial cultures from primary sinusitis cases generally yield single bacterial species, whereas multiple species are often present in secondary sinusitis cases.

Aspiration of relatively acellular, bright yellow fluid from a sinus is suggestive of a sinus cyst.

Haematology

Haematology provides non-specific information in patients with respiratory disease. The following points are offered as a general guide:

12

- Erythrocyte parameters will be depressed by most long-term respiratory diseases
- Total white cell counts are likely to be raised (leukocytosis), largely as a result of neutrophilia, in bacterial respiratory diseases such as strangles (*Streptococcus equi*), postviral infections or pneumonia/pleuropneumonia. Lymphopenia commonly occurs during the acute phase of viral infections but the effect is often transient and it is not a pathognomonic finding. Monocytosis is usually a feature of chronic inflammatory conditions associated with suppuration, granulomatous reactions or tissue necrosis
- Plasma fibrinogen concentration is a sensitive indicator of bacterial inflammation. It is a better prognostic indicator than leukocyte parameters when monitoring the course of bacterial respiratory disease.

Microbial culture

Samples for microbial culture should always be processed as quickly as possible. Swabs should be placed into appropriate media and cultured as soon as possible. Survival of organisms in such culture media will vary between different species and this can affect the final culture results. Fluid aspirates should also be placed into transport medium if there is to be a delay of more than a few hours before culturing takes place. Quantitative culture (which determines the number of colonyforming units for each bacterial species) is helpful when culturing tracheal aspirates. Samples suspected of containing obligate anaerobic bacteria should be cultured immediately. Specimens submitted for virus isolation should be placed into virus transport medium and transported at 4°C (or frozen at -20°C).

CHAPTER APPENDICES

Investigating the causes of nasal discharges and coughing are common problems of differential diagnosis that confront the practitioner. Appendix 12.1 suggests applications of some of the diagnostic techniques covered in this chapter for the investigation of nasal discharges. The nature of the discharge and whether or not it is unilateral or bilateral reflect the type and site of the underlying lesion. Discharges may be variously mucoid, mucopurulent or purulent, and may additionally contain blood or food. Sometimes the discharge may be distinctly malodorous. Unilateral discharges usually indicate lesions associated with the nasal passages or paranasal sinuses. Bilateral discharges suggest a lesion caudal to the nasal septum but an exception is guttural pouch empyema, which often produces a predominantly unilateral discharge. Appendix 12.2 suggests techniques for the investigation of persistent coughing.

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APPENDIX 12.1 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF NASAL DISCHARGE

Discharge	Possible cause	Aids to diagnosis
Mucoid/ mucopurulent	Respiratory virus	Viral isolation; serology
	Bacterial infection	Nasopharyngeal swab
	Paranasal sinus cyst	Endoscopy; radiography
Mucopurulent/ purulent	Sinusitis	Percussion; endoscopy; radiography; centesis (culture); direct sinus endoscopy
	Guttural pouch empyema	Endoscopy; catheterization (culture); radiography
	Fungal rhinitis	Endoscopy; biopsy for histopathology/culture
	Lower airway disease	Auscultation; endoscopy; tracheal aspiration/ BAL for cytology; thoracic radiography
Discharge with food (dysphagia)	Oesophageal obstruction	Endoscopy; radiography
	Pharyngeal paralysis:	
	 guttural pouch mycosis 	Endoscopy
	- trauma of the head and neck	Neurological examination (see Ch. 14)
	- lead poisoning	Lead estimation in: blood; liver/kidney, and topsoil
	– botulism	Check clinical signs and feedstuff (see Ch. 14)
	Pharyngeal mass – abscess/neoplasia	Endoscopy; endoscopic biopsy; radiography; culture
	Pharyngeal foreign body	Endoscopy
	Palatal defect	Endoscopy; radiography
	Guttural pouch tympany	Endoscopy; radiography
	Guttural pouch empyema	Endoscopy; catheterization (culture); radiography
	Grass sickness	Check clinical signs; radiography of the oesophagus (mega-oesophagus and pooling of contrast medium); endoscopy of the oesophagus ('reflux oesophagitis'); post- mortem histopathology of the coeliacomesenteric ganglion

APPENDIX 12.1 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF NASAL DISCHARGE—cont'd

Discharge	Possible cause	Aids to diagnosis
Discharge with dyspnoea	Pharyngeal abscess	Endoscopy; radiography; culture
	Guttural pouch tympany	Endoscopy; radiography
	Guttural pouch empyema	Endoscopy; catheterization (culture); radiography
	RAO	Auscultation; tracheal aspiration/BAL for cytology; thoracic radiography
	Pulmonary abscess/ pneumonia/ pleuropneumonia	Auscultation; chest percussion; endoscopy; tracheal aspiration/BAL for cytology and culture; thoracic radiography; ultrasonography (pleural effusion); thoracocentesis for cytology and culture of effusion
Discharge with necrotic smell	Dental disease	Examine mouth; radiography
	Chronic sinusitis	Percussion; endoscopy; radiography; centesis (culture); direct sinus endoscopy
	Fungal rhinitis	Endoscopy; biopsy for histopathology/culture
	Guttural pouch infection	Endoscopy; culture
	Neoplasia	Endoscopy; endoscopic biopsy; radiography
	Gangrenous pneumonia	Auscultation; chest percussion; tracheal aspiration/BAL for cytology and culture; thoracic radiography; ultrasonography (pleural effusion); thoracocentesis for cytology and culture of effusion
	Inhaled foreign body	Endoscopy
Discharge with blood	Nasal tumour	Endoscopy; radiography
	Guttural pouch mycosis	Endoscopy
	Fungal rhinitis	Endoscopy; biopsy for histopathology/culture
	Foreign body	Endoscopy
Epistaxis	Ethmoidal haematoma	Endoscopy; radiography; haematology (anaemia)
	Turbinate necrosis	Endoscopy; culture
	Guttural pouch mycosis	Endoscopy; haematology (anaemia)
	Exercise induced pulmonary haemorrhage	Endoscopy; thoracic radiography; haematology (anaemia)
	Trauma	Radiography

APPENDIX 12.2 SOME APPLICATIONS OF DIAGNOSTIC TECHNIQUES FOR THE INVESTIGATION OF COUGHING

Possible cause	Aids to diagnosis
Acute coughing	
Viral infection:	Viral isolation; serology
EHV-1 and EHV-4	
Equine influenza	
Equine viral arteritis	
Adenovirus	
Rhinovirus	
'Strangles' (Streptococcus equi)	Nasopharyngeal swab
Choke and other causes of dysphagia	Endoscopy; radiography (see also Appendix 2.1 in Ch. 2)
Acute small airway obstruction in RAO	Auscultation; tracheal aspiration/BAL for cytology; thoracic radiography
EIPH	Endoscopy; thoracic radiography
Pulmonary abscess/pneumonia/ pleuropneumonia	Auscultation; chest percussion; tracheal aspiration/BAL for cytology and culture; thoracic radiography; ultrasonography (pleural effusion); thoracocentesis for cytology and culture of effusion
Inhaled foreign body	Endoscopy
Tracheal collapse	Endoscopy
Chronic coughing/RAO	Auscultation; tracheal aspiration/BAL for cytology; thoracic radiography
Postviral airway disease	History of preceding viral respiratory disease; serology indicating recent infection
Lungworm	Tracheal aspiration/BAL for cytology (eosinophilia) and larvae
Pulmonary abscess/pneumonia/ pleuropneumonia	Auscultation; chest percussion; tracheal aspiration/BAL for cytology and culture; thoracic radiography; ultrasonography (pleural effusion); thoracocentesis for cytology and culture of effusion
Summer-pasture-associated obstructive pulmonary disease	Auscultation; tracheal aspiration/BAL for cytology; thoracic radiography; change environment
Thoracic neoplasia	Endoscopy; thoracic radiography; ultrasonography (pleural effusion); thoracocentesis for cytology; pleuroscopy

CHAPTER 13

Musculoskeletal diseases

Chapter contents

I. Lameness examination 250 History 250 Physical examination 251 The forelimb 251 Examination of the foot 251 Examination of the pastern and fetlock 253 Examination of the metacarpus 253 Examination of the carpus 253 Examination of the elbow and shoulder 254 The hindlimb 254 Examination of the hock 254 Examination of the stifle 254 Examination of the pelvis and hip 255 The back 255 History 255 Examination 256 Radiography 256 Haematology and biochemistry 256 Evaluation of the gait 256 Observation 256 Provocative tests 257 Standardization of the flexion test 258 Interpretation of flexion tests 258 Extension tests 258 Pressure tests 258

II. Local analgesia 259

General considerations in local analgesia 259 Perineural analgesia – general considerations 259

Materials 259 Preparation and restraint of the horse 259 Needle placement 260 Sites of nerve block in the forelimb 261 Palmar digital nerve block 261 Abaxial sesamoid nerve block 261 Palmar and palmar metacarpal nerve block (four-point block) 261 Subcarpal block 263 Median and ulnar nerve block 263 Sites of nerve block in the hindlimb 264 Plantar and plantar metatarsal nerve block (four-point block) 264 Tibial and peroneal nerve block 264 Intrasynovial analgesia - general considerations 265 Intrasynovial analgesia in the forelimb 266 Distal interphalangeal (coffin) joint 266 Navicular bursa 266 Proximal interphalangeal (pastern) joint 267 Metacarpophalangeal (fetlock) joint 267 Digital tendon sheath 267 Midcarpal joint 267 Antebrachiocarpal joint 268 Elbow 268 Shoulder 268 Intrasynovial analgesia in the hindlimb 268 Tarsometatarsal joint 268 Distal intertarsal joint 269 Tarsocrural joint 269

Femoropatellar joint 269 Medial femorotibial joint 270 Lateral femorotibial joint 270

III. Imaging techniques 270

Radiography 271 X-ray machines 271 Intensifying screens and film 271 Digital imaging 271 Supplementary equipment 271 Positioning, centring and collimation 271 Exposure factors 271 Preparation for radiography 272 Personnel 272 Radiographic projections 272 Terminology of projections 272 Interpretation of radiographs 274 Ultrasonography 274 Nuclear scintigraphy 275 Magnetic resonance imaging (MRI) 276 Diagnostic arthroscopy 276

IV. Laminitis 277

Signs of acute laminitis 277 Radiography in laminitis 278 *Interpretation* 278 Signs of chronic laminitis 279

V. Myopathies 280

Acute exertional rhabdomyolysis 280 Recurrent exertional rhabdomyolysis 281 Polysaccharide storage myopathy 281 Clostridial myositis ('malignant oedema') 281 Post-anaesthetic myopathy 281 Atypical myoglobinuria 281 Hyperkalaemic periodic paralysis 282 Nutritional muscular dystrophy ('white muscle disease') 282 Glycogen branching enzyme deficiency 282 Myotonic dystrophy 282 Mitochondrial myopathy 283 Malignant hyperthermia 283 Immune-mediated myositis 283

VI. Clinical pathology 283

Haematology 283 Serum muscle enzymes 283 Selenium and vitamin E 283 Serology 284 Brucellosis 284 Lyme disease (borreliosis) 284 Synovial fluid samples 285 Gross appearance 285 Useful laboratory measurements 285

Further reading 285

I. LAMENESS EXAMINATION

Problems associated with the musculoskeletal system of horses are common and usually present as a physical abnormality or lameness. The diagnostic approach involves a number of basic steps:

- Defining the problem to be investigated
- Localizing the site(s) of abnormality
- Characterizing the nature of the pathological change.

History

Apart from details of breed, age and sex, the essential background information should include:

- The duration of ownership and use (or intended use) of the horse
- Details of recent management including housing, feeding, shoeing and exercise
- Any previous medical problems known to the owner.

The owner should be allowed to describe in his/her own way the reasons for presenting the animal and should then be questioned more specifically to try to define the primary complaint. Once this is achieved, further details can be added, particularly in relation to the owner's observations:

- The limb or limbs thought to be affected
- The timing and nature of the onset of signs
- Details of any associated events or incidents
- The progression of signs since their onset
- Any changes in management (or attempts at treatment) as a result of the problem and their effects
- A summary of the current state of the problem.

Physical examination

Every effort should be made to examine the entire musculoskeletal system, even in the presence of obvious abnormalities at one site. The examination at rest falls into two stages.

1. There should be an overall inspection of the horse from all angles, noting particularly:

- General body condition
- Conformation of the body, limbs and feet (see below)
- Posture and weightbearing on the limbs
- Skeletal and soft tissue symmetry
- Any localized swellings or thickenings
- Any evidence of generalized or systemic disease.

Conformation is the outward appearance of an animal. Ideal conformation is generally accepted to be that which does not bring excessive force(s) to bear on any individual structure. In consequence, it requires symmetry and alignment of the limbs, leading to straight limb flight and even weightbearing. Deviation from good conformation will result in excessive tensile or compressive forces being concentrated on one part of the limb during weightbearing. Over the course of time, the cumulative effect may be injury or disease. Deviation from straight limb flight is inefficient and may result in interference with the gait and self-inflicted injury. The dividing line between conformational variation, adopted posture and pathological deformity is poorly defined in many cases.

2. A detailed evaluation of the individual regions of the limbs should be undertaken by:

- Inspection to reveal deformity, swelling or thickening, skin wounds and muscle wasting
- Palpation to detect heat and pain, as well as characterizing the precise location and consistency of any swellings or thickenings
- Manipulation of limb joints to evaluate their range of movement, i.e. detecting restriction, instability, pain or crepitus.

All three procedures should involve comparison of the region under examination with the contralateral limb to detect potentially significant asymmetries.

The forelimb

Examination of the foot

Initially, the feet should be inspected with regard to their size, shape, symmetry and balance.

Variations in foot shape may be due to conformation, trimming, shoeing, disease or lameness. Not infrequently, abnormalities are actually a result of a complex combination of these factors, which have distorted foot shape over a prolonged time.

The feet should show symmetry between contralateral limbs and between the medial and lateral halves of each foot. The angle between the dorsal wall of the hoof and the ground should be approximately 45–50° in the forelimbs and slightly more upright (50–55°) in the hindlimbs.

The foot is said to be balanced when its shape and position in relation to the limb are such that weight is equally distributed over it. The angle between the dorsal wall of the hoof and the ground should be the same as that of the pastern. This relationship constitutes the *hoof–pastern axis* (Fig. 13.1). A common abnormality, thought to predispose to foot lameness, is the combination of an overlong toe and a low, collapsed heel leading to a 'broken back' hoof–pastern axis. The dorsal wall of the hoof should be parallel to the wall at the heel as viewed from the side of the horse. There should be no rota-

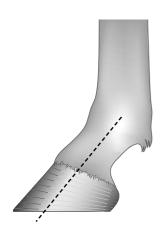


Figure 13.1 The hoof-pastern axis.

tory deformity of the foot ('toe in' or 'toe out') and the heels should be of equal height.

The hoof wall, coronary band and solar surface should then be examined in detail.

The hoof wall should be straight and not splayed out toward the bearing surface. The outer surface of the hoof should be smooth. 'Rings' on the hoof wall may result from nutritional variations, previous episodes of systemic disease, or laminitis. The variations of horn growth associated with nutritional change or systemic disease result in rings that emerge parallel to the coronet and each other. In contrast, laminitic rings appear to diverge at the heel and converge at the dorsal wall, reflecting uneven growth from the coronet. The hoof wall should also be carefully examined for cracks, and their location, depth and any associated lesions of the coronary band should be noted.

The coronary band should be palpated, particularly dorsally, for evidence of pain or 'dipping', which may indicate inflammation or downward displacement of the pedal bone, respectively, as a result of laminitis. Palpation of the coronary band may also reveal local swelling, pain or heat secondary to trauma or infection. The proximal part of the lateral cartilages can be palpated above the coronary band and should be checked at the same time. Distension of the distal interphalangeal joint ('coffin joint') is occasionally palpable just proximal to the coronary band dorsally. The digital pulse at the fetlock should be palpated in association with examination of the foot to see if it is increased in volume on one or both sides. An increased digital pulse suggests inflammation or laminitis in the foot.

The foot is then picked up and the solar surface is examined. The sole should be concave and not flat. In the forelimb, the heel-to-toe length should equal the width of the bearing surface at its widest point, while the hindfoot is generally longer and more pointed. The foot (or shoe) should be inspected for uneven wear and an attempt should be made to correlate this to limb movement and foot placement during motion. The shoe should be evaluated for type, positioning, nail numbers and their placement.

The frog and solar surface of the foot must then be picked clean of bedding and dirt and the superficial layers of horn pared away with a hoof knife before they can be adequately examined. Initial inspection should check for the presence of foreign bodies embedded in the foot or other obvious solar injury. The surface is then carefully searched for discoloration that might indicate bruising (usually red/ purple areas) or possible infection (particularly black spots or lines). Careful attention should be paid in this part of examination to the white line, heels and frog, including the sulci, as these are common sites of infection.

Palpation of the hoof capsule itself usually requires the mechanical advantage provided by hoof testers, unless the sole is very thin and compressible. The hoof testers are used to apply pressure to all parts of the hoof, including the frog, in a systematic manner - always remembering that they exert pressure at two points. Areas that seem to be painful are checked several times to try to localize the painful focus as accurately as possible and ensure that the response is consistent. Suspect areas should be compared with the equivalent site in the contralateral limb if the significance of the horse's response is in doubt. Percussion of the wall and sole with a light hammer or the hoof testers may also be helpful in determining the presence of foot pain. Consistently painful areas should be explored further with the hoof knife to see if deep infection or bruising are the underlying cause.

The distal interphalangeal joint should be flexed and extended and stressed medially, laterally and in rotation, checking for pain, restriction, instability and crepitus. The same manipulations apply in the examination of all limb joints, although they are less easy to perform on the more proximal joints in the horse.

Examination of the pastern and fetlock

The pastern is checked for thickening around the proximal interphalangeal joint ('pastern joint'), or on its palmar aspect, where lie the superficial and deep digital flexor tendons and the distal sesamoidean ligaments.

The fetlock joint should be inspected and palpated for swelling due to synovial effusion. This usually begins as a fluctuant fluid swelling between the palmar aspect of the distal third metacarpal bone and the branches of the suspensory ligament. More severe effusion may produce a swelling that is apparent on the dorsal aspect of the joint. Articular effusion should be distinguished from distension of the digital flexor tendon sheath, which produces a more palmar swelling around the digital flexor tendons at the back of the fetlock, i.e. behind the branches of the suspensory ligament. This is sometimes constricted on the palmar aspect of the proximal sesamoid bones by the annular ligament. The proximal sesamoid bones themselves and the insertions of the branches of the suspensory ligaments are palpated for pain and swelling.

Examination of the metacarpus

The three metacarpal bones are palpated for evidence of pain, heat and swelling; this is particularly relevant to young horses, where dorsal metacarpal periostitis and/or stress fractures (sore shins) and 'splints' are seen most commonly. Palpation of the small metacarpal bones should include their axial (medial), palmar and abaxial (lateral) surfaces as far as possible. Firm, cold, painless splints in middle-aged and older horses are a common incidental finding that is usually of no functional significance. The palmar metacarpal soft tissues are a common source of lameness and should be carefully palpated with the limb bearing weight and then with the foot raised. The individual structures to be checked are the superficial digital flexor tendon, the deep digital flexor tendon, the inferior check ligament and the suspensory ligament.

- Swelling of the superficial digital flexor tendon commonly renders the normally straight palmar surface of the metacarpus convex, i.e. the tendon becomes 'bowed'. Severe swelling makes it impossible to distinguish the deep flexor tendon as a separate structure – however, it is less common for the deep digital flexor tendon to suffer strain injury.
- Inferior check ligament injuries produce a swelling in the proximal and middle thirds of the palmar metacarpus dorsal to the superficial digital flexor tendon and do not usually disturb the straight palmar contour of the region.
- The body and branches of the suspensory ligament are easy to palpate in the mid and distal thirds of the metacarpus. However, the proximal third of the ligament is located between the two small metacarpal bones and is more difficult to assess clinically. Defining the margins of the suspensory ligament using the thumbs is helpful in assessing whether there is any thickening present.

The digital extensor tendons (common digital extensor and lateral digital extensor) should be palpated from their muscular origins above the carpus and followed down the dorsal aspect of the metacarpus.

Examination of the carpus

The dorsal aspect of the carpus ('knee') is examined for synovial swellings owing to effusion in the two more mobile carpal joints (the antebrachiocarpal and midcarpal joints), or tenosynovitis of the extensor tendon sheaths (extensor carpi radialis, extensor carpi obliquus, common digital extensor and lateral digital extensor).

Joint flexion allows palpation of the dorsal margins of the carpal joints for capsular thickening

and alteration in the underlying bony contours. Separated bone fragments can occasionally be palpated within the antebrachiocarpal or midcarpal joints when these are flexed.

The palmar aspect of the joint is examined for swelling of the carpal sheath and stability of the accessory carpal bone. In horses with carpal sheath distension, the palmar aspect of the distal radius is palpated with the carpus flexed to detect radial osteochondromas.

Examination of the elbow and shoulder

Effusions or thickenings of the elbow and shoulder joints are not generally palpable, but regional swelling may occur, particularly with traumatic injury or joint infection.

The stability of the olecranon should be specifically checked by manipulation, especially in horses demonstrating a 'dropped' elbow. Auscultation with a stethoscope over bony prominences during manipulation or movement can be helpful in detecting crepitus associated with proximal limb fractures.

Muscle wasting as a result of lameness is usually most apparent in the largest muscle masses, i.e. around the shoulder and scapula of the proximal limb, even where the precipitating cause is a foot problem. Rapid and severe wasting of specific muscle groups may accompany lower motor neuron injury, e.g. wasting of the supra- and infraspinatus muscles following trauma to the suprascapular nerve.

The hindlimb

Examination of the foot, fetlock and metatarsus of the hindlimb is essentially similar to examination of the lower forelimb.

Examination of the hock

A number of different conditions may give rise to swellings around the hock:

• Effusion of the tarsocrural joint ('bog spavin') is usually most apparent on the dorsomedial aspect of the hock, although smaller fluctuant swellings may also be apparent plantarolaterally and plantaromedially

- Distension of the tarsal sheath ('thoroughpin') produces plantar swellings proximal to the hock on either side of, and slightly cranial to, the Achilles tendon
- Inflammation of the plantar ligament ('curb') causes thickening on the plantar aspect of the hock centred about 10 cm distal to the point of the hock
- Degenerative joint disease of the small hock joints ('bone spavin') may produce thickening on the medial aspect of the distal hock, though this is not commonly detectable.

The Achilles tendon should be examined for thickening indicative of strain injury and the positioning and stability of the superficial digital flexor tendon over the point of the hock should also be checked. Extension of the hock should cause extension of the stifle because of the action of the reciprocal apparatus. If it can be accomplished independently and results in relaxation of the Achilles tendon, then rupture of the peroneus tertius should be suspected.

Examination of the stifle

Effusion of the femoropatellar joint causes palpable swelling between the three distal patellar ligaments. The collateral ligaments can also be palpated medially and laterally.

In cases of suspected intermittent upward fixation of the patella, attempts can be made to lock the joint by reversing and turning the horse in tight circles. Alternatively, locking may be demonstrated by pushing upwards on the patella while keeping the horse's weight on the affected limb by pulling laterally on the tail.

The demonstration of stifle instability due to ligamentous injury is occasionally possible. The integrity of the medial collateral ligament can be tested by placing the shoulder against the lateral aspect of the stifle and abducting the distal limb while palpating the medial aspect of the femoropatellar joint for abnormal widening.

I have never convincingly demonstrated instability as a result of cruciate ligament injury in the horse, but a technique has been described. This involves standing behind the horse with arms brought around the suspect limb and the hands clasped together at the proximal end of the tibia. The clinician's knee is placed in close contact with the plantar aspect of the calcaneus and his/her toe is placed behind the bulbs of the horse's heel. The tibia is pulled sharply caudally and released to allow it to return cranially while feeling for laxity and crepitation. Clearly the temperament of the horse should always be taken into consideration before determining whether it is safe to attempt such a manoeuvre.

Examination of the pelvis and hip

The hip joint is the most deeply buried of the limb joints and the least amenable to direct physical examination. The correct alignment of its components can be evaluated to some extent by checking that the relationships between the greater trochanter, tuber coxae, tuber sacrale and tuber ischii are symmetrical. Hip problems tend to result in outward rotation of the affected limb.

As in the forelimb, muscle wasting as a consequence of hindlimb lameness is usually most noticeable proximally, particularly over the gluteal region, regardless of the site of the problem. The thigh muscles may also show evidence of a loss of bulk as a consequence of persistent or severe lameness.

The pelvis can be evaluated by a combination of inspection and internal palpation of the bony landmarks by rectal examination. In the case of suspected pelvic fractures, rectal examination while the animal is walked slowly forward or rocked from side to side is often helpful in revealing crepitus and movement of bone fragments. Rectal examination also enables assessment of the central alignment of the sacrum and caudal lumbar vertebrae, along with their overlying muscles, and the presence and character of the pulse in the terminal aorta and iliac arteries.

Iliac or aortoiliac thrombosis is an uncommon occlusive condition of the terminal aorta, which is diagnosed by rectal examination. The lesion is associated with ischaemia of one or both hindlimbs and is seen as a lameness or apparent weakness of one or both hindlegs at exercise. In severe cases there may be an acute onset at exercise with severe pain and recumbency. The affected limb(s) feels cold distally and there is reduced pulsation of the common digital artery. When the aortic pulse is detected per rectum and followed caudally with the fingertips, a firm irregular enlargement is felt at the termination of the aorta where it branches into the internal and external iliacs. The enlargement may be unilateral or bilateral (consistent with one or both hindlegs being affected) and of reduced or unappreciable pulse volume. Ultrasonography of the terminal aorta and its iliac branches is a more sensitive diagnostic technique than rectal examination (see 'Ultrasound of peripheral vasculature' in Ch. 9: 'Cardiovascular diseases').

NB: The immediate and much more usual differential for hindlimb pain at exercise is *exertional rhabdomyolysis* (see below under 'Myopathies').

The back

The equine back is an area where objective physical examination remains difficult. Inspection will reveal asymmetries due to swelling, muscle atrophy and curvature deformities. Palpation may be helpful in assessing the presence of pain and muscle spasm – however, it is often difficult to be certain of the significance of mild or moderate responses in relation to the presenting complaint. Some pointers to a diagnostic approach are given below.

History

Clinical signs associated with chronic back pain are extremely varied, the most consistent being a loss of the horse's performance or ability to jump. This may coincide with a distinct alteration of behaviour or temperament, e.g. uncharacteristic resentment of rugging up, grooming, or picking up of the hindlegs. Unfortunately, there is a tendency among owners to seize upon 'a back problem' as a cause of poor performance that may more correctly be attributed to problems of chronic low-grade lameness, schooling or horsemanship. A detailed history of the horse's management, its tack, performance and temperament must also be considered.

Examination

If possible, the dorsal midline of the back should be viewed from above as the horse is standing square, to check that it is correctly aligned. Some degree of lateral curvature suggests a degree of muscle spasm on one side. The symmetry of the pelvis should be checked from behind and the quarters and back should be inspected for muscle wastage; abnormal findings could be indicative of sacroiliac injury. Where there is damage to the muscle or ligaments of the sacroiliac region, it is usually possible to evoke discomfort by exerting pressure over the tuber coxae and/or over the midline at the lumbar region, and/or over the tuber sacrale.

Pinching over the dorsal spinous processes at the withers normally causes 'dipping' of the spine (ventroflexion), while pinching over the sacral region normally causes an extension (dorsiflexion). Reluctance to comply, or a rigidity of the spine, may reflect some underlying back pain. Similarly, pressure with a blunt point over the dorsal spinous processes, caudal sacrum and flank can be used to stimulate spinal flexion away from the stimulus, enabling assessment of the animal's willingness and ability to carry out this movement.

Firm stroking of the longissimus dorsi with a blunt point normally produces lateral flexion of the thoracic and lumbar spine. Resentment suggests painful muscle involvement, but if it is detected on both sides then it could reflect pain in the vertebral column of the mid-back.

In a straight-line walk and trot, back pain can produce a restricted hindlimb action with poor hock flexion and a tendency to drag the toes of one or both hindlimbs. Sharp turning to flex the spine will be resented or appear difficult with clumsy, jerky movements. Backing up is also resented.

Radiography

Radiography of the equine back requires powerful equipment and is associated with considerable radi-

ation scatter. Its use is limited to centres with powerful fixed radiography equipment.

Haematology and biochemistry

Clinical pathology is generally non-specific but serves to rule out other causes of reduced performance (e.g. anaemia, intercurrent infection, chronic rhabdomyolysis).

Evaluation of the gait

Observation

Sophisticated technologies are now available in some centres for the detailed and objective analysis of the gait of the moving horse, including force plates to quantify ground reaction forces and videographic recording of limb and body movements. However, these have yet to find their way into routine use and the clinician's observation of movement remains the mainstay of gait evaluation. The aims should be to identify:

- The presence or absence of a gait abnormality
- The limb or limbs involved
- The character of any abnormality present
- The degree of abnormality.

The gait is usually best evaluated on a hard, level surface. Ideally this should be done in a safe, enclosed area, free of distractions and dangers such as traffic and other horses. The horse should be restrained with a head collar or bridle and bit, and allowed 30–50 cm of head rope to permit sufficient freedom of head movement.

Abnormalities of gait are usually most apparent when the horse is moving at the walk and slow trot. Variations in foot placement and limb movement, e.g. shortening of one phase of the stride in one limb, are generally easiest to appreciate at the walk when limb movement is slower. The abnormalities in head and hindquarter movement resulting from pain during weightbearing are usually most apparent at the slow trot. The horse should be observed moving in a straight line at an even pace from in front, from both sides, and behind.

Lunging the horse in tight circles is a helpful way of demonstrating more clearly lamenesses that are subtle or inapparent when the horse is moving in

13

a straight line. If circumstances do not permit such an examination, trotting the horse around sharp corners may accentuate lameness in a similar although more transient way.

Horses with forelimb lameness owing to pain on weightbearing shift the distribution of weight from the affected limb across to the contralateral forelimb and back to the hindlimbs. This is achieved, at least in part, by raising the head and neck as the lame forelimb takes weight, the head being lowered again as the sound forelimb strikes the ground. This downward nodding of the head as the sound forelimb strikes the ground is generally the easiest abnormality of movement to appreciate and allows identification of the lame (or lamer) forelimb. The sound limb may also be heard to strike the ground with greater force, particularly if the horse is shod.

With hindlimb lameness the gluteal region and tuber coxae on the affected side will rise and fall through a greater range of vertical motion than those of the sound limb. This is often most easily appreciated as a 'hiking up' of the gluteal region on the affected side during weightbearing on that limb.

Several abnormalities of limb movement may also be appreciable in the lame horse:

- Alteration in the relative lengths of phases of the stride. The cranial phase of the stride is that part which occurs in front of the footprint of the contralateral limb, while the caudal phase occurs behind it. If the animal is moving in a straight line the overall stride length in a pair of contralateral limbs must be symmetrical, therefore a reduced cranial phase must always be accompanied by an increased caudal phase. Overall reductions in stride length frequently accompany bilateral orthopaedic conditions leading to a 'pottery' or restricted gait
- Alteration in the arc of foot flight. Lowering of the arc of foot flight may occur as a compensation to reduce impact when the foot lands, or to reduce limb flexion during protraction. If severe it may lead to dragging of the toes. Exaggerated elevation of a foot owing to hyperflexion of the limb joints is occasionally seen, e.g. in 'stringhalt'

• Variations in the path of foot flight and in foot placement. These may occur for similar reasons to those outlined for alterations in the arc of foot flight (above). The foot may be swung medially or laterally during protraction of the limb. The foot may land asymmetrically, contacting the ground first at the toe, heel or on one or other side.

There is tremendous variation in the pattern of foot flight between different horses, some of which can be related to breed and conformation. Bilateral and symmetrical deviations from the ideal, which are unassociated with other evidence of lameness, may not be of clinical significance, e.g. some horses seem to drag the hind toes regularly during protraction without experiencing other evidence of hindlimb dysfunction.

Records of lameness evaluation should contain some assessment of the severity of the problem. This should be recognized as an essentially subjective exercise as spontaneous variations may occur with time, exercise and ground surface. Nevertheless, it can be helpful in communicating findings to other clinicians and when re-evaluating cases. A variety of scoring systems are employed, e.g. 0–5, or 1/10th to 10/10ths lame, with higher scores representing more severe grades of lameness.

Provocative tests

Provocative tests may be used for three basic reasons:

- To demonstrate occult lameness in a horse that appears 'sound' on initial gait evaluation
- To exacerbate a mild lameness
- As an aid to localization of the abnormality causing the lameness.

Various manoeuvres are employed of which the most common are *flexion tests*. A flexion test is performed by holding the joint under consideration in a firmly flexed position for a time – usually about a minute. Once the limb is released, the horse is observed during immediate movement, usually at the trot, to note any change in gait compared with that seen before undertaking the test. Limitations to the technique include the following:

- It is often difficult to flex a single joint in isolation, particularly in the hindlimb, with a resulting lack in specificity of the response
- The response is not necessarily consistent; the same test performed on several different occasions within a single examination may yield varying results
- There are no hard and fast criteria for determining what constitutes a 'positive' response
- Both false-positive and false-negative results may be obtained. Flexion of an abnormal joint may not noticeably influence the gait and, conversely, flexion of a normal joint may produce some degree of lameness when the horse is trotted off, particularly if a severe degree of flexion is maintained for a prolonged period of time.

To reduce these problems it is important to standardize the technique employed and apply caution to the interpretation of results.

Standardization of the flexion test

- Always flex joints for the same period of time; 1 minute is generally considered a satisfactory period.
- Fully flex the joint concerned and hold it in this position with firm pressure. Standardizing the force employed is obviously more difficult than standardizing the time, but it should at least be consistent for each examination performed on one individual.
- Explain to the handler that the horse must be trotted away as soon as the limb is put down and at the same speed as it was moving when the gait was initially evaluated.
- The horse should be trotted away for at least 20–30 m, turned and trotted back again, past the observer, so that the degree and persistence of any response can be fully evaluated.
- Ensure that the response to any individual flexion test has fully subsided before proceeding to perform similar tests on other joints or limbs.

- Perform equivalent tests on the contralateral limb to allow comparison of the response.
- If possible, repeat positive tests to check that the result is consistent.

Interpretation of flexion tests

It is impossible to lay down incontrovertible rules regarding what constitutes a positive response to a flexion test. It should be interpreted in the light of other information about the horse and it is wise to avoid using it as the sole basis for a firm diagnosis.

In general terms the more severe, persistent and consistent the response to a test the more likely the result is to be significant. The extent of the response therefore influences the weight given to it in the final analysis of the results of a lameness investigation. It is important to remember that several joints will be flexed when performing a flexion test and that a positive response may therefore be due to a lesion in one of a number of locations. Trying to eliminate the response to a flexion test by using local analgesia can be helpful in localizing a source of lameness. However, it does presuppose that the response to flexion is not going to change spontaneously with time and repetition.

Extension tests

Extension tests can be performed in a similar manner. The most commonly employed involves extension of the distal limb joints by standing the horse with the toe of the suspect limb elevated on a small wooden wedge and the contralateral limb raised for one minute, followed by trotting the horse off.

Pressure tests

The effect of pressure on a particular area can also be evaluated, e.g. digital pressure on a suspect splint lesion, or frog pressure applied by standing the horse with the frog on the handle of a shoeing hammer. The limitations outlined for flexion tests apply equally to these other provocative tests.

II. LOCAL ANALGESIA

General considerations in local analgesia

'Nerve blocking' is time-consuming, invasive, sometimes hazardous and reliant on subjective evaluation of gait for its interpretation. Despite these disadvantages, in many lame horses it remains the only way to try to answer the question: 'Where does it hurt?'.

Local anaesthetic solutions can be used in a variety of ways to localize the source of pain responsible for lameness. The technique depends on accurate placement of the anaesthetic solution into or around the structure to be anaesthetized, followed by evaluation of its effect on gait.

The results of local analgesia are most easily and reliably interpreted when the horse is showing an adequate and consistent degree of lameness in the first instance. Interpretation is more difficult and less reliable if the initial lameness is very slight or inconsistent. Techniques for accentuating the degree of lameness, e.g. lunging the horse in tight circles on hard ground, have been described previously. In the case of chronic low-grade lameness, it may be helpful to try to exacerbate the problem by exercising the horse for a few days to make the lameness more apparent, prior to the use of local analgesia.

Conversely, caution should be exercised with the use of local analgesia in acute lameness if there is a possibility that the cause may be an injury that could be exacerbated by the abolition of pain under local anaesthesia. An example would be an animal with an undisplaced fracture where the fracture line may extend and displace with the increased weightbearing promoted by relieving the associated pain. Initial radiographic and/or scintigraphic examination (see later) may be prudent in such cases.

Local anaesthetics may be used for:

- Perineural infiltration around specific nerves to desensitize the regions of the limb supplied by that nerve
- Intrasynovial analgesia of joints, tendon sheaths or bursae

Local infiltration around suspect superficial lesions.

Perineural analgesia – general considerations

Perineural analgesia in the diagnosis of lameness is confined to the distal limb, i.e. below the elbow and stifle. The peripheral nerves in the distal limb are largely sensory since the muscles supplied by motor nerves are located proximally. In general, blocking of nerve conduction at these more distal levels does not significantly interfere with the horse's ability to move the limb normally.

Materials

A number of different local anaesthetic solutions can be used for perineural analgesia. It has been suggested that mepivacaine and prilocaine cause less inflammatory reaction than lidocaine and they are commonly employed for this reason. *Solutions* without adrenaline (epinephrine), corticosteroid, antibiotic or other additivies should be used.

It is advisable to use fresh bottles of sterile solution for each examination, although not necessarily for each block. Fresh sterile needles should be used for each injection site. The needle diameter and the length required vary depending upon the block to be performed; my preferences are outlined below. Perineural analgesia should ideally be performed in a clean, quiet, well-lit, relatively confined area without bedding (in which displaced or dislodged needles can easily be lost).

Preparation and restraint of the horse

Opinions vary on the necessity for clipping the hair at the site of perineural analgesia. It is undoubtedly possible to clean the site for needle puncture more effectively if the hair is clipped. However, the incidence of infection appears to be low if the hair and underlying skin are simply scrubbed with antiseptic solution and rinsed with surgical spirit prior to injection.

Clipping is recommended in horses with thick coarse hair or 'feather' around the distal limb, as this

allows palpation of the appropriate landmarks for injection. A tail bandage is helpful when performing blocks on the hindlimb to keep the tail hair out of the way. Alternatively, the tail should be held to one side by an assistant.

The degree of restraint necessary will vary depending on the temperament of the horse under examination. The use of a bridle and bit together with a twitch are helpful in uncooperative animals. In very uncooperative horses, short-acting sedation with xylazine or romifidine can be used, but there is always the possibility that the drug will interfere with the subsequent interpretation of the block and they are best avoided if at all possible.

Needle placement

As small a volume of local anaesthetic as will reliably block the nerve is deposited adjacent to it with as much accuracy as possible. The more proximal nerve trunks are thicker and more deeply situated within the limb tissues, making accurate placement more difficult. In general, the volume of local anaesthetic used is therefore greater for the more proximal nerve blocks. For proximal blocks requiring long needles (which are therefore of relatively wide gauge), it is helpful to place a small bleb of local anaesthetic subcutaneously using a fine-gauge needle before positioning the larger needle.

A sterile needle held by the hub is placed rapidly through the skin. The tip is then repositioned to the required site and depth. Once any movement of the horse has ceased, the syringe is attached and injection is made. *The syringe is attached to the needle firmly enough to prevent loss of fluid during injection but loosely enough to enable it to be removed rapidly should the horse move, thus leaving the needle in position*. Excessive resistance to injection usually means that the tip of the needle is buried in dense connective tissue and that repositioning is required.

The limb to be injected may be weightbearing or held in a flexed position by the clinician or an assistant at the time of injection. The exact routine employed is largely a matter of personal preference. Holding the limb flexed for injection gives good control of the limb and this approach is used by me for those distal limb blocks where the nerves themselves can usually be palpated, e.g. the palmar digital block and the abaxial sesamoid block in the forelimb. Injecting with the limb in a weightbearing position has the advantage of tensing the regional soft tissues, which makes identification of landmarks easier and this approach is used by me for all regional blocks proximal to the fetlock. If the limb to be injected is weightbearing, the opposite limb can be held up as a form of restraint. The disadvantage to this approach is that some horses will suddenly withdraw and flex the limb being blocked when the needle is placed through the skin. If the opposite forelimb is raised, the horse may collapse on its carpi. This risk may be reduced by placing the needle through the skin with both limbs on the ground and then lifting the contralateral limb for restraint during injection once the needle is in the correct position.

The peripheral nerves generally run in association with an artery and a vein as a neurovascular bundle. If a needle inadvertently enters a blood vessel on initial placement it should be withdrawn slightly and repositioned (usually a little more caudally). It is also prudent to withdraw on the syringe plunger prior to injection to double-check that the needle is not in a blood vessel.

Distal limb blocks will usually take effect within 5-10 minutes. Because of the increased thickness of proximal nerve trunks, the more proximal blocks take longer to become effective and should generally be allowed at least 20 minutes. The efficacy of distal perineural analgesia can be tested to some extent by evaluating skin sensation distal to the block. This is best done using a blunt point such as a ballpoint pen. The response should be compared with that in the equivalent region of the contralateral limb assuming that this has not been previously blocked. Some horses are very stoical about such stimulation and require quite considerable pressure even on unanaesthetized skin to elicit a response. In contrast, some horses, particularly after multiple nerve blocks, become extremely sensitive to any approach to the distal limb and withdraw the limb before it has even been touched. In these cases it is helpful to shield the horse's eye so that it cannot see the

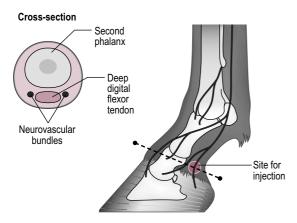


Figure 13.2 Site of palmar digital nerve block.

approach of the examiner and also to approach from the opposite side of the animal.

Perineural analgesia should be carried out in a sequential manner starting distally and working proximally if the lameness is still present. If a specific joint is suspected to be the source of the lameness from the initial physical examination, it may be better to proceed to an intra-articular block of this joint first rather than a regional block proximal to the joint. The intra-articular block will not interfere with subsequent, more distal, regional blocks if the response is negative.

Sites of nerve block in the forelimb

Palmar digital nerve block (Fig. 13.2)

The palmar digital neurovascular bundle is palpable in most horses on the palmarolateral and palmaromedial aspects of the pastern. The nerve is the most palmar structure within the bundle.

The site for injection is subcutaneously on the palmarolateral/medial border of the digital flexor tendons just proximal to the margin of the lateral cartilage. A volume of 1–2 ml local anaesthetic is delivered through a 1-inch 23G (25 0.65 mm) needle. It is usual to block both medial and lateral nerves but they can be done independently if uniaxial foot lesions are suspected as the cause of lameness.

The palmar digital block is traditionally regarded as anaesthetizing the palmar part of the foot.



Figure 13.3 Site of abaxial sesamoid nerve block.

However, while skin desensitization is usually confined to the heels, deep sensation at more dorsal sites within the foot may also be lost since the nerves run forward within the hoof capsule. As a consequence, some cases of distal interphalangeal joint pain and even laminitis and other causes of sole pain will show improvement in response to a palmar digital block.

Abaxial sesamoid nerve block (Fig. 13.3)

The neurovascular bundle is usually palpable with ease where it runs over the abaxial surface of the proximal sesamoid bones, making this technique the easiest regional block to perform.

The site of injection is subcutaneously on the palmar aspect of the neurovascular bundle over the abaxial surface of the proximal sesamoid bones. A volume of 2 ml local anaesthetic is infiltrated on each side using a 1-inch 23G (25 0.65 mm) needle.

Skin sensation is lost over the palmar pastern and the distal dorsal pastern. Deep sensation is lost from the foot and proximal interphalangeal joint. Partial desensitization of the palmar fetlock may occur.

Palmar and palmar metacarpal nerve block (four-point block)

To desensitize the fetlock joint and all structures distal to it, the medial and lateral palmar nerves are

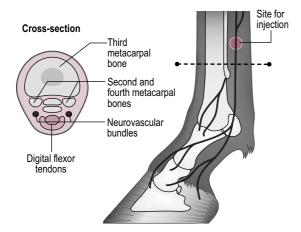


Figure 13.4 Site for blocking the palmar nerves.

usually blocked together with the medial and lateral palmar metacarpal nerves.

Palmar nerves (Fig. 13.4)

The palmar neurovascular bundles run dorsolateral and dorsomedial to the deep digital flexor tendon in the metacarpus.

The site for injection is therefore subcutaneously just dorsal to the deep flexor tendon about 8 cm proximal to the fetlock. A volume of 3 ml local anaesthetic is infiltrated on each side using a 1-inch

23G (25 0.65 mm) needle. In determining the optimal proximal-to-distal level for injection, consideration must be given to the presence of two anatomical structures:

- The digital flexor tendon sheath, which surrounds the digital flexor tendons in the distal quarter of the metacarpus.
- A communicating nerve branch that runs from the medial palmar nerve distally and superficially around the palmar aspect of the flexor tendons to join the lateral palmar nerve. This is usually palpable on the palmar aspect of the superficial digital flexor tendon in the mid-metacarpal region.

The injection sites for the palmar nerves should be above the level of the digital sheath but below the communicating branch.

NB: In the hindlimb the communicating branch is usually situated more distally and is generally

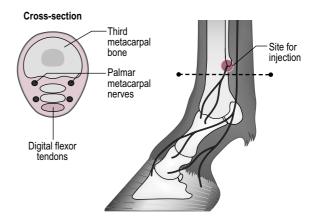


Figure 13.5 Site for blocking the palmar metacarpal nerves.

more difficult to palpate. This leaves less room between the branch and the digital sheath and in this instance the injection may be better made above the communicating branch.

The required number of needle penetrations of the skin can be reduced by injecting around both lateral and medial palmar nerves from the lateral side. This is done by performing the lateral injection as described above and then pushing the needle deeper right across the limb just in front of the deep digital flexor tendon to inject around the medial palmar nerve.

Palmar metacarpal nerves (Fig. 13.5)

The medial and lateral palmar metacarpal nerves are derived from the lateral palmar nerve at the level of the distal carpus and run distally, axial to the second and fourth metacarpal (splint) bones. They emerge from under the distal button of the splint bone to supply the dorsal aspect of the fetlock joint.

The site for injection is subcutaneously just distal to the button of the splint bone on each side. A volume of 2 ml local anaesthetic is infiltrated on each side using a 1-inch 23G (25 0.65 mm) needle.

NB: In the hindlimb the situation is complicated by the presence of medial and lateral dorsal metatarsal nerves (derived from the deep peroneal nerve), which contribute to the innervation of the dorsal aspect of the fetlock. Opinions vary on the necessity for specifically blocking these nerves to achieve ade-

Musculoskeletal diseases

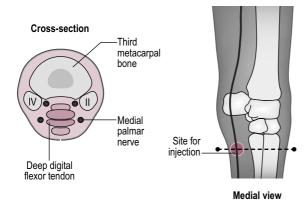


Figure 13.6 Site of medial palmar nerve block.

quate fetlock desensitization. The dorsal metatarsal nerves can be blocked via the same needle placement employed for the plantar metatarsal nerves by redirecting the needle one to two centimetres dorsally just under the skin and injecting a further 2 ml of local anaesthetic.

Subcarpal block

The subcarpal block involves anaesthetizing the medial and lateral palmar nerves and the medial and lateral palmar metacarpal nerves just below the carpus. This will desensitize the structures on the palmar aspect of the metacarpus in addition to the fetlock and digit.

Medial palmar nerve (Fig. 13.6)

The site for injection is on the dorsomedial margin of the deep flexor tendon beneath the carpal fascia just distal to the carpus. A volume of 6–8 ml local anaesthetic is infiltrated at a depth of approximately 1 cm using a 1-inch 20G (25 0.90 mm) needle.

Lateral palmar nerve and palmar metacarpal nerves (Fig. 13.7)

The lateral palmar nerve gives off a deep branch in the proximal metacarpus, which in turn gives rise to the medial and lateral palmar metacarpal nerves. By blocking proximal to this branch the palmar metacarpal nerves are also blocked without the necessity for separate injections.

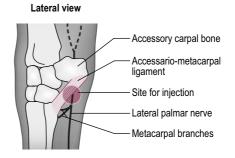


Figure 13.7 Site for blocking the lateral palmar nerve and the palmar metacarpal nerves.

The site for injection is midway along and just distal to the ligament linking the palmar border of the accessory carpal bone to the head of the fourth metacarpal bone (the accessario-metacarpal ligament) and deep to the carpal flexor retinaculum. A volume of 10 ml of local anaesthetic is injected at a depth of 1–2 cm using a 1-inch 20G (25 0.90 mm) needle.

Median and ulnar nerve block

Median and ulnar nerve blocks will desensitize the carpus and structures distal to it. As with tibial and peroneal blocks in the hindlimb, skin desensitization is not complete distal to the block but only affects certain areas (see below). It is therefore advisable to perform these proximal limb blocks on a different occasion from the more distal regional blocks in order to be able to demonstrate that the appropriate skin desensitization has occurred.

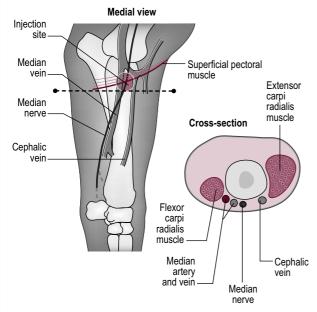
Median nerve (Fig. 13.8)

The site of injection is the caudomedial border of the radius just distal to the superficial pectoral muscle. The nerve lies cranial to the median artery and vein. A volume of 15 ml of local anaesthetic is injected at a depth of 3–4 cm using a 2-inch 19G (51 1.0 mm) needle. NB: Skin desensitization involves only the medial aspect of the pastern.

Ulnar nerve (Fig. 13.9)

The site for injection lies in the groove on the palmar aspect of the antebrachium between the ulnaris lateralis and the flexor carpi ulnaris muscles, 10 cm proximal to the accessory carpal bone, at a depth of

Diagnostic techniques in equine medicine



Site for injection Cross-section Lat Ulnaris muscle Ulnar inerve

Figure 13.9 Site of ulnar nerve block.

Figure 13.8 Site of median nerve block.

1–2 cm. A volume of 10 ml local anaesthetic is injected using a 1-inch 20G (25 0.90 mm) needle. NB: Skin desensitization occurs on the dorsolateral aspect of the proximal metacarpus.

Sites of nerve block in the hindlimb

The distal hindlimb can be desensitized in very much the same way as the forelimb, as outlined above. When dealing with chronic hindlimb lamenesses, many clinicians will start with an abaxial sesamoid block, or even a four/six-point block above the fetlock, as specific chronic foot lameness is generally less common in the hindlimb than in the forelimb. If a positive response is obtained to such a block, more specific regional or intrasynovial blocks distal to this level may be performed on a later occasion. Extra care should always be taken when performing hindlimb blocks owing to the greater potential for injury to the clinician.

Plantar and plantar metatarsal nerve block (four-point block)

Details relevant to this hindlimb block are included under the distal forelimb four-point block (above).

Tibial and peroneal nerve block

Tibial and peroneal blocks will eliminate deep sensation from the hock and structures distal to it. As with median and ulnar blocks in the forelimb, loss of skin sensation is limited to certain areas and may be inconsistent.

Tibial nerve (Fig. 13.10)

The site for injection is just caudal to the deep digital flexor tendon and cranial to the Achilles tendon about 10 cm proximal to the top of the tuber calcis on the medial aspect of the limb beneath the fascia. A volume of 15–20 ml local anaesthetic is deposited at a depth of 1 cm through a 1-inch 20G (25 0.90 mm) needle. Skin sensation is usually lost between the bulbs of the heel.

Peroneal nerve (Fig. 13.11)

The site for injection is between the long and lateral digital extensor tendons on the lateral aspect of the crus, 10 cm proximal to the lateral malleolus. The peroneal nerve has deep and superficial branches. A volume of 15 ml local anaesthetic is loaded into a syringe for delivery through a 2-inch

19G (51 1.0 mm) needle. Ten millilitres is injected around the deep branch at a depth of about 2–3 cm and 5 ml is delivered around the superficial branch during withdrawal of the needle. Skin sensa-

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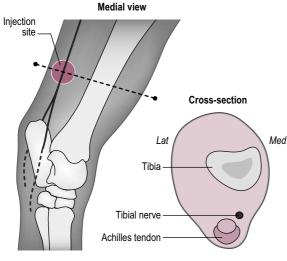


Figure 13.10 Site of tibial nerve block.

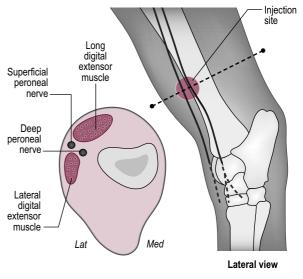


Figure 13.11 Site of peroneal nerve block.

tion is usually lost over the lateral aspect of the distal hock.

Intrasynovial analgesia – general considerations

The general remarks made above in relation to perineural analgesia also apply for the most part to intrasynovial analgesia. However, a few additional points should be borne in mind. The consequences of inadvertent introduction of infection into a synovial cavity can be disastrous and it is therefore extremely important that all practical steps are taken to minimize this risk. The hair at the site of needle placement should, therefore, always be clipped and the skin thoroughly scrubbed with antiseptic solution prior to needle placement. The clinician should wear sterile gloves and fresh bottles of local anaesthetic solution should always be used for each injection.

The most reliable sign that the needle has been accurately placed within the synovial cavity is the appearance of synovial fluid at the hub of the needle. However, this will not always happen immediately for a number of reasons:

• Some of the smaller joints and bursae contain only a very small amount of synovial fluid

(e.g. the navicular bursa or the distal intertarsal joint)

• Synovial villi may be sucked into the end of the needle and effectively prevent any synovial fluid escaping through it.

Twisting or slightly repositioning the needle may help to obtain synovial fluid and thus confirm accurate needle placement. It may also be possible to aspirate yellow synovial fluid into the syringe once a small amount of local anaesthetic has been injected. In some small synovial cavities local anaesthetic (sometimes tinged yellow by the synovial fluid) will reflux spontaneously into the syringe when pressure is taken off the needle, indicating that injection has been made into a closed cavity. In some situations the depth and direction that the needle has penetrated indicate that it must have entered the joint space (e.g. penetration of the tarsometatarsal joint). Other, less satisfactory, indicators of correct placement include the palpable contact of the needle with the cartilage of the articular surface and the lack of resistance to injection of the anaesthetic solution.

There is no reliable way of testing whether an intrasynovial block has taken effectively and thus it is important to be sure that needle placement is

Diagnostic techniques in equine medicine

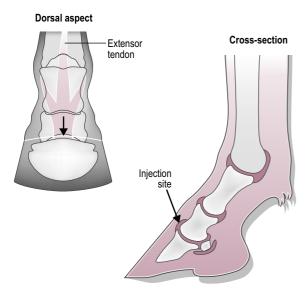


Figure 13.12 Position and direction of needle insertion to anaesthetize the coffin joint.

accurate in the first instance. In those cases where a positive response is seen, the time taken to achieve soundness varies from as little as 5 minutes for small distal joints (such as the distal interphalangeal joint) to up to an hour for large, complex joints such as the stifle.

Intrasynovial analgesia in the forelimb

Distal interphalangeal (coffin) joint (Fig. 13.12)

The site for injection is in the dorsal midline approximately one centimetre proximal to the coronary band with the needle angled slightly more steeply than at right angles to the skin. The needle is advanced through the extensor tendon and correct placement is usually associated with an immediate flow of synovial fluid. A volume of 5–8 ml local anaesthetic is delivered through a 1-inch 20G (25 0.90 mm) needle.

Navicular bursa (Fig. 13.13)

The site for injection is at the midline of the palmar aspect of the pastern between the bulbs of the heel. It is helpful to place a bleb of local anaesthetic sub-

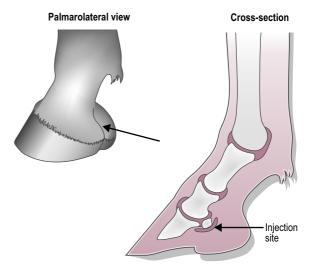


Figure 13.13 Position and direction of needle insertion to anaesthetize the navicular bursa.

cutaneously using a fine needle before positioning a larger needle. A 3.5-inch 19G (90 1.0 mm) disposable spinal needle is aimed to run through the digital cushion to the flexor cortex of the navicular bone. If the site of needle entry is just above the coronary band it is usual to have to aim for the dorsal surface of the hoof wall midway between the coronary band and the toe.

The exact angle at which the needle needs to be inserted varies depending on the foot conformation of the horse. Radiographic guidance may be helpful in determining this – at specialist centres insertion can be monitored periodically by fluoroscopy. In practice, a plain film can be taken prior to needle placement with a small metallic marker taped to the site of intended skin puncture. This allows estimation of the necessary angle of insertion relative to the weightbearing surface of the foot.

Synovial fluid does not often appear at the hub of the needle initially, although passive reflux of local anaesthetic plus fluid usually occurs if pressure is released from the plunger after the injection of 2 ml of local anaesthetic. It is possible to inject a mixture of local anaesthetic and a small amount of a water-soluble, non-ionic, organic iodine contrast material, e.g. iohexol (Omnipaque:



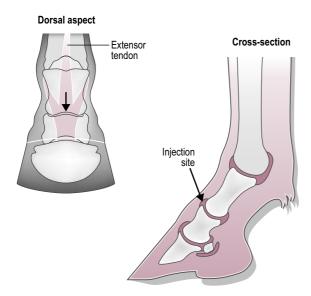


Figure 13.14 Position and direction of needle insertion to anaesthetize the pastern joint.

Nycomed) and subsequently take a further lateromedial radiograph of the foot. This should demonstrate the contrast material within the confines of the navicular bursa if the injection has been made correctly.

Proximal interphalangeal (pastern) joint (Fig. 13.14)

The site for injection lies in the dorsal midline approximately 3 cm proximal to the coronary band with the needle angled slightly more steeply than at right angles to the skin. Firm palpation may reveal the level of the dorsal joint margin and provide a further guide to the level for insertion of the needle. The needle is advanced through the extensor tendon until bone is reached. Synovial fluid usually emerges following successful placement. A volume of 5 ml local anaesthetic is introduced using a 1-inch 20G (25 0.90 mm) needle.

Metacarpophalangeal (fetlock) joint (Fig. 13.15)

I inject the palmar pouch of the fetlock joint with the limb picked up and the joint flexed. The needle

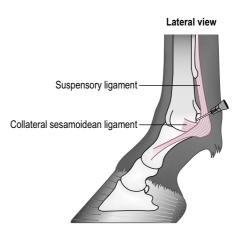


Figure 13.15 Site for anaesthetizing the fetlock joint.

is then placed into the gap created by flexion between the lateral sesamoid bone and the third metacarpal bone. The palmar pouch is often distended in the presence of fetlock joint disease. A volume of 10 ml local anaesthetic is introduced through a 1-inch

20G (25 0.90 mm) needle. A dorsal approach to the joint is also possible and is favoured by some clinicians.

Digital tendon sheath

Analgesia is most commonly performed only in the presence of synovial distension of the sheath. The sheath may be injected from palmarolateral or palmaromedial sites just distal to the annular ligament or via any prominently distended part. A volume of 10 ml local anaesthetic is introduced through a 1-inch 20G (25 0.90 mm) needle.

Midcarpal joint (Fig. 13.16)

The site for injection with the carpus flexed lies on the dorsal surface of the joint just lateral to the extensor carpi radialis tendon between the proximal and distal rows of carpal bones. The midcarpal joint usually communicates with the carpometacarpal joint, which will also be anaesthetized by this procedure. A volume of 10 ml local anaesthetic is introduced using a 1-inch 20G (25 0.90 mm) needle.

Diagnostic techniques in equine medicine

Dorsal view of flexed carpus

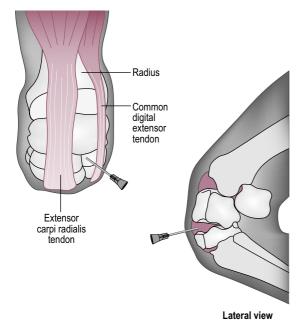


Figure 13.16 Site for anaesthetizing the midcarpal joint.

Antebrachiocarpal joint (Fig. 13.17)

The site for injection with the carpus flexed lies on the dorsal surface of the joint just lateral to the extensor carpi radialis tendon between the distal radius and the proximal row of carpal bones. A volume of 10 ml local anaesthetic is injected using a 1-inch 20G (25 0.90 mm) needle.

Elbow (Fig. 13.18)

The site for injection lies just cranial or caudal to the lateral collateral ligament. The level of the joint space can usually be appreciated with careful palpation. A volume of 15 ml local anaesthetic is injected through a 2-inch 19G (51 1.0 mm) needle.

Shoulder (Fig. 13.19)

The site of needle insertion lies horizontally between the cranial and caudal prominences of the lateral tuberosity of the humerus at 45° to the long axis of the horse. A volume of 20 ml local anaesthetic is

Dorsal view of flexed carpus

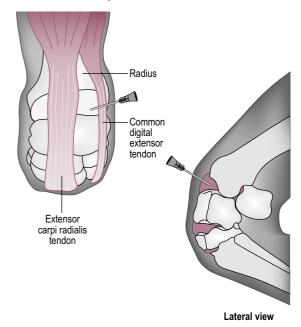


Figure 13.17 Site for anaesthetizing the antebrachiocarpal joint.

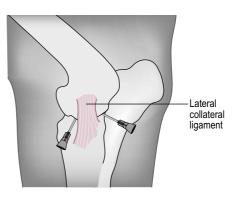


Figure 13.18 Site for anaesthetizing the elbow joint.

introduced through a 3.5-inch 19G (90 1.0 mm) spinal needle.

Intrasynovial analgesia in the hindlimb

Tarsometatarsal joint (Fig. 13.20)

The site for injection lies over the head of the fourth metatarsal bone, between this point and the fourth

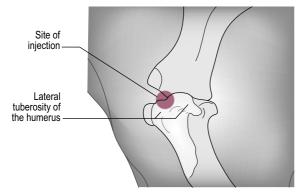


Figure 13.19 Position and direction of needle insertion to anaesthetize the shoulder joint.

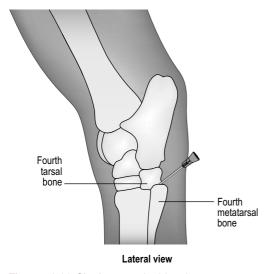


Figure 13.20 Site for anaesthetizing the tarsometatarsal joint.

tarsal bone. A 1-inch 20G (25 0.90 mm) needle is directed at 45° distally and slightly axially. A volume of 5–8 ml local anaesthetic is introduced.

Distal intertarsal joint (Fig. 13.21)

The site for injection lies 1 cm proximal and slightly dorsal to the junction between the head of the second metatarsal bone, the third metatarsal bone and the distal row of tarsal bones. A volume of 5–8 ml local anaesthetic is introduced through a 1-inch 20G (25 0.90 mm) needle. This injection

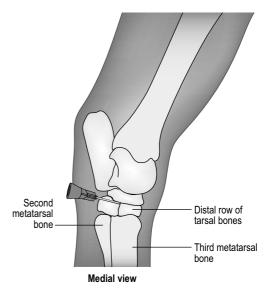


Figure 13.21 Site for anaesthetizing the distal intertarsal joint.

is considerably more technically challenging than the tarsometatarsal joint injection described above, which may serve to anaesthetize both joints as a result of diffusion of the local anaesthetic in many cases.

Tarsocrural joint (Fig. 13.22)

The site for injection is into the dorsomedial pouch of the joint just medial or lateral to the saphenous vein. A volume of 15 ml local anaesthetic is delivered through a 1-inch 20G (25 0.90 mm) needle.

Femoropatellar joint (Fig. 13.23)

The site for injection is either medial or lateral to the middle patellar ligament. The needle is directed inwards and proximally. The femoropatellar joint communicates with the medial femorotibial joint in at least 65% of horses. A volume of 20 ml local anaesthetic is injected through a 2-inch 18G (51 1.2 mm) needle.

An alternative approach involves injection into the lateral cul-de-sac of the femoropatellar joint, just caudal to the lateral patellar ligament and 5 cm proximal to the lateral condyle of the tibia.

13

Diagnostic techniques in equine medicine

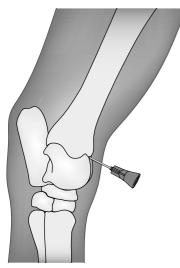
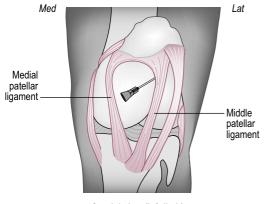




Figure 13.22 Site for anaesthetizing the tarsocrural joint.



Cranial view (left limb)

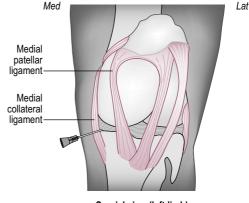


Medial femorotibial joint (Fig. 13.24)

The site for injection lies between the medial patellar ligament and the medial collateral ligament just proximal to the tibial plateau. A volume of 20 ml local anaesthetic is delivered through a 2-inch 18G (51 1.2 mm) needle.

Lateral femorotibial joint (Fig. 13.25)

The site for injection lies between the tendon of origin of the long digital extensor and the lateral collateral



Cranial view (left limb)

Figure 13.24 Site for anaesthetizing the medial femorotibial joint.

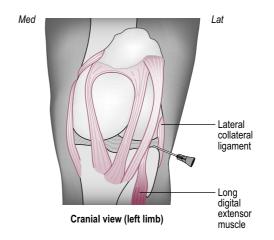


Figure 13.25 Site for anaesthetizing the lateral femorotibial joint.

ligament of the femorotibial joint just proximal to the tibia. A volume of 20 ml local anaesthetic is delivered through a 2-inch 18G (51 1.2 mm) needle.

III. IMAGING TECHNIQUES

Imaging techniques are most usefully employed once the site or sites of abnormality have been definitively established by physical examination and, if necessary, local analgesia. Radiography, ultrasonography and nuclear scintigraphy have found routine clinical application in the horse and magnetic resonance imaging is now being used with increasing frequency.

Radiography

X-ray machines

Modern portable X-ray machines are capable of producing diagnostic images of most of the equine limb, particularly if used in conjunction with rare earth cassette screens for the more proximal regions.

The capacity to produce a reasonably high tube current (e.g. 60 mA) will enable short exposure times to be employed, which will reduce the chance of movement blur. The machine should be easily and quietly manoeuvrable around the patient and the tube head should be capable of going down to the floor, to radiograph feet, and up to the height of the upper cervical spine and head. A light beam diaphragm is essential for accurate collimation of the X-ray beam.

Intensifying screens and film

Rare earth intensifying screens are more sensitive than older calcium tungstate screens, enabling shorter exposure times and extending the capabilities of lower-powered equipment. High-definition rare earth screens should be used for distal limb radiography to produce highly detailed images. Single-screen cassettes with single-emulsion films are available, which will give even better detail, but at the expense of slightly longer exposure times.

Digital imaging

Digital imaging systems remove the need for film and processing equipment and supplies. Images can be manipulated, stored and transmitted as digital files much more easily than on film, and image detail may be improved.

Supplementary equipment

Grids are employed to reduce the effects of scattered radiation on the film. They are most helpful when

radiographing the thicker proximal parts of the horse that produce more scattered radiation, e.g. shoulders, spine and pelvis.

Cassette holders should be employed wherever possible to support the cassette while a film is being exposed. *There is no reason why a cassette need ever be hand-held for radiography of the carpus, hock or more distal limb structures.* For radiography of the feet, cassettes may be supported in wooden boxes which, for certain views, need to be strong enough to support the weight of the horse. Blocks to raise the foot off the ground are also essential, e.g. for lateromedial views of the feet. Cassettes may be placed in a bag supported by a drip stand for radiography of the shoulder, spine or head.

Films should always be permanently labelled with the date, owner and animal identification, limb under examination, projection, and identification of lateral/medial aspects.

Positioning, centring and collimation

The part to be radiographed should be as close as possible to the cassette and parallel to it – thereby avoiding magnification and distortion of the resultant image. The beam should be centred on the area of primary interest – usually at the level of the joint space in the case of joint examination. The beam should be collimated to include only the area of interest and in such a way that it does not extend beyond the margins of the cassette at any point. This not only improves radiation safety but also image quality by reducing the amount of unnecessary scatter.

Exposure factors

Production of an exposure chart or log based on experience with a particular machine and film/ screen combinations is an invaluable aid to the consistent production of high-quality radiographs. As many factors as possible should be standardized, including films, screens, tube-film distance and development procedures and materials. The only variable to take into account should then be patient size.

Preparation for radiography

The degree of restraint that is required will depend upon the temperament of the patient and the projections required. Manual restraint with a head collar or bridle and bit will be sufficient for most horses. Sedation with detomidine or a combination of detomidine and butorphanol will help in more difficult animals. Restraint should be sufficient to enable high-quality films to be produced safely and quickly. In particular, it should be sufficient to eliminate patient movement, which will otherwise degrade film quality and increase the physical and radiation hazards to personnel and subject.

Dirt on the coat and, in particular, the foot will cast confusing shadows on the film. The hair coat over the area of interest should be washed or brushed clean prior to radiography. Prior to radiographing the feet the shoes should be removed, the feet cleaned, superficial horn pared away and the frog sulci packed with a material of soft-tissue density (e.g. soft soap or 'play dough') to even out the density of the soft tissues.

Personnel

All personnel involved in the radiographic examination should wear leaded rubber aprons – other people should be excluded from the area. Staff regularly involved in radiographic examination should wear a monitor badge. Everyone involved should be aware of the procedure to be undertaken and how to achieve the optimal result quickly and safely.

Radiographic projections

Equine bones are relatively thick and dense. This results in difficulty in appreciating subtle abnormalities when they are superimposed on the normal bone mass. Many abnormalities are easiest to see when 'skylined' on the edge of the bone.

A radiograph is a two-dimensional representation of a three-dimensional object. In order that any abnormality may be fully appreciated, it must be imaged from at least two directions. This explains the necessity for using multiple projections in equine limb radiography. In very general terms it is preferable to obtain views from in front and from the side, and at least two additional oblique views taken at 45° when radiographing distal joints.

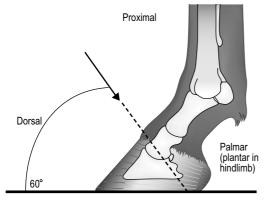
There is no limit to the number of projections that may be obtained of any particular area of the equine limb. The decision as to which are regarded as routine and used in every examination of a particular area and which are used only in specific circumstances remains a matter of clinical judgement and to some extent personal preference. A balance must be struck between the risk of missing or incompletely understanding a lesion by obtaining too few views and the additional time, expense and radiation exposure if every conceivable projection is employed in every case under examination. The following list is therefore not intended to be comprehensive but to act as a guide as to which views might be regarded as routine and what specific additional options are commonly used.

Not infrequently, additional views are obtained once the routine views have been examined. Slight variations in exposure factors or the angle of obliquity are attempted in order to demonstrate more clearly a lesion seen, or suspected, on the initial films. To some extent each radiographic examination is therefore unique and tailor-made for the circumstances surrounding the individual horse and should not be constrained by formal lists of possible views.

Terminology of projections

To avoid imprecision and consequent confusion, a specific nomenclature has been developed to describe radiographic projections. The terms dorsal and palmar (forelimb) or plantar (hindlimb) are used to describe the front and back of the limbs respectively, up to and including the carpus and hock. Above this level the terms cranial and caudal are employed instead. Together with the self-explanatory terms lateral, medial, proximal and distal, this enables a complete description of the projection to be made by outlining the path taken by the X-ray beam as it passes through the animal. The following





Distal

Figure 13.26 Dorso 60° proximal–palmarodistal oblique projection (D60Pr–PaDiO). This can be centred and collimated to view either the navicular bone or the distal phalanx.

terms are applied to projections at different levels of the limbs.

Foot

Lateromedial (LM)

Dorso 60° proximal-palmarodistal oblique (D60Pr-PaDiO) – Figure 13.26

- (a) Centred just above the coronary band and collimated and exposed for the navicular bone
- (b) Centred just below the coronary band and collimated and exposed for the distal phalanx

Palmaroproximal-palmarodistal oblique (PaPr-PaDiO) (flexor view)

Additional views may be:

True dorsopalmar (DPa)

Oblique variations of the D60Pr-PaDiO or DPa

Pastern

Lateromedial (LM)

Dorsopalmar (DPa)

Dorsolateral-palmaromedial oblique (DL-PaMO) - Figure 13.27

Palmarolateral-dorsomedial oblique (PaL-DMO) - Figure 13.27

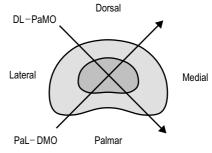


Figure 13.27 Projections of the pastern: dorsolateral– palmaromedial oblique (DL–PaMO) and palmarolateral– dorsomedial oblique (PaL–DMO).

Metacarpo/metatarso-phalangeal (fetlock) joint

Lateromedial (LM)

Dorsopalmar (DPa)

Dorsolateral-palmaromedial oblique (DL-PaMO) Palmarolateral-dorsomedial oblique (PaL-DMO)

Additional views may be:

Flexed lateromedial

Various 'skyline' views of the metacarpal condyles

Metacarpus/metatarsus

Lateromedial (LM) Dorsopalmar (Dpa) Dorsolateral-palmaromedial oblique (DL-PaMO) Palmarolateral-dorsomedial oblique (PaL-DMO)

Carpus

Lateromedial (LM) Dorsopalmar (DPa) Dorsolateral-palmaromedial oblique (DL-PaMO) Palmarolateral-dorsomedial oblique (PaL-DMO) Flexed lateromedial (flexed LM) Dorsoproximal-dorsodistal oblique (skyline) (DPr-DDiO) – Figure 13.28

Elbow

Flexed mediolateral (flexed ML) Craniocaudal (CrCa)

Diagnostic techniques in equine medicine

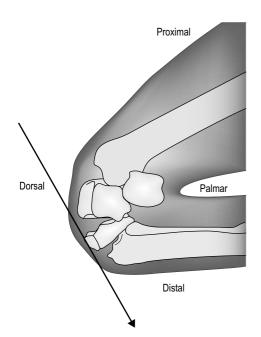


Figure 13.28 Dorsoproximal–dorsodistal oblique (skyline) projection of the carpus (DPr–DDiO).

Shoulder

Extended mediolateral (extended ML) Craniomedial-caudolateral oblique (CrM-CaLO)

Hock

Lateromedial (LM) Dorsoplantar (DPl) Dorsolateral–plantaromedial oblique (DL–PlMO) Plantarolateral–dorsomedial oblique (PlL–DMO) Additional views may be: Flexed plantaroproximal–plantarodistal oblique (PlPr–PlDiO)

Stifle

Lateromedial (LM) Caudocranial (CaCr) Additional views may be: Cranioproximal-craniodistal oblique (CrPr-CrDiO) Oblique variations on the CaCr

Hip

Ventrodorsal

Proximodistal oblique views of the pelvis in the standing horse

Interpretation of radiographs

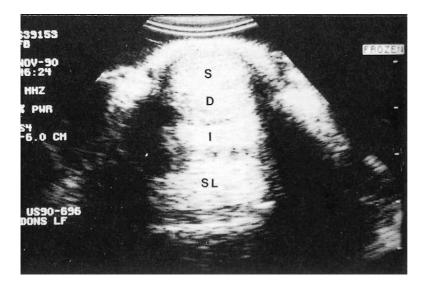
With the increasingly detailed and early clinical evaluation of equine lameness cases has come the realization that quite severe or protracted lameness may be associated with either very subtle radiological changes or no radiological changes at all (e.g. lameness associated with the distal interphalangeal joint). In contrast, very obvious radiological findings such as advanced ossification of the lateral cartilages of the foot may be of no clinical significance. Accurate interpretation involves awareness of normal radiographic anatomy and its variations with age and type of horse as well as the abnormalities that may occur with disease or injury.

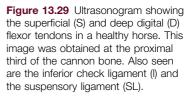
Good conditions and equipment for viewing films help to make the job easier. They should include viewing boxes and a bright light source for evaluation of relatively overexposed parts of the film. It is also a great advantage to have available atlases of normal radiographic anatomy, together with bone specimens, to help resolve uncertainties of interpretation. In the case of unilateral problems, radiographs of the opposite limb may be helpful for comparison.

Ultrasonography

Diagnostic ultrasound provides a useful means of imaging soft tissue structures and is employed in the examination of the equine musculoskeletal system; particularly in the evaluation of flexor tendon and ligamentous injuries. However, the technique is equally applicable to other structures, including muscle and to some extent joints.

The tendons and ligaments most commonly imaged are those on the palmar/plantar aspect of the metacarpus/tarsus. These are fairly superficial structures and are therefore best imaged using a high-frequency transducer to give good image detail – at least 7.5 MHz transducers are most commonly





employed. Small linear array transducers are probably the best option as they give better longitudinal images than sector probes. A 'stand-off' to separate the transducer from the skin is essential when imaging the superficial digital flexor tendon in order to bring it within the focal zone of the transducer.

Restraint of the patient is as described for radiography. Preparation involves clipping the hair over the site to be 'scanned' and thoroughly cleaning the underlying skin. Acoustic coupling gel is then applied to the skin and the transducer to enable sound to travel easily between the two.

When scanning the palmar metacarpus/metatarsus, both transverse and longitudinal images may be produced throughout the region. The individual structures to be identified include the superficial and deep digital flexor tendons, the accessory head of the deep digital flexor tendon (inferior check ligament) and the suspensory ligament (Fig. 13.29).

Each structure should be evaluated for its size, shape, position, echogenicity and margination. Slight alterations in the angle or pressure with which the transducer is held against the skin can markedly alter the image. In consequence, attempts should be made at each level to optimally image each structure in turn as it is difficult to produce an optimal image of all of them at once. If possible, hard copy images or a digital archive should be made and kept as permanent records of the images produced and these should be suitably labelled with the animal and owner identification, date, limb and level of the scan.

Any suspect abnormalities should be re-imaged several times to check that the appearance is consistently abnormal. 'Lesions' that can be made to disappear by altering the angle of the transducer are usually artefactual. As usual, comparison with the contralateral limb is helpful in interpreting images in many cases.

Nuclear scintigraphy

This technique involves the administration of a radioactive nuclide that is conjugated to a compound that becomes preferentially located in a particular body system or lesion. The level of uptake at a particular site can then be determined using a detecting or imaging system that records the radiation emitted from that site at a particular time after administration.

In evaluation of the skeletal system the isotope most commonly employed is technetium-99m. This emits gamma radiation at 140 keV with a half-life of 6 hours. This is usually linked to methylene diphosphonate (Tc99m-MDP), which is taken up into the mineral lattice of bone at a concentration dependent upon the rate of bone turnover at a particular site. Sites that have a high rate of bone turnover for physiological or pathological reasons will therefore accumulate more of the nuclide than normal and this will be reflected in an increase in the amount of gamma radiation being emitted from that particular site.

The radiopharmaceutical is normally administered as an intravenous injection and the scan for bone uptake is performed approximately 3 hours later. Earlier scans can be performed to note the distribution of the isotope in the vascular and soft tissue phases. These earlier examinations are more meaningful if the site of abnormality has been previously determined – for example as a result of nerve blocks. Tc99m-MDP is excreted via the urinary tract, which has consequences for both imaging and radiation safety. Urine within the bladder and when voided contains a high concentration of nuclide.

Scintigraphy is probably most useful in the detection of relatively acute bone injuries that may be difficult to define in other ways in their early stages, e.g. stress fractures. More chronic entities such as degenerative joint disease and enthesiopathies (pathology of muscle and ligament attachments) may also result in variations in uptake, but these may be more subtle and therefore less easy to interpret.

Magnetic resonance imaging (MRI)

Magnetic resonance imaging has been increasingly used over the last 10 years in the imaging of the distal limbs of horses. Both high-field units requiring general anaesthesia and low-field units for standing horses are currently being used. For the limbs, the techniques are principally employed in the imaging of the lower limbs from the carpus and hock distally. MRI allows cross-sectional imaging in multiple planes as well as threedimensional reconstructions. The use of different radiofrequency pulse sequences and T_1 or T_2 weighting allows highlighting of different tissues or pathological changes. Pathological changes that can be determined include bone oedema, necrosis, inflammation, trabecular microdamage and fibrosis, tendon and ligament injuries and cartilage damage. MRI has been particularly useful in studying changes within the equine foot, which is difficult to explore by other means (such as ultrasound) because of the surrounding hoof capsule. Lesions in the bones and soft tissues within the foot, including the deep digital flexor tendon, impar ligament and articular cartilage, which have defied definition in the living animal to date, can now be demonstrated by means of MRI. The limitations remain the necessity for general anaesthesia for high-field systems, limitations on resolution and problems of movement blur in low-field standing systems, the expense of installation and the time needed to obtain and interpret MRI images. Advances in this technology are likely to increase further its applicability to equine lameness diagnosis over the next 10 years.

Diagnostic arthroscopy

Arthroscopy allows direct inspection of synovial cavities, including joints and tendon sheaths. It therefore allows assessment of cartilage surfaces, synovial membranes and intra-articular ligaments; all of which may be difficult to assess fully by other means.

The drawbacks and limitations are firstly that the procedure necessitates general anaesthesia and therefore the usual attendant risks and expense. Secondly, while the range of areas that can be inspected using the arthroscope is constantly expanding, there are still limitations as to the joints that can meaningfully be evaluated and it is rarely possible to examine any joint in its entirety.

Within these limitations the technique can still be useful as a purely diagnostic aid. It is particularly useful in the examination of joints known through the use of intra-articular analgesia to be a site of pain, but where no explanation has been forthcoming using radiography or other imaging techniques (e.g. ligamentous injuries in the carpus or femorotibial joints).

13

IV. LAMINITIS

Laminitis has been mentioned earlier in this chapter but merits a separate section. The acute form represents an emergency that requires immediate diagnosis to enable appropriate treatment; otherwise separation of the laminae predisposes displacement of the pedal bone within the foot. A high percentage of horses with pituitary adenoma (pituitary pars intermedia dysfunction ('equine Cushing's disease')) develop laminitis (see Ch. 5: 'Endocrine diseases').

Signs of acute laminitis

The extent of the injury, and therefore the clinical signs, varies from mild and reversible to severe and progressive. In severe forms there is separation of the pedal bone from the hoof wall. The following signs are collectively diagnostic of the acute case:

- The animal is unwilling to move and may lie down for protracted periods
- The stance may reflect the horse's attempt to throw its weight back on to its heels in order to reduce the pain, i.e. to oppose the separation of the greatest area of laminae at the front of the pedal bone. If only the front feet are affected, the body's weight is thrown back over the hindlegs, producing a characteristic 'backward lean' stance. If all four feet are affected, the weight is transferred to the mid-back region and the stance appears more normal, but the back may be roached
- In progression, the stride and arc of foot movement are reduced. There is a characteristic action in which the heel of an affected foot is always brought to the ground before the toe
- There is a bounding digital pulse palpable over the proximal sesamoids behind the fetlock of each affected foot (Fig. 13.30)
- In early stages there may be heat in the coronet and upper hoof, but this is a variable and unreliable sign.

Within 24–48 hours of acute onset the pedal bone may start to displace within the hoof, giving rise to further dramatic signs:



Figure 13.30 Location of the finger and thumb over the proximal sesamoids of the fetlock to appreciate pulsation of the digital artery.

- If the shape of the coronet at the dorsal wall has changed from a normal rounded bulge to a sunken channel, then it is likely that the pedal bone has displaced, dragging the coronary corium with it. This distal movement of the front of the pedal bone is often termed *founder*. If the coronet is palpably sunken beyond the dorsal wall, around the quarters and heels, then it is likely that there has been a total separation of interlaminar bonds with displacement of the entire pedal bone (*sinker*)
- Extensive laminar exudate may track upwards and break out at the front of the coronet
- A pliability of the sole in front of the point of the frog indicates that the animal's weight on its displaced pedal bone has induced a pressure

necrosis of the soft tissues at its toe, and that it has impinged on the solar horn. In extreme cases the sole is penetrated and the toe of the pedal bone may be visible.

The speed and severity of these changes varies enormously between cases. *In acute cases pedal movement can begin within 24 hours of onset and for this reason the condition constitutes an emergency*. More chronic cases may deteriorate gradually over weeks or months. If the pain of the acute case exceeds 48 hours then lateral radiographs should be taken to assess pedal movement.

Radiography in laminitis

To be of diagnostic use, radiographs must show the relationship of the pedal bone to the hoof wall and sole in order to judge whether it has moved from its normal position. A lateral view of the whole hoof is required with markers indicating the outside of the wall, the relative position of the frog to the base of the pedal bone, and the ground surface.

To achieve this a wooden block is required to raise the foot approximately 8 cm from the ground. A wire is embedded into the bearing surface of the block to provide a radiopaque ground reference.

The sole and frog are trimmed lightly to remove excess or flaking horn. A stiff wire of known length is then used to delineate the front and top of the hoof wall on the radiograph. This is fastened in position using adhesive tape with the top of the wire placed at the point where the wall starts to change from hard to soft horn (Fig. 13.31). Because the wire is of known length, it can be used as a reference for measurements on the radiograph, thus overcoming any artefacts of magnification. In this way the 'displacement distance' (see below) can be measured and compared with subsequent radiographs.

The position of the point of the frog in relation to the base of the pedal bone can be judged by inserting a shortened drawing pin into the frog approximately 2 cm behind its apex. Its position in the foot is marked by a line drawn across the trimmed sole with a felt pen, thus enabling cross reference between the pin image on X-ray and its position in the patient's foot. This is of particular



Figure 13.31 Placement of a wire marker at the front of the hoof prior to radiographic assessment of laminitis.

relevance if it is intended that a 'heart bar' shoe will be fitted as part of the management of the condition.

The radiograph is taken with the leg in a vertical standing position. This usually requires the opposite leg to be raised so that the horse stands straight on the foot under investigation. The beam should be parallel to the top of the block and perpendicular to the axis of the limb.

Interpretation

In the healthy foot the phalanges are in a straight line and the wire marker at the front of the hoof is parallel to the front of the pedal bone. The top of this wire is usually just above the top of the pedal bone at the extensor process (Fig. 13.32).

In pedal displacement there is an increased vertical distance between the top of the wire and the

Musculoskeletal diseases

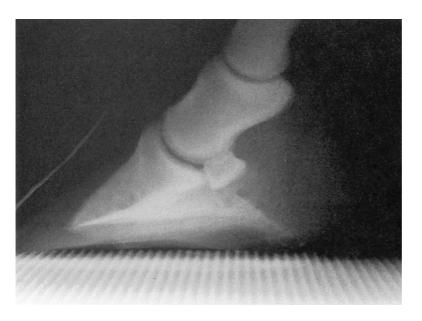


Figure 13.32 Radiograph showing the relationship of the wire marker to the pedal bone in a healthy foot.

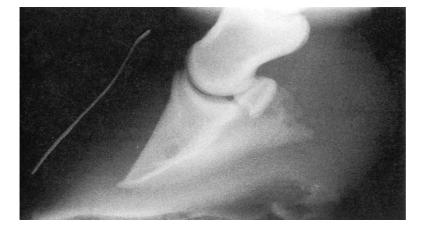


Figure 13.33 Radiograph showing pedal bone displacement. Note that the marker wire is not parallel with the front of the pedal bone and that the extensor process is well below the top of the wire.

extensor process (displacement distance), and the front of the pedal bone is no longer parallel with the marker wire (Fig. 13.33). If the pedal bone is tipped out of straight alignment with the first and second phalanges, it is probably because of the pull of the deep digital flexor tendon – a condition frequently referred to as 'rotation' of the pedal bone. In the extreme case of total pedal displacement, the vertical distance between the top of the wire and the extensor process increases greatly and the toe of

the pedal bone is brought close to the inside of the solar horn and may be seen pressing on to it.

Signs of chronic laminitis

A number of characteristic hoof changes are associated with chronic laminitis:

• There is a disproportionate growth between the heel, quarter and front regions of the coronet. Because the blood supply at the front of the

coronet is usually under pressure from pedal displacement during the acute episode, the subsequent growth of hoof wall at that region is slower than that of the heel

- The disproportionate growth rates cause hoof rings to grow that are divergent at the heel and close together at the toe ('laminitic rings'), unlike the parallel rings seen in the wall of the healthy foot
- Looking at the sole, the white line becomes wider at the toe as a result of the detachment of interlaminar bonds at the front of the foot. This thickening, combined with differential hoof wall growth from the coronet, provokes a 'curling' of the toe in the chronic laminitis patient
- The normal concavity of the sole is lost and it assumes a flattened or even convex shape as a result of chronic pedal displacement
- Radiographs will reveal the various disparities considered in 'Radiography in laminitis' (above).

V. MYOPATHIES

Contributed by Philip Ivens and Richard Piercy

The commonest myopathy encountered in equine practice is exertional rhabdomyolysis (synonyms: 'azoturia', 'tying up', 'set fast' or 'Monday morning disease'). Exertional rhabdomyolysis is a syndrome that may occur in animals with an underlying hereditary predisposition, or it may be an acquired problem – for example, in horses that are physically over-exerted. Underlying myopathies that are sometimes associated with exertional rhabdomyolysis include recurrent exertional rhabdomyolysis, polysaccharide storage myopathy and idiopathic chronic exertional rhabdomyolysis.

Other myopathies or related disorders include: clostridial myositis; post-anaesthetic myopathies; atypical myoglobinuria; hyperkalaemic periodic paralysis; nutritional muscular dystrophy; glycogen branching enzyme deficiency; myotonic dystrophy; mitochondrial myopathy; malignant hyperthermia and immune-mediated myositis.

Acute exertional rhabdomyolysis

The history often includes exercise following a recent reduction in training, or exercise without a concurrent decrease in carbohydrate feeding. The acute onset occurs during exercise (sometimes low-grade) and interferes with the normal gait. The pelvic limbs become stiff, there is considerable pain and the horse may stagger or, in the extreme, collapse. Clinical examination sometimes reveals firm and painful gluteal and lumbar muscles.

Diagnosis

- The clinical signs associated with exercise are highly indicative. The immediate differentials are laminitis, colic, tetanus, vertebral or pelvic fracture associated with a fall, and iliac thrombosis (see above under 'Hindlimb – examination of the pelvis and hip').
- Horses are tachycardic, sweating and often distressed.
- Concentrations of the serum muscle enzymes creatine kinase (CK) and aspartate aminotransferase (AST) are elevated as a result of muscle cell degeneration (see later).
- In severe cases the urine will show a red to dark brown discoloration owing to release of the muscle respiratory pigment myoglobin (myoglobinuria).

Comments

- Serum muscle enzymes are an excellent monitor of recovery, but gentle, consistent and regular exercise can resume when clinical signs have resolved. The horse should be kept on a low plane of nutrition.
- Differential causes of urine discoloration include haematuria and haemoglobinuria. In the case of haematuria, red blood cells will settle (or centrifuge) out of a urine sample, whereas haemoglobin and myoglobin will not. Because haemoglobinuria is invariably a result of haemolysis, a blood sample will show red discoloration of the plasma (haemoglobinaemia) if allowed to settle for a

few minutes. In contrast, horses suffering rhabdomyolysis do not show discoloration of the plasma, despite myoglobinuria.

 Marked myoglobinuria indicates levels of myoglobin in the circulation that are potentially nephrotoxic. If myoglobinuria persists it is wise to check serum urea and creatinine concentrations, and also urine cytology as indicators of renal failure.

Recurrent exertional rhabdomyolysis

Recurrent exertional rhabdomyolysis describes a disease reported in Thoroughbreds with aberrant myofibre calcium regulation. It should not be assumed that the syndrome is common to all Thoroughbreds with intermittent exertional rhabdomyolysis, but the clinical signs and diagnosis are similar to acute exertional rhabdomyolysis. Between episodes, an exercise test (20 minutes of moderate lunged exercise) with pre- and 6-hour post-exercise plasma CK and AST measurement can be indicative. A twofold rise or more in CK activity is supportive of a diagnosis.

Polysaccharide storage myopathy

Polysaccharide storage myopathy is a heritable form of exertional rhabdomyolysis first characterized in Quarter Horses and related breeds and since reported in a variety of other breeds including draught horses, cobs and warmbloods, but not Thoroughbreds. Clinical signs include muscle pain, stiffness, exercise intolerance and a reluctance to move after exercise. Less frequently, gait abnormalities, weakness, back pain and muscle atrophy are seen. Plasma CK and AST activities may be mildly-markedly elevated and an exercise test (see above) may be useful. A polymerase chain reaction (PCR)-based DNA test on hair roots or whole blood in EDTA (University of Minnesota) identifies a dominantly inherited glycogen synthase mutation (GYS1) in many affected horses. However, there is evidence that alternative forms exist and, for these, muscle biopsy is required.

Muscle biopsy is useful for all forms of muscle disorders and for exertional rhabdomyolysis or

polysaccharide storage myopathy the semimembranosus is preferred for surgical biopsy and the middle gluteal for needle biopsy. However, processing and interpretation of muscle histopathology are specialized techniques and for this reason biopsies are best undertaken at specialist centres (see 'Further reading').

Clostridial myositis ('malignant oedema')

This is an acute, severe inflammatory necrosis of muscle that may follow within a few hours of a local intramuscular injection. Contamination of the medication or skin with anaerobic bacteria (often *Clostridium* sp.) is the likely cause. Signs include muscle pain and swelling, gas crepitus on palpation, or shadowing on an ultrasound image. There may be marked dependent oedema and systemic changes characteristic of shock. Diagnosis is based on the history and clinical signs. Gram stain of an aspirate from the affected muscle may reveal spore-forming Gram-positive anaerobic rods.

Post-anaesthetic myopathy

This is an uncommon complication of general anaesthesia. During anaesthesia the weight of the horse's body on its underlying musculature can cause myositis associated with reduced blood perfusion. The postoperative result is a localized muscular swelling and pain, often with disability of the affected limb(s). The clinical findings, associated with recumbency under general anaesthesia and elevated serum muscle enzyme concentrations, are diagnostic.

Atypical myoglobinuria

Atypical myopathy is an acute, acquired form of severe (often fatal) disease that affects grazing horses and foals. It is believed to be caused by an environmental toxin. Most cases occur in the autumn or early winter and several animals in a group may be affected. Clinical signs include muscle weakness, recumbency, myoglobinuria, tachycardia and

sometimes cardiac dysrhythmia. Death occurs within 48 hours in approximately 90% of cases, despite intensive treatment.

Diagnosis

- Serum muscle enzyme concentrations are massively increased and hypertriglyceridaemia is often present.
- Muscle biopsy (see 'Further reading') reveals extensive myonecrosis and sarcoplasmic lipid accumulation in muscles, with a high proportion of type 1 fibres.
- The differential causes of discoloured urine (haematuria and haemoglobinuria) are easily investigated (see above under 'Acute exertional rhabdomyolysis').

Hyperkalaemic periodic paralysis

Hyperkalaemic periodic paralysis is associated with a co-dominantly inherited mutation in the alpha subunit of the skeletal muscle sodium channel. It is seen in certain Quarter Horses and other breeds that are related through their pedigree to the founder stallion 'Impressive'. Clinical signs include intermittent episodes of brief myotonia with fasciculations and weakness, sometimes with respiratory distress from laryngeal paresis. Patients may become recumbent. Serum potassium is usually elevated during an episode and electromyogram (EMG) reveals pseudomyotonic discharges ('dive bomber-type') between and during episodes. Affected horses often have prominent, hypertrophied musculature. Genetic testing on DNA is available in the USA.

Nutritional muscular dystrophy ('white muscle disease')

Nutritional muscular dystrophy is a non-inflammatory degenerative disease of the skeletal and cardiac muscle of ruminants, horses and pigs. It is common in selenium-deficient areas worldwide but can also occur with vitamin E deficiency. Selenium is an essential cofactor for the antioxidant enzyme glutathione peroxidase, which together with vitamin E acts as a biological antioxidant. The deficiency is seen in young or rapidly growing animals. There is sometimes acute cardiac disease that rapidly progresses to cardiovascular collapse, or subacute insidious skeletal muscle disease, characterized by profound muscular weakness.

Diagnosis

- The clinical signs in the young animal are suggestive: failure to suck, weakness, difficulty in standing, falling when exerted. In acute cases tachycardia and hyperpnoea are usual and foals may die of exhaustion associated with heart and circulatory failure.
- Serum muscle enzyme concentrations are elevated, reflecting myopathy in skeletal and cardiac muscles.
- Muscle biopsy (see 'Further reading').
- At post-mortem examination the muscles show generalized pallor or white 'streaking'.
- The glutathione peroxidase activity of whole (heparinized) blood is usually low.

Glycogen branching enzyme deficiency

This is a fatal multisystem autosomal recessive disease that affects Quarter Horse fetuses and neonates, causing abortion and premature death. Signs include recumbency, weakness, seizures, flexural deformities and cardiac failure. Diagnosis is supported by finding leukopenia with elevated liver and muscle enzyme concentrations. Muscle biopsy reveals abnormal amylopectin accumulation and a definitive diagnosis can be achieved by genetic testing of DNA for the *GBE1* mutation.

Myotonic dystrophy

This is a rare condition described in a variety of breeds associated with abnormal contracture of muscles. Affected horses are usually recognized when young; they often have pronounced musculature and may have flexural contractures. Muscle enzyme activities are usually moderately elevated and various endocrine abnormalities may be recognized. Diagnosis is achieved by muscle biopsy and EMG.

Mitochondrial myopathy

This rare condition has been described in an Arabian horse with poor performance and markedly elevated serum lactate concentrations following only mild exercise. Diagnosis is highly specialized and involves measuring muscle mitochondrial enzyme activities.

Malignant hyperthermia

Malignant hyperthermia describes the clinical signs that may be induced by halogenated inhalation anaesthetics, depolarizing skeletal muscle relaxants and, occasionally, stress or exercise. The collective signs are a rapid rise in core body temperature (often above 43°C), profuse sweating, tachycardia, tachypnoea, dysrhythmias, rhabdomyolysis and muscle rigidity, myoglobinuria and often death. A ryanodine receptor (*RYR1*) skeletal muscle calcium release channel mutation has been described in affected Quarter Horses. Diagnosis is based on the clinical signs and a PCR-based DNA test.

Immune-mediated myositis

This relatively rare condition occurs most commonly in Quarter Horses. It is associated with symmetrical muscle atrophy over the back and gluteal musculature and marked elevations in serum CK and AST concentrations. Many horses have a history of previous exposure to *Streptococcus equi* subsp. *equi* infection. Diagnosis is by biopsy of affected muscles.

VI. CLINICAL PATHOLOGY

Haematology

Haematology has limited application in the precise diagnosis of musculoskeletal disease except for the non-specific indicators of inflammation. The total white cell count may be raised in inflammatory conditions and an increase in the plasma fibrinogen or serum amyloid A concentration suggests inflammation or infection.

Serum muscle enzymes

Skeletal and heart muscle cells release enzymes into the circulation following injury. The concentration of these serum muscle enzymes can be measured and in general terms there is good correlation between the severity of injury and the muscle enzyme profile.

The two enzymes most commonly monitored are AST and CK. AST is present in the mitochondria and cytoplasmic fluid of all cells but it is particularly concentrated in liver, heart and skeletal muscle cells. The serum concentration may therefore be raised by many forms of soft tissue injury but is markedly increased following liver, heart or skeletal muscle injury. In contrast, CK is mainly found in heart and skeletal muscle and can be regarded as muscle-specific.

Following hard exercise in a healthy horse there is a physiological increase in the circulating concentrations of both enzymes. AST concentration peaks at about 24–48 hours and returns to normal range within 10–21 days. CK increases to a peak at about 4–6 hours and returns to normal range within 3–4 days. These physiological changes amount to a fourfold increase in the concentration of their upper ranges, but in conditions of myopathy the concentrations increase by a factor of 10 to many thousands. Persistent high concentrations indicate an ongoing myopathy; the enzymes will only return to their normal ranges once the lesion has resolved.

In cases where exertional rhabdomyolysis is suspected as a recurrent problem, serum muscle enzymes should be measured before and after exercise. The exercise regimen should mimic a normal hard day's work for the horse at its current level of fitness. In a healthy horse the samples obtained before exercise and at 6 and 24 hours thereafter should compare with the physiological variations described above. Massive increases that are persistently raised for days afterwards are consistent with rhabdomyolysis.

Selenium and vitamin E

Selenium is an essential mineral nutrient whose metabolism is closely linked with that of vitamin E.

It has been suggested that the function of vitamin E is to prevent the oxidation of selenium. Although a deficiency of selenium has been associated with myopathy in many species, this has not been demonstrated unequivocally in the horse, except in the singular case of nutritional muscular dystrophy – which is a rare condition.

Tocopherol concentrations in the blood and liver provide good information on the vitamin E status of an animal but are difficult to assay and are not commonly undertaken. A deficiency of vitamin E is assumed if the animal is of low selenium status.

Selenium is incorporated into the glutathione peroxidase (GSH-Px) of red blood cells during erythropoiesis. In horses it has been shown that a direct relationship exists between erythrocyte GSH-Px activity and selenium levels in the blood and tissues. Consequently, GSH-Px is a sensitive indicator of dietary selenium intake and/or the response to selenium administration. However, increases in GSH-Px activity as a result of selenium administration will not be detectable until 5–6 weeks later. Whole blood should be submitted in heparin for GSH-Px estimation.

GSH-Px activity in foals reflects the amount of selenium taken up by the mare during pregnancy.

Serology

Brucellosis

Brucellosis in horses is now a very rare disease, probably because of the success in eradicating the causal agent *Brucella abortus* from cattle.

As eradication in cattle proceeded in the UK, there were marked shifts in the clinical manifestations of equine brucellosis. Purulent bursitis of the supraspinous bursa ('fistulous withers') and/or occipital bursa ('poll evil'), gave way to inflammation of the joints and/or tendon sheaths. A systemic form of the disease, similar to that in humans, was also recognized and was characterized by a fluctuating body temperature ('undulant fever'), with a generalized stiffness and lethargy.

Nowadays equine brucellosis is hardly recognized, but it is worth investigating in cases of shifting lameness associated with synovial inflammation and cases of generalized stiffness where other (more likely) causes have been eliminated. In tick areas, patients with these clinical signs should also undergo serological investigation for Lyme disease (see below).

Diagnosis

Serological confirmation of circulating antibody is still undertaken by some commercial veterinary laboratories and, by arrangement, via government laboratories. A positive serum agglutination test (SAT) is indicative but requires confirmation using more sensitive antibody detection systems: the complement fixation test (CFT) and the Coombs' test.

NB: It is possible to obtain positive SAT titres without associated clinical signs. A definitive diagnosis is indicated by a high SAT titre with a confirmatory CFT and Coombs' test, or a rising SAT titre over 3–4 weeks of disease.

Lyme disease (borreliosis)

Lyme disease is caused by the spirochaete *Borrelia burgdorferi*, which is transmitted by a tick vector. The disease is well characterized in humans and dogs, but its importance in horses is still unclear. In the UK a serological survey has indicated that asymptomatic infection in horses is probably quite common, particularly in tick areas, but there is little evidence of associated clinical disease.

In the USA borreliosis has been reported in association with diverse clinical signs such as arthritis, myositis, weight loss and fever. A number of unexplained lamenesses associated with fever and/or tick infestation have also shown positive titres in the UK, but it is difficult to prove disease when so many clinically healthy horses are also seropositive.

In clinical practice it is almost impossible to demonstrate the presence of the organism in cases of suspected disease. Culture is notoriously difficult and although the presence of *B. burgdorferi* DNA can be detected by PCR, this test is not routinely available. Diagnosis therefore relies upon the ambiguity of the serological test. Commercial laboratories offer an enzyme-linked immunosorbent assay (ELISA) test, for which a serum sample is required.

Comments

- A raised titre to *B. burgdorferi* does not necessarily indicate active infection.
- Cross-reacting antibodies produced by other infections may interfere with the specificity of the test.

Synovial fluid samples

The approaches used for arthrocentesis of individual joints for the collection of synovial fluid samples have been described earlier under 'Intrasynovial analgesia'. The remarks on adequate restraint and careful site preparation apply equally to fluid collection as to injection of local anaesthetic. Fluid is aspirated using a sterile needle and syringe.

Gross appearance

Synovial fluid is normally yellow, clear, translucent and viscous. Gross evidence of discoloration (e.g. with blood), opacity or turbidity, low viscosity or clotting would be suggestive of abnormality. Recent trauma or infection will produce marked changes in gross appearance. Other joint diseases, e.g. osteoarthritis, do not generally have a marked effect on the gross appearance of the fluid.

Useful laboratory measurements

Cytology

Total and differential white cell counts are particularly useful; especially in the diagnosis of septic arthritis or tenosynovitis.

Normal: 0.2 10⁹/l; <10% neutrophils, some lymphocytes and mononuclear cells.

Trauma: 0.5–10 10⁹/l; neutrophil ratio increased.

Degenerative joint disease: $0.5-1 \quad 10^{9}$ /l. Infection: >10 10^{9} /l; >90% neutrophils.

Total protein

Normal: 10–20 g/l. Inflammation: 20–40 g/l. Infection: >40 g/l.

Gram stain and culture

Samples for culture should be submitted in sterile containers with a minimal air gap to support anaerobes. A better alternative is to inoculate a commercial culture medium that will sustain both aerobic and anaerobic growth. Sample collection and inoculation must be done under strict aseptic conditions to avoid contamination.

In approximately 50% of septic synovitis cases, no organisms are found in synovial fluid because bacteria are sequestrated in the synovial membrane and/or antibiotics have been administered previously. A higher culture rate can be obtained by using enrichment broths, submitting anaerobic as well as aerobic culture samples, and culturing synovial membrane biopsies in addition to synovial fluid samples.

Markers of cartilage degeneration

The main disadvantage of synovial fluid analysis is that it does not allow for the recognition of cartilage damage. The search for a marker of osteoarthritis has led to the attempt to analyse joint fluid for the presence of cartilage particles after filtration of the synovial fluid sample. Although this has proven to be an inconsistent technique, new and more promising biochemical markers of proteoglycan and collagen breakdown are presently under investigation.

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CHAPTER 14

Neurological diseases

Chapter contents

I. Neurological examination 288 The head and cranial nerves 288 CN I: Olfactory nerve 288 CN II: Optic nerve 288 CN III: Oculomotor nerve 288 CN IV: Trochlear nerve 291 CN V: Trigeminal nerve 291 CN VI: Abducens nerve 291 CN VII: Facial nerve 291 CN VIII: Vestibulocochlear nerve 291 CN IX (Glossopharyngeal nerve)/CN X (Vagus nerve)/CN XI (Spinal accessory nerve) 292 CN XII: Hypoglossal nerve 292 Neck, trunk and limbs 292 Gait assessment 292 II. Specific conditions 293 The brain and cranial nerves 293 Head trauma 293 Space-occupying lesions 293 Meningitis 293 Viral encephalitides 294 Narcolepsy/cataplexy 294 Diffuse cerebral disease 295 Seizures 295 Horner's syndrome 296 Head shaking 296 Dysphagia 296 Vestibular syndrome 297

The spinal cord 297 Cervical vertebral malformation/ stenosis 297 Equine protozoal myeloencephalitis 297 EHV-1 myeloencephalopathy 298 Polyneuritis equi 298 Equine degenerative myeloencephalopathy 299 Ryegrass staggers 299 Generalized neuropathies 299 Equine motor neuron disease 299 Botulism 299 Tetanus 300 Equine grass sickness 300 Localized neuropathies 300 Suprascapular nerve injury 300 Radial nerve injury 301 Peroneal nerve injury 301 Stringhalt 301 Shivering (shivers) 301

III. Practical techniques 301

Cerebrospinal fluid collection 301 Atlanto-occipital puncture 302 Lumbosacral puncture 303 Cerebrospinal fluid analysis 304

Further reading 304

I. NEUROLOGICAL EXAMINATION

The aims of a neurological examination are to determine the presence or absence of neurological disease and, if present, to localize neuroanatomical lesion(s). It should always follow a thorough clinical examination. Information concerning the horse's clinical history and the nature of the complaint should be obtained and comment is often required from the owner/groom as to what is considered normal for an individual animal. Distinguishing horses with primary neurological disease from those showing neurological signs secondary to generalized or systemic disease is important (e.g. signs of hepatic encephalopathy associated with hepatic failure). In most cases, an examination starting at the head and moving caudally is preferable.

The head and cranial nerves

The animal's behaviour should be observed both at rest and when being handled. Any inappropriate or unusual behaviour, especially if repeated, should be noted. Mental status is a feature of both the animal's temperament and state of arousal, but it may be altered by systemic factors (e.g. exhaustion, pain or weakness). Abnormalities of behaviour or mental state (e.g. head pressing, turning or aimless circling) usually relate to cerebral dysfunction.

The cranial nerve examination enables evaluation of the brainstem and afferent (sensory) and efferent (motor) limbs of reflex pathways, and the responses that involve the peripheral cranial nerves. The nerves are numbered according to their anatomical arrangement and it is helpful to evaluate them in a logical manner. The details given below are summarized in Table 14.1.

CN I: Olfactory nerve

This carries sensory afferents associated with smell and deficits are rarely detected. A positive response to a familiar smell such as the owner's hand or a hand containing feed, or a negative response to an unfamiliar smell such as a spirit-soaked swab can be assessed with the horse blindfolded.

CN II: Optic nerve

This carries visual afferents. Observing the animal in its normal environment may assess its function or when negotiating an obstacle course (e.g. straw bales placed in an indoor arena), through which the horse is encouraged to walk. Unilateral deficits can be evaluated by blindfolding one eye. Additionally, the menace response, which is absent in neonates, is evaluated by making a threatening movement towards the eye, but without touching it. Care should be taken to avoid generating air currents that may be sensed by a visually impaired horse. A normal menace response includes abrupt closure of the eyelids and occasionally moving the head away and indicates a functional pathway that includes the ipsilateral refractory media of the eyeball, the retina, the optic disc and chiasm, and primarily the contralateral optic tract, the lateral geniculate nucleus (thalamus), the optic radiation and the cerebral occipital cortex. Modifying pathways involve the cerebellum. The efferent pathway is a branch of the ipsilateral facial nerve (CN VII). The pupillary light reflexes (PLRs) are assessed by shining a bright light into each eye in turn, ideally in a darkened environment. The normal response is a reflex constriction of the ipsilateral and contralateral (consensual) pupil (CN III) - the latter being more difficult to evaluate. Further details, including the full ophthalmic examination, are described in Chapter 15: 'Ocular diseases'.

CN III: Oculomotor nerve

This is responsible for innervation of the pupillary constrictor muscles and extraocular muscles (excluding the dorsal oblique (CN IV) and lateral rectus (CN VI)). A lesion affecting this nerve will not affect vision but usually produces a dilated pupil (mydriasis). In addition, the PLR will be absent when light is directed at the eye on the affected side and the consensual PLR will be absent when light is directed at the contralateral eye. Lesions of CN III result in ventrolateral strabismus. However, mild ventrolateral strabismus is seen commonly when a normal horse's head is elevated.

Table	14.1 Summary of fur	Table 14.1 Summary of functions and tests for cranial nerves	nerves		
CN	Nerve	Function	Clinical test	Normal response	Abnormal response
CN	Olfactory	Smell	Challenge	Horse sniffs	No response
CN II	Optic	Vision	Menace response	Eyelids close	Eyelids do not close
			Pupillary light reflex (PLR)	Pupils constrict	Pupils do not constrict
			Obstacle course	Horse avoids obstacles	Obstacles not avoided
CN	Oculomotor	Pupillary constriction	PLR	Pupils constrict	Affected eye – no response Normal eye – pupil constricts
		Extraocular muscles	Observe ocular position and move head	Normal eye position	Ventrolateral strabismus Mydriasis
CN I<	Trochlear	Dorsal oblique muscle	Observe ocular position	Normal eye position	Dorsomedial strabismus
CN V	Trigeminal	Sensory to the head	Stimulate skin of the head	Behavioural avoidance response	No response
			Palpebral reflex	Blink	No blink
		Motor to the muscles of mastication	Jaw tone and observe muscle symmetry	Resistance to jaw opening	Flaccid jaw and muscle atrophy
CN VI	Abducens	Lateral rectus muscle	Observe ocular position	Normal eye position	Medial strabismus
		Retractor bulbi muscle	Retractor oculi reflex	Eyeball retraction	No response
					Continued

Neurological diseases

Table -	Table 14.1 Summary of fun	functions and tests for cranial nerves-cont'd	nervescont'd		
CN	Nerve	Function	Clinical test	Normal response	Abnormal response
CN VII	Facial	Motor to muscles of facial expression.	Observe facial symmetry	Ear and lip movement	Ear droop, muzzle deviation and inability to blink
		Innervates lacrimal and certain salivary glands	Corneal reflex Palpebral reflex	Blinks Blinks	No response No response
CN VIII	Vestibulocochlear	Auditory	Hand clap	Behavioural response	No response
		Vestibular system	Observe ocular position and move head	Normal eye position	Spontaneous or positional nystagmus. Head tilt
			Blindfolding	Normal gait	Exacerbates vestibular signs
CN IX	Glossopharyngeal	Sensory/motor to the pharynx	Gag or swallowing reflex	Swallows	No swallowing
CN X	Vagus	Sensory/motor to the pharynx and larynx	Gag or swallowing reflex	Swallows	No swallowing
			Laryngeal endoscopy	Anytenoid abduction	Arytenoid paresis
CN XI	Spinal accessory	Motor to certain neck muscles	Palpation of the neck muscles	Normal	Muscle atrophy
CN XII	Hypoglossal	Motor to the tongue	Retract tongue	Retracts and symmetric	Paresis or unilateral atrophy

Diagnostic techniques in equine medicine

Neurological diseases

CN IV: Trochlear nerve

CN IV innervates the dorsal oblique extraocular muscle. Lesions are rare, but may result in strabismus.

CN V: Trigeminal nerve

This provides the sensory innervation for most of the head via its three divisions (mandibular, maxillary and ophthalmic). In addition, the mandibular branch provides the motor innervation for the muscles of mastication. Unilateral abnormalities of motor function may be seen as unilateral muscle wastage of the masseter and temporalis muscles. If a bilateral lesion is present, a dropped jaw and dysphagia may be evident in addition to muscle atrophy. Sensory function may be assessed by pinching the skin over the head in the regions supplied by each of the three branches of the trigeminal nerve. Gentle stimulation will usually produce local twitching of the ears, eyelids or lips, mediated via the facial nerve (CN VII). More sustained stimulation will usually induce a behavioural (withdrawal) response. In addition, assessing the palpebral reflex can test the ophthalmic branch. Light digital pressure just medial to the eyelids should normally result in a closure of the palpebral fissure via the facial nerve (CN VII).

CN VI: Abducens nerve

This is responsible for innervation of the lateral rectus extraocular and retractor bulbi muscles. Dys-function may result in a medial strabismus together with inability to retract the globe and is assessed by placing steady digital pressure through the eyelid on the cornea (sensory CN V), and feeling for reflex retraction.

CN VII: Facial nerve

This nerve supplies motor function to the muscles of facial expression and innervates the lacrimal and certain salivary glands. It therefore controls movements of the eyelids, ears, lips and nostrils and is assessed during the menace response and the palpebral reflex. Facial paralysis is usually recog-

Figure 14.1 This horse with temporohyoid osteopathy had a lesion in the facial nerve (CN VII) causing an ear droop, eyelid paralysis and decreased lacrimation (subpalpebral lavage in place).

nized by drooping of the ear and lips on the affected side with ptosis of the upper eyelid (Fig. 14.1) and a deviation of the muzzle and nares towards the normal side. Inability to close the eyelids, together with reduced or absent tear production, may result in corneal ulceration. If there is only weakness of the lips and a deviated muzzle, without an ear droop and ptosis, there is probably only involvement of the buccal facial nerve branches and a peripheral lesion is more likely (e.g. following trauma).

CN VIII: Vestibulocochlear nerve

CN VIII includes the vestibular (balance) and cochlear (auditory) nerves. Hearing loss, unless bilateral and complete, is difficult to assess. Vestibular disease may present as abnormalities of balance, a head tilt or pathological nystagmus. The eyes are examined for normal vestibular nystagmus when the head is moved from side to side. Spontaneous nystagmus (horizontal, rotary or vertical) is abnormal. In general, disorders of the peripheral vestibular nerve (or inner ear) result in a head tilt (down on the



affected side) and, in acute disease, the fast phase of nystagmus being directed away from the side of the lesion. Ataxia, but not weakness, is usually observed. Signs of vestibular disease may be exacerbated by blindfolding the animal to remove any compensatory visual input, but take care because this may result in the animal falling. Central vestibular disease can result in any form of nystagmus, or nystagmus that alters its form, and may be associated with ataxia and weakness (due to damage to the descending brainstem upper motor neuron pathways). It is important to distinguish a head tilt from a head turn: in the former, the rotation occurs around the axial plane, and the eye and ear on the affected side are lower; head turns result in the head/ neck being turned away from the long axis of the horse (see above).

CN IX (Glossopharyngeal nerve)/ CN X (Vagus nerve)/ CN XI (Spinal accessory nerve)

These nerves collectively provide the sensory and motor input for normal pharyngeal and laryngeal function. Disorders of these nerves therefore result in pharyngeal and laryngeal paralysis. Normal pharyngeal and laryngeal function can be assessed by observing and listening during normal breathing and swallowing, and/or during an (unsedated) endoscopic examination of the pharynx and larynx. Pharyngeal paralysis usually results in signs of dysphagia (nasal return of food; see below), and laryngeal paralysis results in signs of inspiratory dyspnoea. In both cases bilateral involvement is associated with more severe clinical signs.

CN XII: Hypoglossal nerve

This nerve provides motor function to the tongue. The tongue should therefore be evaluated for normal movement, tone and signs of atrophy.

Neck, trunk and limbs

The symmetry of muscle mass and bony landmarks over the body should be assessed. Neck flexibility may be evaluated by encouraging the animal to turn

and lower the head when being offered food. Back flexibility can be examined by looking for adoption of a lordotic stance on stimulating the skin over the epaxial musculature with haemostats. Localized or asymmetrical muscle loss may be caused by neurogenic atrophy caused by a local spinal cord lesion (ventral grey matter), or spinal nerve damage. Clearly delineated regions of cervical and thoracic sweating can be useful indicators of regional loss of sympathetic nerve supply to sweat glands (see 'Horner's syndrome', below) and, similarly, areas of loss of cutaneous sensation can be helpful in localizing lesions. However, cutaneous sensory innervation of the limbs is hard to evaluate, but strength in the thoracic and pelvic limbs can be evaluated by lifting the contralateral limb and, in the thoracic limb, by hopping the horse laterally. The cutaneous trunci (panniculus) reflex can be useful in delineating the cranial extent of a thoracic spinal lesion. This involves stimulating the thoracolumbar sensory dermatomes with haemostats, working in a caudocranial direction and eliciting a twitch in the cutaneous trunci muscle of the flank. Abnormalities are easier to recognize if they are asymmetrical. Total absence of the response may indicate loss of motor function to the cutaneous trunci muscle resulting from a lesion at spinal segments C8-T1. The tail and perineum should be assessed for tail tone and the presence of a normal perineal reflex. The latter involves pricking the skin of the perineum and watching for normal reflex anal sphincter contraction and clamping down of the tail. Loss of tail tone and sensation of the perineum suggests damage to the cauda equina nerves. A rectal examination may be used to assess any abnormal faecal retention or bladder distension, in addition to local pelvic or sacral trauma.

Gait assessment

Gait evaluation is a major part of the routine neurological examination. However, deficits associated with lameness must be differentiated from neurological disease by thorough lameness investigation and, if necessary, with appropriate regional analgesia or administration of analgesic medication. Furthermore, musculoskeletal and neurological disease occurring in the same animal make interpreting diagnostic tests for either more difficult. Gait is evaluated by observing the animal when walking, trotting, circling and backing. Signs of ataxia (erratic limb and foot placement and truncal sway) and paresis (stumbling and toe dragging) are common. In particular, it is helpful to determine whether deficits are symmetrical or asymmetrical and to grade them (scale 0-5) for each limb with '1' being undetectable by an untrained eye and '5' recumbent. Mild neurological deficits (grade 1) may only become apparent with more specific investigations such as walking up and down a slope, walking with the head and neck raised and walking blindfolded. In addition, signs of weakness may be determined by attempting to pull (via the tail) or push the animal off balance while it is standing still or walking forwards.

II. SPECIFIC CONDITIONS

The brain and cranial nerves

Head trauma

If head trauma is witnessed then a direct report of the nature and extent of trauma may be available. In many cases however, head trauma is only suspected, perhaps due to local cutaneous abrasions or haemorrhage (e.g. epistaxis or bleeding from the external ear). Signs vary depending on the site(s) of injury and its severity, but depression and dementia are the most common general signs associated with cerebral damage. Certain particular deficits occur with specific injuries: for example, flipping over backwards and striking the poll may result in visual deficit (usually permanent) caused by optic nerve damage. In addition, vestibular and/or facial nerve signs are frequently caused by basisphenoid bone fracture and damage to the brainstem. Lateral radiography of the head and endoscopy of the guttural pouches is usually diagnostic. Worsening neurological status occurring over several hours since injury suggests oedema and/or local haematoma formation. Cerebrospinal fluid (CSF) analysis may be

helpful in confirming suspected head trauma (see later), but avoid cisterna magna aspirate, since raised intracranial pressure may cause prolapse of the brain into the foramen magnum. Advanced imaging such as magnetic resonance imaging (MRI) and computed tomography (CT) can be useful in defining lesions and planning surgical intervention if indicated.

Space-occupying lesions

Space-occupying lesions produce clinical signs related to damage of adjacent structures. These are most apparent where skeletal structures restrict local expansion of a lesion, as occurs within the cranial vault. The most common causes in horses are haematoma formation following trauma, abscess formation (usually involving Streptococcus equi subsp. equi), cholesterol granuloma and neoplasia. Onset of clinical signs is often acute, despite the insidious nature of the underlying lesion, but signs may also wax and wane. A thorough neurological examination will often allow accurate localization of the lesion and CSF analysis may be informative. Advanced imaging such as MRI and CT are very useful if available. In many cases a definitive diagnosis is reached only at post-mortem examination.

Meningitis

Bacterial meningitis is rare in adult horses, except when infection has been introduced through ascending contamination following direct trauma (e.g. fracture of the cranial vault), or via a fractured petrous temporal bone in temporohyoid osteoarthropathy. Meningitis is more common in young animals, particularly neonatal foals, as a sequel to sepsis. Other causes of meningitis (viral or fungal) are uncommon. The main clinical signs include depression and ataxia/weakness. Stiffness, particularly of the head and neck, hyperaesthesia, muscle fasciculations and seizures are also sometimes observed, but pyrexia is an inconsistent finding. Haematology may reveal neutrophilia and there are generally markedly raised numbers of neutrophils in the CSF. The latter may be turbid with raised protein and decreased glucose concentrations.

Viral encephalitides

Viral encephalitides include rabies virus, Borna virus and the arboviruses. The latter comprise the Togaviridae (eastern/western/Venezuelan equine encephalitis viruses (EEE, WEE, VEE)) and the Fla-viviridae (Japanese encephalitis virus and west Nile virus (WNV)).

Rabies has a worldwide distribution, but some islands such as the UK, Australia and New Zealand are free from the disease. Dogs, foxes, bats, raccoons and skunks are reservoirs and transmit the disease through bites. Clinical signs can vary from muzzle tremors and lethargy to severe ataxia, aggression (e.g. furious form), seizures, colic and lameness. Rabies should be on the differential list of any unexplained central nervous system (CNS) disease, so that care should be exercised when handling possibly rabid animals (wear gloves). Rabies is usually fatal within 5-10 days, but there are no reliable definitive ante-mortem diagnostic tests. CSF of infected animals may be normal but may have increased protein and nucleated cell count, usually consisting of lymphocytic pleomorphic cells. The gold standard diagnostic test is at postmortem examination using an immunofluorescent antibody test (IFAT) and histopathology of the hippocampus.

Togaviridae (EEE, WEE, VEE) are endemic to various parts of North, Central and South America. Bird species are the primary reservoir, with small rodents serving as an additional reservoir for VEE. Mosquitoes transfer virus to equids and humans and therefore the disease occurs in the summer in temperate regions. Some infected animals remain subclinical but, in affected animals, pyrexia occurs initially, followed by variable neurological signs including dullness, ataxia, cortical blindness, propulsive walking and head pressing. Clinical signs are not diagnostic and differentials include bacterial, viral and verminous encephalitides. CSF may show a neutrophilic pleocytosis (in particular with EEE) and an increased protein concentration. Antigenspecific antibody detection in serum or CSF (IgM and IgG) is possible and IFAT is available on brain tissue post-mortem.

West Nile virus (WNV) is endemic to Africa, the Middle East and, since 1999, North America. Sporadic outbreaks in France and Italy have been reported and the disease represents a threat to non-endemic areas, such as the UK, as highlighted by the North American epidemic. WNV replicates in birds and is transmitted by mosquitoes. The disease is therefore seasonal and is seen generally in summer months when vector activity is at its highest. Humans and equids are accidental hosts for the virus. There is widespread subclinical infection in both humans and equids, but some animals show pyrexia, depression, anorexia and sometimes colic without neurological signs. Others develop acute neurological signs that may be progressive. The major hallmarks of WNV encephalomyelitis are muscle fasciculations and changes in behaviour/ mentation. Affected horses may have a slow, stilted, paretic and ataxic gait that may be confused with lameness if asymmetrical. Cranial nerves may also be affected. WNV-infected horses often have an absolute lymphopenia on haematology. CSF usually shows a mononuclear pleocytosis in combination with an increased protein concentration and the colour of the fluid can be mildly xanthochromic. Other differentials to be excluded include other viral encephalitis, equine protozoal myeloencephalitis (see below), equine herpesvirus (EHV)-1, botulism and verminous encephalomyelitis. Serological testing for IgM by the IgM-capture enzyme-linked immunosorbent assay (ELISA) is available in the USA. Infected horses develop a reliable IgM response on exposure, lasting for 6 weeks. The test has a sensitivity of 81% and a specificity of 100% and is not affected by the vaccination status of the horse. A fourfold change in neutralizing antibody titre (e.g. the plaque-reduction neutralization test) is supportive of a diagnosis of WNV, although results are confounded by prior vaccination.

Narcolepsy/cataplexy

This syndrome is characterized by sudden onset of sleep (narcolepsy) and collapse (cataplexy) and by the inappropriate episodic loss of almost all striated muscle tone. Two syndromes are recognized, the first affecting neonates (in particular a familial problem of unknown aetiology in miniature Shetland ponies and Suffolk Punch horses) and the second affecting adults as an acquired condition. Less severe signs, particularly in the acquired condition, include sudden lowering of the head, buckling of the knees and stumbling. Both forms are sometimes initiated by a precipitating circumstance (e.g. grooming, feeding, tacking up or leading out of the stable). Diagnosis is based on the description or observation of an attack. Clinical and neurological examinations should eliminate any other neurological disorder. Differentiating cataplexy from other causes of episodic collapse (such as syncope) is important. Pharmacological testing using physostigmine salicylate as a provocative agent has been advocated, but its reliability is questioned. The test involves the slow intravenous infusion of 0.06–0.08 mg/kg physostigmine salicylate to induce an attack within 10 minutes in an affected horse. Care is needed with the test as the cholinergic effects of physostigmine may induce colic, diarrhoea, bronchospasm and bradycardia. Some cases of adult-onset narcolepsy may be attributable to sleep deprivation, caused for example by chronic pain.

Diffuse cerebral disease

Hepatic encephalopathy (HE) may occur in horses with severe liver disease or complete hepatic failure. Signs may be sudden in onset (e.g. acute hepatic necrosis) or a terminal event in more chronic liver disease such as that caused by the long-term ingestion of pyrrolizidine-alkaloid-containing plants. Clinical signs of HE include dullness, yawning, blindness, ataxia, aimless wandering, bilateral laryngeal paresis, head-pressing and, terminally, manic behaviour and seizures. Although the precise pathophysiology is unclear, gastrointestinal-derived metabolites, such as ammonia, act as false neurotransmitters, augmenting the activity of gammaaminobutyric acid (and other chemicals) in the brain. Diagnosis is based on the exclusion of other encephalopathies, confirming clinicopathological and ultrasonographic evidence of hepatic disease,

and measuring blood ammonia concentration (see Ch. 4: 'Liver diseases').

Non-hepatic hyperammonaemia syndromes: hyperammonaemia resulting in clinical signs similar to those described for HE has been reported in horses with enteric disease (colic and diarrhoea). Idiopathic hyperammonaemia has been reported in Morgan foals at weaning. Hyperammonaemia is also rarely seen in foals with portosystemic shunts: these animals generally have elevated serum bile acid concentrations but normal liver enzymes.

Mouldy corn poisoning (leukoencephalomalacia) occurs in the eastern and midwestern USA. Although it is rare in the UK, it is seen in various other locations worldwide. Clinical signs include abrupt onset of cerebral signs including depression and central blindness. Other cerebral diseases should be excluded, including viral encephalitis, meningitis, cerebral abscessation, hepatic encephalopathy and trauma. The disease is caused by a fumonisin mycotoxin, which may be apparent as a reddish-brown mould on the maize.

Seizures

A seizure is a paroxysmal event that arises as a result of excessive activity of cerebrocortical neurons. Clinical signs vary from mild alterations in consciousness and focal muscle fasciculation (often starting at the muzzle or lips) to recumbency with tonicclonic convulsions and loss of consciousness. There may be a prodromal phase where the horse is restless or appears distracted. Postictal signs include depression and central, but usually transient, blindness. Conditions that may result in seizure activity include the following disorders: developmental (e.g. hydrocephalus); metabolic (e.g. hypoglycaemia); neoplastic; toxic; infectious (e.g. meningitis); iatrogenic (e.g. intracarotid injection); and idiopathic. A familial disorder (with a good prognosis) is described in young Arabian foals. Other causes of collapse, such as cardiac syncope and narcolepsy, should be considered. Diagnosis relies on careful history taking, ruling out other potential causes, MRI or CT, CSF analysis and, if available, electroencephalography.

Horner's syndrome

Horner's syndrome is the collection of clinical signs associated with dysfunction of the sympathetic nerve supply to the head. The neurological pathway starts in the hypothalamus, descends in the cervical spinal cord and exits in the cranial thoracic spinal segments to join the paravertebral sympathetic trunk via the cranial thoracic stellate ganglion. The sympathetic trunk travels cranially in the neck to the cranial cervical ganglion, which is situated in the caudodorsal surface of the medial compartment of the guttural pouch. The classical signs of Horner's syndrome include the triad of ptosis (most obvious by examining the angle of the eye lashes to the cornea), miosis (usually mild) and enophthalmos with associated protrusion of the third eyelid. Sweating as a result of acute sympathetic denervation and occasionally hyperaemia of the nasal and conjunctival mucous membranes may also be seen. The sympathetic supply may be damaged at any level but is often associated with guttural pouch disease or damage to the sympathetic trunk (e.g. perivascular jugular injection) or trauma at the thoracic inlet. Sweating of the head and neck down to the level of C2 is most consistent with guttural pouch disease and a pre- (cranial/cervical) ganglionic lesion or a lesion affecting the cervical sympathetic trunk, whereas postganglionic lesions result in sweating that may only extend to the level of C1. Cervical spinal cord injury or a space-occupying brainstem lesion can cause sympathetic damage with Horner's syndrome and sweating over the whole body on the affected side; however, other neurological signs such as ataxia would also be evident. Brachial plexus trauma and cranial thoracic lesions (abscesses/neoplasia) can also cause preganglionic lower motor neuron Horner's syndrome, with sweating over the whole neck. Equine grass sickness (EGS) can cause signs consistent with bilateral Horner's syndrome and patchy sweating.

Head shaking

Head shaking is the term used to describe a condition involving excessive and spasmodic movements of the head and neck. Although such movements are probably normal if they are only occasional, persistent, repetitive movements are considered abnormal. Signs may be apparent at rest and/or at exercise and in severe cases may render the horse unrideable. Signs include vertical head nodding, horizontal head flicking, rotary head movements and rubbing the nostrils on the ground, the forelegs, or the rider's legs. Increased snorting and a mucoid nasal discharge may also be seen. Signs may be variable but in most cases increase in severity with further exercise. Signs may also be seasonal, occurring during summer months with an apparent respite during the winter, or may vary in severity with the location and the prevailing weather conditions. Diagnosis is based on clinical signs and the exclusion of physical causes of head pain such as sinusitis, dental disease, temporomandibular joint disease, ear or eye inflammatory disease or injury. The aetiology is unknown, but a trigeminal neuralgia triggered by various stimuli, photic head shaking, vasomotor rhinitis or allergic rhinitis has been suggested. Accordingly, further diagnostic investigations have included infraorbital/trigeminal or maxillary nerve blocks, applying a nose net or mask, applying tinted contact lenses and administration of various pharmacological agents such as cyproheptadine and carbamazepine. Results are inconsistent and the cause in most cases is unrecognized.

Dysphagia

Dysphagia is a common clinical sign associated with disorders of prehension, mastication and swallowing. Clinical signs include inability/unwillingness to eat, pain on eating, dropping food while chewing ('quidding'), drooling of saliva/food, nasal return of saliva/food and coughing. There are numerous potential causes that are not primarily neurological disorders, including a foreign body in the tongue, oral ulceration, oesophageal impaction ('choke'), oesophageal stricture, dental disease and cleft palate. Neurological diseases that may cause dysphagia include grass sickness, cranial nerve damage (IX, X) associated with guttural pouch empyema/mycosis, retropharyngeal abscessation (*S. equi*), polyneuritis equi, botulism, tetanus and lead poisoning. Severe cerebral disease (e.g. meningitis, viral encephalitis, HE) and hypoxic ischaemic encephalopathy in neonates may also cause dysphagia. Investigation may include a clinical and neurological examination, oral examination, haematology and biochemistry, radiography of the tongue and larynx, and upper airway and guttural pouch endoscopy (see Appendix 2.1 in Ch. 2: 'Alimentary diseases').

Vestibular syndrome

Vestibular syndrome (head tilt and ataxia, with or without nystagmus) occurs with both peripheral and central vestibular disease (see above), although central accommodation can cause an improvement in signs over several weeks. Peripheral disease is often the result of trauma, bacterial otitis interna/ media in foals (which may follow bacteraemia), polyneuritis equi or temporohyoid osteoarthropathy (THO) in adults. Vestibular disease in THO, often acute in onset, is usually associated with fracture of the petrous temporal bone due to ankylosis of the tympanohyoid joint between the petrous temporal bone and the stylohyoid bone. The facial nerve is commonly also affected. Diagnosis of THO is achieved with guttural pouch endoscopy (looking for evidence of enlargement of the proximal stylohyoid bone) and radiography and/or CT. A CSF aspirate is important to rule out ascending meningitis. Central vestibular disease may be caused by brainstem lesions due to basisphenoid fracture, equine protozoal myeloencephalitis, viral encephalitis or space-occupying lesions. Additional signs, including other cranial nerve signs, weakness and depression (due to reticular formation involvement) are likely. Brainstem auditory evoked responses can be useful in helping to differentiate central from peripheral disease.

The spinal cord

Cervical vertebral malformation/ stenosis (CVM)

Cervical vertebral malformation/stenosis is a wellrecognized cause of 'wobbler syndrome' in horses and is associated with chronic compression of the spinal cord in the neck. Although it is seen in any age and breed, young, fast-growing Thoroughbreds (6 months-2 years) are prone to a dynamic compressive form (type 1), often associated with osteochondrosis lesions elsewhere in the appendicular skeleton. A static form (type 2) typically affects older horses in the caudal cervical region and is caused by osteoarthritic enlargement of the cervical articular processes. Variable symmetrical ataxia and weakness are the common clinical signs, usually with the pelvic limbs being more severely affected than the thoracic limbs. Neck pain may also be present and, not infrequently, the disorder is first recognized after a traumatic episode. Diagnosis is based on clinical signs and exclusion of other differentials of spinal ataxia. Plain (true) lateral cervical radiography and assessment of intra- and intersagittal ratios can be supportive of the diagnosis. Myelography can help determine the site of spinal cord compression (essential if surgery is being considered) but is prone to false-positive and false-negative results. Details of the abnormal radiographic findings and ratio measurement are well reported and beyond the scope of this book (see 'Further reading').

Equine protozoal myeloencephalitis (EPM)

EPM is one of the more common neurological infectious diseases of horses in North America, but the disease may be seen in Europe in imported horses. Most cases are caused by Sarcocystis neurona and less commonly Neospora hughesi, both protozoan apicomplexan parasites. The life cycle is complex: S. neurona's definitive host is the opossum and there are various intermediate hosts including skunks, raccoons and domestic cats. The horse is an aberrant host. Many normal horses in the USA demonstrate serological conversion to S. neurona, indicating prior exposure. There is low seroprevalence to N. hughesi. Localized inflammatory infiltrate within the CNS associated with sarcocysts causes highly variable clinical signs that are dependent on the neuroanatomical location(s) of the parasite. Common clinical signs include localized and often asymmetric muscle atrophy, cranial nerve signs, paresis and/ or ataxia that may be acute or insidious in onset.

Ante-mortem diagnosis of EPM remains difficult and must be considered tentative. A thorough clinical and neurological examination should confirm neurological disease and help rule out musculoskeletal problems. If signs localize to the neck, cervical radiography is indicated to assess possible CVM. CSF cytology and protein concentration, often collected from the lumbosacral site, is usually normal. Immunodiagnostics such as Western blot, immunofluorescence and ELISA can demonstrate antibodies to the parasite. Presence of antibodies in the serum only indicates exposure and not necessarily active disease, but a negative result is helpful in ruling out the disorder. The presence of antibodies in the CSF is more suggestive of infection but is not definitive because false-positive results may be caused by iatrogenic (or other) blood contamination.

EHV-1 myeloencephalopathy

Certain strains of EHV-1 occasionally cause neurological disease. Signs vary in severity but may involve acute onset ataxia and paresis, often beginning in the pelvic limbs and quickly leading to recumbency with the need for euthanasia. There is often tail paresis associated with faecal and urinary retention leading to constipation, bladder distension and dribbling of urine. A short pyrexic episode may precede neurological signs. The disease may occur in outbreaks, especially at breeding establishments, where concurrent signs of respiratory disease, pyrexia and abortion may be present. Diagnosis is based on the clinical signs and ruling out other problems such as sacral trauma, polyneuritis equi and CVM. A high EHV-1 antibody titre in the patient or a rising titre in in-contact animals provides strong supportive evidence of EHV-1 involvement, as does virus isolation from respiratory secretions, CSF or the buffy coat of a heparinized blood sample. Real-time polymerase chain reaction (PCR) on these samples is possible. CSF may be xanthochromic with a raised protein concentration, but often features a normal cell count (see below). Rectal examination combined with nuclear scintigraphy or radiography can be useful in differentiating cases from sacral trauma.

Polyneuritis equi

This condition (formerly known as 'neuritis of the cauda equina') is uncommon and is caused by a progressive immune-mediated lymphocytic infiltration and demyelination of the sacrococcygeal and lumbar nerve roots of the cauda equina. Clinical signs include faecal and urinary retention and overflow, tail rubbing, colic and flaccid paralysis of the tail, anal sphincter and penis. There is usually sensory loss around the perineum (Fig. 14.2), surrounded by a ring of hyperaesthesia. Individual cranial nerve involvement (particularly CN V, CN VII and CN VIII) may also be present. Diagnosis is based on the clinical signs, particularly if cranial nerves are additionally involved. Many affected horses have been shown to have raised circulating antibodies against P2-myelin protein. An ELISA, which provides supporting evidence, can detect these antibodies but the test is not available commercially.



Figure 14.2 View of the perineum of a horse with polyneuritis equi showing an area of perineal analgesia (forceps), loss of anal sphincter tone and faecal retention.

Equine degenerative myeloencephalopathy (EDM)

EDM is a rare condition of young horses (6 months-2 years) primarily reported in the USA but also seen occasionally in Europe. The disease is non-compressive, symmetrical and degenerative, and is associated with tetraparesis, ataxia and spasticity. Typically, the pelvic limbs are significantly more severely affected than the thoracic limbs. There is no muscle atrophy. The aetiology remains obscure; however, a familial tendency is suspected and low serum vitamin E (alpha-tocopherol) concentration has been shown to be a risk factor. The history of a lack of access to fresh pasture, or access to poor quality pasture, and a low plasma vitamin E concentration is supportive. Cervical radiographs should be obtained to evaluate possible CVM. Histopathology at post-mortem is diagnostic and (among other changes) shows neuronal degeneration of white matter tracts throughout the spinal cord and the caudal brainstem.

Ryegrass staggers

Ryegrass staggers is reported in North America (especially the Pacific northwest), Australia, New Zealand and Europe. The disease typically occurs in pastured horses on ryegrass infected with an endophytic fungus that produces a neurotoxic tremorgen (lolitrem). Hot, dry weather with drought, stress and overgrazing are epidemiological risk factors. Muscle tremors are seen, especially of the head and neck, which progress to stiffness, ataxia, hypermetria, opisthotonus and seizures. Multiple animals are often affected and most recover within a week after removal from the pasture. Diagnosis is based on clinical signs and elimination of other causes. Analysis of affected grass for the presence of the endophyte is possible.

Generalized neuropathies

Equine motor neuron disease (EMND)

EMND is an acquired neurodegenerative condition of adult horses that is recognized worldwide. Severe symmetrical and generalized weight loss and weak-

ness occur despite a normal or increased appetite. Muscle tremors and fasciculations are usually seen. Affected animals appear unable to lock their stifles and shift their weight. An abnormal stance (the so-called 'elephant on a tub'), low head carriage, increased periods of recumbency and excessive sweating are often seen and many affected horses have a raised tail head. Ataxia is not a feature and affected horses move better than they stand. In some horses, ophthalmic examination reveals varying degrees of a mosaic pattern of dark brown to yellow pigment (lipofuscin) in the tapetal and non-tapetal fundus. There may be mild-moderate increases in serum muscle enzyme activities. Plasma vitamin E concentration is usually low and oral glucose or xylose absorption may be reduced. Electromyography (EMG) reveals fibrillation potentials and positive sharp waves. Diagnosis is achieved through muscle biopsy of the sacrocaudalis dorsalis medialis muscle, which contains a high proportion of the type 1 fibres that are principally affected (see 'Further reading'). Fresh and 10% formalin-fixed tissue should be submitted to a specialist muscle histopathology laboratory. Positive results indicate changes consistent with denervation muscle atrophy and fibrosis.

Botulism

Botulism is a progressive muscular paresis caused by exotoxins of Clostridium botulinum that prevent acetylcholine release from nerves at the neuromuscular junction. Compared with other species, the horse appears especially sensitive. A 'toxicoinfectious' form is seen in foals, associated with production of toxin by enteric C. botulinum following ingestion of spores, whereas adult horses are usually affected following ingestion of preformed toxin from soil- or animal-carcass-spoilt forage or poorly ensiled hay. With big bale silage, contamination should be suspected if the product is alkaline, mouldy, or exhibiting an ammoniacal smell. A rare form, 'wound botulism', is occasionally seen associated with wound contamination. The severity of the condition is variable and dependent upon the amount of toxin present, but typically the animal presents

with a progressive, muscular weakness with normal mentation and absence of CNS signs. Lingual and pharyngeal paralysis cause dysphagia and a flaccid tongue may loll from the mouth. In mild cases poor tongue tone may be apparent when there is delay or inability in retraction after it is pulled from the mouth. Mydriasis, sluggish PLRs, ptosis, muscle fasciculations, poor tail and anal tone are also seen. In severe cases the muscle paresis leads to recumbency and death follows paralysis of the respiratory muscles. Diagnosis is based on the history and clinical signs while excluding other differentials such as hyperkalaemic periodic paralysis (Quarter Horses), electrolyte abnormalities and grass sickness (in affected areas). A toxin test can be performed on serum, gut contents or suspected feed, but it is rarely successful because of the toxin's labile nature and its usually low concentration.

Tetanus

Tetanus is usually associated with deep puncture wounds with local tissue necrosis and contamination by the spores of Clostridium tetani in the relatively anaerobic conditions that favour their germination. Clostridial spores are abundant in herbivore faeces and in the soil. The vegetative state of the bacterium produces a potent exotoxin that is transported both in the circulation and by retrograde axonal migration to the central nervous system. The toxin prevents neurotransmitter release from inhibitory interneurons in the ventral horn, causing inappropriate increased peripheral motor nerve activity and muscle spasticity. An early sign is dysphagia due to spasm of the masseter muscles ('lockjaw'), as is prolapse (and spasm) of the third eyelid. A stilted, stiff gait with a rigidly extended head and neck is also seen (Fig. 14.3). An anxious expression to the face appears as a result of eyelid retraction, nostril flaring and pricking of the ears. The tail head may be raised. In severe cases the extensor spasm of the limbs becomes more apparent and may lead to recumbency. Tachycardia is commonly observed. Diagnosis is based on the clinical signs and may be supported by evidence of a wound, but the primary source is often not identified. Laboratory detection



Figure 14.3 Typical appearance of a pony with tetanus, showing extensor rigidity of the limbs, raised tail head and extended head and neck.

of either the organism or the toxin is difficult and rarely warranted.

Equine grass sickness (EGS)

EGS is seen commonly in southern Scotland and less commonly in other parts of the UK and northern Europe, South America (*mal seco*), the Falkland Islands and Chile. EGS, classically described as a dysautonomia, is a polyneuropathy of unknown aetiology affecting peripheral (including enteric) and central nervous systems. Clinically the disease may occur in acute, subacute and chronic forms. Clinical signs include dysphagia, generalized ileus, colic, patchy sweating, salivation, ptosis, rhinitis sicca, muscle fasciculations and variable tachycardia (see 'Clinical evaluation of the colic patient' in Ch. 2: 'Alimentary diseases').

Localized neuropathies

Suprascapular nerve injury

Damage to this nerve usually follows injury over the shoulder, most often caused by collision with the side of a doorway or passageway. The lack of collateral support to the shoulder from the denervated supraspinatus muscle results in lateral subluxation of the shoulder when weightbearing. After a few weeks, neurogenic atrophy of the supraspinatus and infraspinatus muscles leads to a prominence of the scapular spine, so-called 'sweeney'.

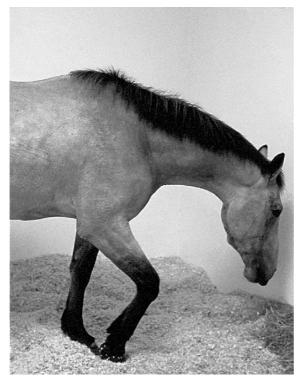


Figure 14.4 'Dropped elbow' stance in a horse with radial nerve dysfunction following prolonged general anaesthesia in lateral recumbency.

Radial nerve injury

Damage to this nerve may accompany a humeral fracture, but it is frequently the most prominent sign associated with brachial plexus avulsion-type injury or following prolonged lateral recumbency on either limb during general anaesthesia. A 'dropped elbow' is characteristic. The limb is often held slightly pro-tracted or maintained with the carpus and fetlock semi-flexed (Fig. 14.4). In the absence of an accompanying orthopaedic injury, manual extension of the carpus will usually not be resented and may allow the horse to bear partial weight.

Peroneal nerve injury

Damage to this nerve may occur in conjunction with a sciatic nerve injury or may follow an injury to the lateral stifle. Peroneal neuropathy may also be seen after prolonged recumbency. There is an inability to flex the hock and extend the digit, so that the horse will often bear weight on the dorsal surface of the hoof and fetlock. The fetlock is usually dragged along the ground when the horse attempts to walk.

Stringhalt

This condition occurs as a sporadic disease in individual horses worldwide, usually affecting one pelvic limb, or as outbreaks (typically bilaterally). The former may follow hock injury and the latter is believed to be associated with the ingestion of certain plants (Australian flatweed). Damage to neurons associated with normal myotactic reflex pathways follows. Typically, there is an acute onset of hyperflexion of one or both pelvic limbs during movement, the animal being normal at rest. The severity of the signs varies and may resolve with time. In extreme cases the pelvic limb may hit the ventral body wall when the horse moves. Diagnosis depends on observing the classic clinical signs and differentiating from other musculoskeletal gait disorders, such as upward fixation of the patella and fibrotic myopathy.

Shivering (shivers)

This is believed to be a manifestation of a reflex hypertonia affecting primarily the flexor muscles of the pelvic limbs similar to stringhalt. The aetiology is unknown. The disease is characterized by involuntary flexion of the pelvic limbs and extension of the tail. The limb may be held flexed and abducted with muscle tremors. Signs may be exacerbated by backing the horse and may be seen in one or both pelvic limbs. Draught horses appear especially prone to this progressive condition.

III. PRACTICAL TECHNIQUES

Cerebrospinal fluid collection

CSF may be obtained via the atlanto-occipital (AO) or lumbosacral (LS) spaces and several factors influence the clinician's choice. For example, a LS tap is

more likely to provide information about diseases caudal to the foramen magnum because of the caudal CSF flow. AO collection requires general anaesthesia in adults. AO samples are usually contraindicated when the clinician considers that intracranial pressure may be raised, as the sudden drop in CSF pressure can cause a fatal herniation of the cerebellum (e.g. following trauma, or as a result of a space-occupying lesion). Rapid clinicopathological analysis with specialized equipment is necessary, as cell morphology quickly deteriorates. If immediate analysis is not possible, the sample can be divided, with one part fixed by adding an equal volume of 50% ethanol. Any lesion that is confined to the grey or white matter of the CNS (i.e. that does not impinge on the subarachnoid space) is unlikely to cause the leakage of pigments or protein, or the exfoliation of cells into the CSF. Space-occupying lesions such as neoplasia, abscessation or haematoma may produce only scant haemorrhage or non-specific abnormal cytology and most toxic and metabolic neurological diseases are associated with normal CSF analysis. However, infectious diseases and trauma of the spinal cord are often associated with increased protein concentrations and cellularity.

Atlanto-occipital puncture

Adults under general anaesthesia or sedation, or foals that are moribund, are positioned in lateral recumbency. Padding should be used to ensure that the long axis of the cervical spine and the head are horizontal and parallel with the ground. The skin over the dorsal aspect of the AO joint should be clipped and surgically prepared and sterile gloves should be worn. The head is held in 90° flexion and a 3 inch 18G (76 1.2 mm) spinal needle with stylet (for adults) or a 1.5 inch 20G (38 0.9 mm) disposable needle with a clear hub and no stylet (for foals) is inserted perpendicular to the long axis of the cervical spine and parallel to the ground through a point in the midline along an imaginary line drawn between the cranial borders of the atlas wings. It is useful to mark these with white tape outside the sterile area (Fig. 14.5). The needle is



Figure 14.5 Landmarks for cerebrospinal fluid collection via the atlanto-occipital space. The tape joins the cranial borders of the wings of the atlas and the rectangular marker identifies the external occipital protuberance.



Figure 14.6 Atlanto-occipital puncture and CSF collection. The neck is flexed by 90° and the needle is inserted perpendicular to the long axis and directed axially, towards the horse's lower lip.

inserted carefully and gradually in the direction of the horse's lower lip to an approximate depth of 3–5 cm in a 450 kg horse, or 1.5–2.5 cm in a foal. Every few millimetres the stylet is removed to check whether the subarachnoid space has been entered (CSF should flow). If not, the stylet is replaced and the needle is advanced a few millimetres further. Occasionally, rotating the needle is helpful. When the needle enters the subarachnoid space, CSF will drip rapidly from the needle as soon as the stylet is removed (Fig. 14.6). If CSF spurts from the needle

Neurological diseases



Figure 14.7 Site of needle insertion for collection of cerebrospinal fluid via the lumbosacral space in a standing horse. The midline point between the tuber sacrale (arrows) is identified.

this indicates increased pressure and the needle should be withdrawn immediately. The sterile stylet is replaced for needle withdrawal. Fluid can be collected into both EDTA and plain containers for cytology, culture and sensitivity and/or biochemistry, respectively.

Lumbosacral puncture

Collection of fluid from the LS space is usually performed in the standing animal, although it can be performed in the recumbent patient. Moderate sedation with an alpha-2-agonist and butorphanol is recommended. With the animal standing squarely, the landmarks are palpated. These include a line drawn over the rump between the caudal borders of the tuber coxae and the point between the cranial aspects of the tuber sacrale in the midline, where there is usually a palpable dip (Fig. 14.7) This is often close to, or at, the highest point of the rump of a standing horse. A 20 20 cm area of skin is clipped and surgically prepared. Sterile gloves should be worn. A bleb of local anaesthetic is inserted in a sterile manner under the skin at this site. A 6-inch

18G (150 1.2 mm) spinal needle with stylet is ideal for most adult horses, although a shorter needle (10 cm) with stylet may be sufficient in a pony. The clinician should stand to one side of the animal and insert the needle with the wrists firmly positioned against the animal's back to prevent any inadvertent



Figure 14.8 Collection of cerebrospinal fluid via the lumbosacral space. Caudal view during insertion of the needle to ensure that a vertical orientation is maintained.

movement of the needle if the patient moves unexpectedly. It is useful to have one assistant standing back from the horse, observing the direction and angle of the needle to ensure that it maintains a vertical position (Fig. 14.8). Beneath the dermis there is usually little resistance to passage of the needle to a depth of 12-13 cm. At this level, penetration of the lumbosacral interarcuate ligament is met with an apparent loss of resistance or a subtle 'pop'. Simultaneous penetration of the dura mater and arachnoid usually produces a mild local response from the horse (movement or tail twitch). Occasionally, more violent movements may be exhibited. If bone is encountered, the needle should be almost fully withdrawn before being angled either cranially or caudally. Occasionally, lateral angulation is required in horses with an unbalanced pelvis. Once the dura has been penetrated the stylet can be withdrawn. CSF rarely flows without the application of slow, steady aspiration using a 5 ml syringe (Fig. 14.9). If no CSF flows, the needle should be gently rotated through



Figure 14.9 Collection of cerebrospinal fluid via the lumbosacral space. Gentle aspiration with a syringe attached to the needle.

90° or repositioned. Occluding both jugular veins may also increase flow (Queckenstedt's phenomenon). Occasionally, several attempts are required. latrogenic (mild) blood contamination is common at the LS site, but whole blood is obtained occasionally when an epidural vein is punctured. Gentle and steady CSF aspiration reduces iatrogenic contamination and breaking the seal on the syringe plunger before the procedure reduces excessive aspiration pressures. Taking several small aliquots (about 2 ml) of sample into successive syringes will ensure that the least contaminated sample can be selected for cytological analysis. For selection of sample tubes see AO puncture, above.

Cerebrospinal fluid analysis

Normal CSF appears clear and colourless. Xanthochromia (a yellow hue) that persists after centrifugation is caused by either red blood cell lysis from subarachnoid haemorrhage, pathological conditions where the protein is high as a result of vasculitis (e.g. from EHV-1 myeloencephalitis), trauma or hyperbilirubinaemia. Mild xanthochromia is a

normal finding in neonates. Free red cells are seen with trauma and iatrogenic haemorrhage - the former is more likely to be the case if erythrocytophagy is observed. A differential white count is performed on cytocentrifuged sediments. Normal CSF contains no blood and low numbers of white cells (<5 WBC/ 1: small unreactive lymphocytes and very occasional monocytes). Any other leukocytes are considered abnormal. The protein concentration is generally less than 0.8 g/l. Increased protein concentration, a non-specific change, is seen in any condition that increases the blood-CSF barrier permeability. Bacterial diseases generally produce a neutrophilia, whereas viral diseases may be associated with a mononuclear or neutrophilic pleocytosis.

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CHAPTER 15

Ocular diseases

Chapter contents

I. Examination techniques 305 Ocular examination protocol 305 Restraint and local anaesthesia 306 Sedation 306 Topical anaesthesia 306 Local nerve blocks 306 Examination in the light 308 Assessment of vision in horses 308 General examination of the eye in the light 309 Examination in the dark 311 Pen light examination 311 Slit lamp biomicroscopy of the anterior segment 312 Indirect and direct ophthalmoscopy 313

II. Supplementary techniques 316

Swabs, scrapes, smears and biopsies 316 Topical ophthalmic stains 317 *Fluorescein* 317 *Rose Bengal* 318 Schirmer tear production test 318 Investigation of nasolacrimal drainage 318 *Visual inspection* 318 *Cannulation/catheterization* 319 *Fluorescein drainage* 319 *Dacryocystorhinography* 319 Tonometry 320 Diagnostic imaging 321 *Radiography* 321 *Ocular ultrasonography* 321

Further reading 322

I. EXAMINATION TECHNIQUES

Ocular examination protocol

This chapter describes the techniques for clinical examination of the eye and the routine sequence in which they should be undertaken. In all cases it is essential to keep accurate and sequential records of ocular findings and this is most simply done using standard annotated forms and diagrams. The eye and adnexa (i.e. the globe, eyelids, nictitans, lacrimal apparatus, orbit and paraorbital areas) are first examined in the light with a focal light source such as a penlight or transilluminator. The lens, vitreous and retina are then examined in the dark. A light source and magnification are necessary, but the examination of the lens, vitreous and retina will also require the use of indirect and direct ophthalmoscopy. These techniques and the associated equipment are available to the non-specialist.

Restraint and local anaesthesia

Examination of the eye and adnexa may require twitch restraint and/or sedation. These can be augmented by the use of local anaesthesia; either topical and/or regional.

Sedation

Active or intractable patients can be sedated with 10–30 µg/kg detomidine hydrochloride (Domosedan: SmithKline Beecham) by slow intravenous injection. Xylazine (Rompun: Bayer) at 0.5–1 mg/kg and romifidine (Sedivet: Boehringer Ingelheim) at 40–100 µg/kg are suitable intravenous alternatives. If pain is prominent the sedative can be combined with the analgesic butorphanol (Torbugesic: C-Vet) given by slow intravenous injection at a dose rate of 25–50 µg/kg. However, head jerking movements associated with the use of butorphanol may make ocular examination and obtaining cytological specimens difficult.

Topical anaesthesia

If the eye is painful, topical anaesthesia may be required to densensitize the corneal and conjunctival surfaces. A topical ophthalmic anaesthetic such as 0.5% proxymetacaine or proparacaine (Ophthaine: Squibb) should be administered via a 1 ml syringe (Fig. 15.1). Alternatively, 1% amethocaine hydrochloride or tetracaine (Minims Amethocaine: Smith & Nephew) may be used.

Local nerve blocks

Auriculopalpebral nerve block

This motor nerve block is used when pronounced blepharospasm, which may be associated with handling or ocular pain, makes examination of the eye difficult. It is also useful when there is a risk of expulsion of the intraocular contents in the presence of a deep corneal ulcer, or because of an existing full-thickness penetrating injury. It prevents upper eyelid movement (akinesia), but slight lower eyelid movement is present. Corneal and conjunctival sensation via the trigeminal nerve is not eliminated and



Figure 15.1 A 1 ml syringe being used to deliver local anaesthetic to the eye. The same technique can be used to deliver other types of liquid medication. Most horses tolerate this method of application, but a small proportion may react violently.

invasive procedures involving the cornea will therefore require topical anaesthesia.

The auriculopalpebral nerve is a branch of the facial nerve, which may be blocked at a number of sites (Fig. 15.2). It originates deep to the parotid gland and passes dorsally to innervate the ear muscles. The palpebral branch passes anteriorly and obliquely over the zygomatic process of the squamous temporal bone and then courses forwards in subcutaneous tissues along the dorsomedial edge of the zygomatic arch towards the upper eyelid. In a small proportion of animals, sedation will be required prior to administering the palpebral nerve block.

Using a 1 inch \times 25G (25 \times 0.7 mm) needle, 5–7 ml (up to 10 ml) of 1% lidocaine hydrochlo-

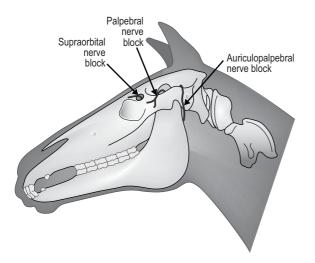


Figure 15.2 The sites for auriculopalpebral, palpebral and supraorbital nerve block.

ride, 2% prilocaine hydrochloride or 2% mepivacaine hydrochloride (Carbocaine) is injected deep to the skin in one of the following sites.

- ٠ Anaesthesia of the auriculopalpebral nerve is achieved by infiltrating local analgesic into the depression formed by the base of the ear cartilage and the vertical ramus of the caudal mandible approximately one inch (2.5 cm) below its highest point (Fig. 15.3). If the eye is particularly painful or the horse is fractious, this is a slightly easier and safer site for injecting local anaesthetic, but it may rarely produce mild reversible facial paralysis. The needle is directed obliquely upwards and inwards towards the highest point of the zygomatic arch. A successful block is indicated by mild ptosis of the upper eyelid, lid akinesia and eversion of the lower eyelid. The ipsilateral ear may also temporarily droop in rare cases.
- *The palpebral branch of the nerve* is palpated and blocked by infiltrating a similar volume of anaesthetic immediately medial and slightly rostral to the highest point of the zygomatic arch, approximately halfway between the eye and the ear (Fig. 15.4). The palpebral nerve is palpable at this point as it crosses the zygomatic arch in a ventromedial direction. The efficacy of



Figure 15.3 The site for auriculopalpebral block at the base of the ear.

the block is indicated within 5–10 minutes by ptosis and a much reduced or absent palpebral reflex (Fig. 15.5).

Supraorbital nerve block

The supraorbital or frontal nerve is a branch of the trigeminal nerve and supplies sensory innervation to the nasal and middle two-thirds of the upper eyelid. The supraorbital block is useful for minor eyelid surgery, including biopsies. It does not provide anaesthesia to the cornea or conjunctiva.

The supraorbital nerve is blocked by injecting 3-5 ml of 1% lidocaine or 2% mepivacaine using a 1-inch × 25G (25×0.7 mm) needle placed through the supraorbital foramen to a depth of



Figure 15.4 The site for palpebral nerve block.

approximately 1 cm (Fig. 15.6). The foramen can be palpated as a depression in the frontal bone on the dorsal aspect of the orbit. The efficacy of the block is indicated by desensitization of the upper lid within 5–10 minutes of injection. This block may also produce some slight motor paralysis of the palpebral branch of the facial nerve. A small blood vessel near the foramen should be avoided if possible.

Examination in the light

Assessment of vision in horses

Complaints concerning the visual capacity or ability of horses present a major diagnostic problem. Vision tests in horses are necessarily empirical. Ophthalmic examination may define ocular lesions, but their significance in terms of the animal's ability to see is often uncertain. There is at present no means of truly assessing vision or assigning a specific level of visual capability or disability unless the horse is completely blind.

The amount of vision as determined by the ability of a horse to negotiate unfamiliar surroundings may

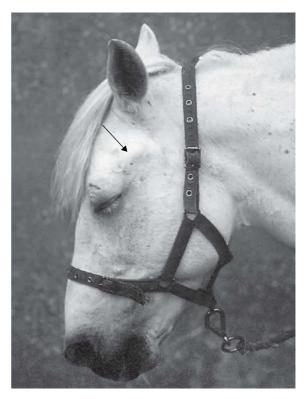


Figure 15.5 Mild ptosis of the upper eyelid and eversion of the lower eyelid denote a successful block of the palpebral nerve. The arrow denotes the site of injection.

be tested in a simple obstacle course with blinkers or a cloth blindfold tucked under one side of the halter, covering each eye alternately. The test should be undertaken in both light and dim conditions. Although this procedure will detect most horses with blindness, the horse's use of nonvisual sensory cues make this a highly unreliable method of estimating the true extent of any visual disability in the horse.

The pupillary light reflex (PLR; direct and indirect) evaluates the integrity of the retina, optic nerve, midbrain, oculomotor nerve, and iris sphincter muscle. The normal equine pupil responds somewhat sluggishly and incompletely unless the stimulating light is particularly bright. Stimulation of one eye normally results in the constriction of both pupils. The PLR is valuable in testing potential retinal function in eyes with severe corneal or lens



Figure 15.6 The site for supraorbital sensory nerve blocks.

opacity, but is not a true test of vision as it is a subcortical reflex (see later).

Making a quick, threatening motion toward the eye to cause a blink response and/or a movement of the head away from the motion tests the menace response. Care must be taken not to create air currents towards the eye, which may stimulate other sensory pathways to cause blinking or shying. A positive menace response requires normal function of both peripheral and central visual pathways and therefore confirms some degree of visual capacity, but it is at best a crude test of vision. A negative menace may be associated with retinal pathology.

Veterinarians should be cautious when giving opinions on the functional consequences of minor ocular pathology. Correlating the degree of functional vision to the amount of anatomic ocular pathology is difficult, as some horses with what appear to be major eye problems function quite well, while other horses with what appear to be minor ocular lesions have severe vision problems.



Figure 15.7 Initial assessment of the eye and adnexa should concentrate on the symmetry of the right and left sides of the face, including the obvious depression of the supraorbital fossa (arrowed), and whether or not any abnormal ocular or nasal discharges are present.

General examination of the eye in the light

Appearance of the eye and adnexa

The general appearance of the eye and the adnexa (globe, eyelids, lacrimal apparatus, orbit and paraorbital areas) should be assessed and the symmetry of each side compared. Abnormal elevations, depressions or deviations of the skull or soft tissues of the head should be evaluated. In particular, it is important to check that the depth of the supraorbital fossa is normal and symmetrical bilaterally (Fig. 15.7). Swelling in this region may indicate a retrobulbar or orbital space-occupying lesion. The quantity and



Figure 15.8 (Plate 14 in colour plate section) Closer naked eye examination of the eye and adnexa should note external details shown here such as the angle of the cilia on the upper eyelid, the dorsal and ventral orbital sulci (which divide the eyelids into tarsal and orbital portions), the position of the third eyelid and caruncle, and the amount of pigmentation present on the eyelids and conjunctiva. The limbus should be clearly defined; note the pigmented rim in this animal. The cornea should be transparent, allowing the fine structure of the iris to be clearly visualized. In this horse the grey line which marks the insertion of the pectinate ligament into Descemet's membrane and the cornea is very obvious laterally, less so medially. The pupil should be an almost symmetrical horizontal ellipse and granula iridica are usually obvious on the dorsal pupillary border, less so on the ventral pupillary border. In order to appreciate internal details beyond the pupil, examination must be continued in the dark.

quality of ocular or nasal discharges should be evaluated for increased or decreased tear production (associated with inflammation), and tear overflow (epiphora) from blockage of the lacrimal drainage system. Purulent discharge may be associated with infection.

The angle of the upper eyelashes with respect to the cornea should be examined. Normally they should be at almost 90° to the cornea (Fig. 15.8 (Plate 14)). Downward deviation may indicate *enophthalmos* (backward displacement of the eye into the orbit) due to ocular pain. Enophthalmos may be normal in ponies. Alternatively, an upward deviation may indicate *exophthalmos* (abnormal protrusion of the eye).

The lacrimal apparatus

The presence of the upper and lower lacrimal puncta and the nasal ostium should be confirmed (see below under 'Investigation of nasolacrimal drainage'). The pre-ocular tear film causes the cornea to be shiny and should be assessed visually. A Schirmer tear test (see later) can be performed if there is any suggestion of abnormality.

The eyelids

The margins, outer and inner surfaces of the upper and lower eyelids should be examined. The position of the eyelashes on the upper lid is noted. Nonpigmented eyelids should be examined carefully as they are more susceptible to squamous cell carcinoma. The outer surface of the third eyelid can be easily protruded and examined by applying thumb pressure on the dorsolateral globe through the upper eyelid. The inner surface of the third eyelid is examined by everting it using atraumatic tissue forceps following the application of a local anaesthetic. This examination should be undertaken when neoplasia (e.g. squamous cell carcinoma) or a foreign body is suspected.

The ocular surface

The cornea and conjunctiva of the anterior ocular surface have a continuous epithelium that begins at the lid margins, extends on to the back of the lids into the conjunctival fornices and then moves across the limbus to the cornea. The conjunctiva of the eve and third eyelid should be shiny from the tear film and must be examined with particular care, especially when non-pigmented, as this is a site where squamous cell carcinoma can develop. The limbus or junction between the 'white' of the eye (bulbar conjunctiva with underlying episclera and sclera) and the clear cornea is well defined by a narrow rim of pigment. The cornea should be lustrous and transparent, allowing the fine structure of the iris to be visualized clearly. In shape the cornea is a horizontally elongated ellipse and the medial cornea is slightly wider than the lateral cornea. In most horses there is an obvious grey line on the corneal side of the medial (nasal) and lateral (temporal) limbus, which represents the insertion of the pectinate ligaments into the posterior cornea at the termination of Descemet's membrane (Fig. 15.8 (Plate 14)).

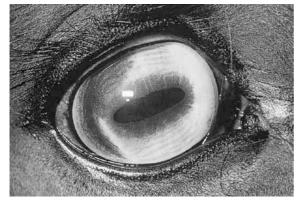


Figure 15.9 (Plate 15 in colour plate section) This horse shows a normal variant of iris coloration known as heterochromia iridis, whereby different sectors of the iris are of different colours, reflecting the degree of iris pigmentation. When the deficiency of pigment is marked and generalized the heterochromic iris may be so hypoplastic that it is possible to view details of the underlying lens equator and zonule.

The anterior chamber and iris

These can be examined briefly with a pen light at this stage but it is easier to examine them in the dark. The pupil should be an almost symmetrical horizontal ellipse and black-pigmented masses (the granula iridica or corpora nigra) are usually obvious on the dorsal pupillary border (Fig. 15.8 (Plate 14)). Variations in iris pigmentation are common (Fig. 15.9 (Plate 15)).

Examination in the dark

At this stage specific abnormalities detected in the first part of the examination can be looked at in more detail without the presence of distracting reflections. The eye, the adnexa and the anterior and posterior segments are examined in sequence. A penlight or transilluminator, magnifying lens, condensing lens of +5.5 to +30 dioptres (D) and a direct ophthalmoscope are required.

Pen light examination

Eye and adnexa

The eye and adnexa are examined by pen light using magnification if necessary. Corneal opacities and lesions such as foreign bodies, abrasions, lacera-

Figure 15.10 Illumination and magnification provided by using an otoscope with the speculum removed (mainly performed in darkness).

tions, ulcers or puncture wounds should be apparent using this technique. A useful light source with low-power magnification can also be obtained by using an otoscope with the speculum removed (Fig. 15.10).

Anterior segment

The anterior segment (the cornea and internal structures of the globe up to and including the lens) is examined using the same equipment. The light should be shone from a number of different angles for evaluation of the anterior chamber, iris, lens and aqueous humour. The anterior chamber and iris should be examined for evidence of foreign bodies, cysts, neoplasia, uveitis or complications of uveitis such as synechiae. The latter may cause an irregular pupil shape and size.

Pupillary light reflex

The pupillary light reflex (PLR) and the position, shape and size of the pupils are checked. The normal





Figure 15.11 Slit lamp biomicroscopy (performed in darkness) for examination of the cornea and anterior segment.

equine pupil responds somewhat sluggishly and incompletely, especially in comparison with that of the cat and dog, unless the light is particularly bright. The presence of a PLR is not synonymous with vision, nor does the absence of a reflex necessarily indicate that the horse is blind. A reduced or absent PLR is usually indicative of an ocular problem or a subcortical lesion. In more centrally located central nervous system disease, the PLR may remain intact despite blindness. A fixed and dilated pupil is associated with retinal and optic nerve disease, visual cortex problems and glaucoma.

Lens, vitreous and retina

For comprehensive examination of the lens, vitreous and retina, a mydriatic is necessary following evaluation of the pupillary light reflexes. Atropine should not be used as its topical effects are very long-lasting in the normal equine eye (\approx 14 days). Topical application of 1% tropicamide (Mydriacyl: Alcon) is perfectly satisfactory and will produce a dilated pupil within 20–30 minutes; its effects last for 8–12 hours.

The pen light or transilluminator may be used to demonstrate the anterior and posterior surfaces of the lens. An image of the light source may be readily identified on the anterior cornea and with decreasing clarity on the anterior lens capsule and the posterior lens capsule. These are the *Purkinje–Sanson images* and their relative movement in relation to the light source (parallax) is a simple way of establishing the depth of anterior segment opacities. In cataract formation, there may be a loss of one or both lens images, depending on the site and size of the cataract.

Slit lamp biomicroscopy of the anterior segment

The hand-held or transilluminator-mounted slit lamp biomicroscope (Fig. 15.11) provides a detailed means of examining the cornea, anterior chamber and lens of the equine anterior segment utilizing high magnification. Its use may be essential to a definitive diagnosis but for the most part it is usually employed at specialist centres. It consists of a light source that can produce diffuse illumination (or a narrowed slit beam illumination of 0.8–1.0 mm) and a binocular microscope that can move independently with respect to the light source.

Diffuse illumination is used initially to detect gross lesions involving the cornea, anterior chamber, iris, lens or anterior vitreous. The beam is then narrowed to a slit and directed obliquely so that a

15

magnified optical section of the corneal epithelium, stroma and endothelium can be assessed. The anterior chamber contains optically clear aqueous humour. Increased protein levels in the anterior chamber can be noted clinically as *aqueous flare*. White cells in the anterior chamber are called *hypopyon*, and red cells in the anterior chamber are called *hyphaema*. Aqueous flare, hypopyon and hyphaema indicate uveitis.

The lens should be checked for position and any opacities or cataract. There are a number of lens opacities that may be regarded as normal variations: prominent lens sutures, the point of attachment of the hyaloid vessel, refractive concentric rings of optical discontinuity, fine 'dust-like' lens opacities and sparse 'vacuoles' within the lens substance.

Cataracts are lens opacities and are associated with varying degrees of blindness. They can be congenital or secondary to previous uveitis, and may be progressive or non-progressive. In some horse breeds they may be hereditary. Normal ageing of the horse lens will result in cloudiness of the lens nucleus (nuclear sclerosis) beginning at 7–8 years of age, but this is not a true cataract. The suture lines and the lens capsule may also become slightly opaque as a normal feature of ageing. However, the adult vitreous should be free of obvious opacities. Vitreal 'floaters' can develop with age, or may be a sequel to *equine recurrent uveitis* and are generally benign in nature.

Indirect and direct ophthalmoscopy

The posterior segment (the internal structures of the globe behind the lens) consists of the vitreous, retina and optic nerve and is examined using indirect ophthalmoscopy followed by direct ophthalmoscopy. The two methods are complementary rather than exclusive.

The normal appearance of the equine fundus requires considerable practice for correct interpretation, because there is much normal variation. Most pathological lesions of the fundus are identified near and below the optic nerve head (optic disc), and typically involve hyperpigmentation or depigmentation.

Indirect ophthalmoscopy

This is a useful technique for screening the ocular fundus and can be performed most simply using a bright pen light or transilluminator, and a condensing lens. The system produces a low magnified, reversed, inverted, virtual image, such that a large field of view is produced. Mydriasis, a bright light source and darkness are essential for detailed fundic examination. A strong lens (high plus: 30 D) produces a small, bright image whereas a weaker lens (lower plus: 5.5 D) produces a larger, less bright image.

Monocular indirect ophthalmoscopy. A condensing lens is held some 2–8 cm from the horse's eye and the light source is held level with the bridge of the observer's nose (Fig. 15.12). The aim is that the observer's eye, the light source, the lens and the patient's pupil should all lie in the same axis. The plane of the lens must be parallel to that of the horse's iris and pupil. The light is directed into the horse's eye so that the tapetal reflection is obtained and the lens is moved to and fro until a sharp, clear image is produced. The observer–patient distance is approximately 50–75 cm.

Binocular indirect ophthalmoscopes have an integral light source and a prism system for delivery of separate images to the observer's eyes (Fig. 15.13). They are mounted on a headpiece or spectacle-type frame. The working principle is the same as for monocular indirect ophthalmoscopy, but the instruments have the benefits of stereopsis and more powerful light sources. They also allow the observer a free hand.

Direct ophthalmoscopy

The use of a standard direct ophthalmoscope produces an upright image of greater magnification than is possible with the indirect ophthalmoscope when used close to the patient's eye. However, viewing the fundus directly along a beam of light necessarily restricts the field of view. The direct ophthalmoscope provides the most magnified view of the fundus in the horse, with a lateral magnification of 7.9× and an axial magnification of 8.4×. Both distant direct ophthalmoscopy and close direct ophthalmoscopy should form part of direct ophthalmoscopic examination.



Figure 15.12 Monocular indirect ophthalmoscopy using a pen light and condensing lens. The condensing lens is held 2–8 cm from the equine patient's eye. The observer to patient distance is approximately 50–75 cm (performed in darkness).



Figure 15.13 Binocular indirect ophthalmoscopy using a commercial instrument and condensing lens (performed in darkness).

Distant direct ophthalmoscopy. This technique uses the tapetal fundus as a means of retro-illuminating the structures anterior to it. The ophthalmoscope is set to 0 D (no magnification) and directed to find the tapetal reflex in the pupil at an observer-patient distance of 25–40 cm (Fig. 15.14). It is a useful way of assessing whether there are any opacities between the observer and the fundus and is usually used as a quick screening method prior to more detailed assessment. Any opacities present in the ocular media (cornea, aqueous, lens, vitreous) will appear as black forms against the fundus reflex. Assessment of comparative pupil sizes may also be made using this technique.



Figure 15.14 Distant direct ophthalmoscopy (performed in darkness).

Close direct ophthalmoscopy. Direct ophthalmoscopy can be performed with direct or Panoptic ophthalmoscopes. The Panoptic ophthalmoscope (American Optical Company) has an intermediate level of magnification between direct and indirect ophthalmoscopes. Direct ophthalmoscopy can be used to examine all aspects of the eve and adnexa but is most commonly used to examine the retina and optic nerve head. The aperture of the instrument must be held as close as possible to the observer's eye and the horse's eye (Fig. 15.15). The lens range is approximately +30 D (magnifying lenses) to -30 D (reducing lenses). As the examiner reduces the strength of the plus lenses, the focus of observation gradually extends posteriorly, so that magnified details of the lids, cornea, aqueous, iris, lens and vitreous are successively visualized until features of the fundus are brought into focus. For detailed examination darkness is essential and mydriasis is helpful.

For examination of the external eye and adnexa a setting of +20 to +15 D (green numbers are plus) is required. The iris may be examined with a setting of +15 to +12 D. For the lens, the setting will be about +12 to +8 D depending on whether the anterior or posterior parts are being examined. Intermediate settings will be required for the aqueous and vitreous. Close examination of the fundus is usually performed with the ophthalmoscope placed some 2 cm from the eye and a setting of between +2 D or -2 D (usually 0) is required (red numbers are minus). The examination is often made easier and safer if the hand holding the ophthalmoscope is rested lightly against the horse's head, so that sudden movements do not damage the eyes of the horse or the examiner.

The fundus should be examined in logical fashion: the tapetal fundus (when present), non-tapetal fundus, optic disc and visible vasculature should all be assessed and both eyes should be compared and contrasted (Figs 15.16 (Plate 16), 15.17 (Plate 17)). The fundus should be examined for any signs of equine recurrent uveitis, such as peripapillary depigmentation. The non-tapetal region ventral to the optic disc should be carefully examined with a direct ophthalmoscope, as this is the area where focal retinal scars are seen. Retinal detachments may be congenital, traumatic or secondary to equine recurrent uveitis, and are serious faults because of their association with complete or partial vision loss.

Diagnostic techniques in equine medicine



Figure 15.15 The Panoptic ophthalmoscope combines the direct and indirect techniques and provides an erect image, but at less magnification than the direct ophthalmoscope.

II. SUPPLEMENTARY TECHNIQUES

Swabs, scrapes, smears and biopsies

Swabs, scrapes and smears are most usefully taken from the eyelid margins, conjunctiva (Fig. 15.18) and cornea. Precise sampling of corneal lesions requires topical anaesthesia and is recommended for the conjunctiva and eyelid margins as well.

Cultures of corneal ulcers using microbiological culture swabs should be obtained prior to placing any topical medications with preservatives in the eye. The swabs should gently touch the cornea as they are rolled across the corneal ulcer surface.

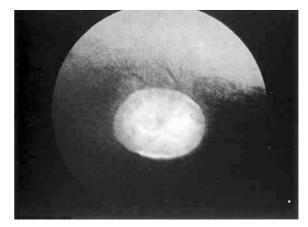


Figure 15.16 (Plate 16 in colour plate section) Normal fundus in a horse with a heavily pigmented iris. The optic disc (optic nerve head or papilla) is located within the non-tapetal fundus. Note the fine peripapillary retinal vessels which radiate from the optic disc; they are deficient in the 6 o'clock position (a normal variation which marks the site of the original fetal fissure). Choroidal vessels are visible dorsal to the optic disc because of suprapapillary hypopigmentation. The discrete black dots ('stars of Winslow') distributed throughout the green tapetal fundus represent choriocapillaris vessels viewed end on.

Bacteria and fungi may not be superficial in a corneal lesion. Corneal scrapings for cytology specimens to detect bacteria and deep fungal hyphal elements can be obtained at the edge and base of a corneal lesion with topical anaesthesia and the handle end of a sterile scalpel blade. Superficial swabbing cannot be expected to yield the organisms in a high percentage of cases, so removing the superficial debris can be helpful prior to collecting the sample. Cytology of eyelid and conjunctival masses can also be diagnostic.

Impression smears can be used to establish the nature of lesions that involve the ocular or adnexal surface, such as squamous cell carcinoma. A clean dry glass slide is pressed gently, but firmly, against the abnormal area and the preparation is air-dried, fixed in methanol and submitted to a reliable histopathologist for staining and interpretation. At least two smears should be prepared.

Biopsies may be taken from the eyelids and conjunctiva following adequate topical anaesthesia.

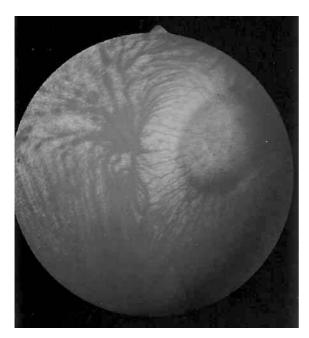


Figure 15.17 (Plate 17 in colour plate section) Normal fundus in a horse with a pale (subalbinotic) iris (china eye or wall eye). In this subalbinotic fundus there is no tapetum and very little pigment, hence both retinal and choroidal vessels are clearly visible against the creamy white of the sclera. The confluence of the choroidal vessels to form a vortex vein are very obvious in this animal.

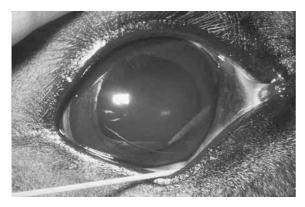


Figure 15.18 Conjunctival culture. The swab is placed in the lower conjunctival sac and rotated firmly against the palpebral conjunctiva.

Several applications of local anaesthetic will be required before the biopsy is performed. Fineneedle aspiration or surgical excision (partial or complete) may be used. For surgical excision the



Figure 15.19 (Plate 18 in colour plate section) Fluorescein has been used to stain the large ulcer which is present in this horse's cornea.

normal tissue at the edge of the lesion is grasped with fine-toothed forceps and an adequately sized sample is snipped off with fine pointed scissors or excised with a scalpel. It is important to avoid crushing and distorting the tissue when obtaining the sample and the correct orientation is most easily maintained if the sample is placed on very thin card before immersion in fixative. Neutral buffered formaldehyde is acceptable for routine light microscopy and immunohistochemistry, whereas 2.5% glutaraldehyde in 0.1 mol/l cacodylate buffer is the fixative of choice for electron microscopy. Always consult the laboratory before taking the samples to ensure which fixative to use.

Topical ophthalmic stains

Fluorescein

This is an orange dye that changes to green in alkaline conditions. In horses it is primarily used to detect corneal ulceration as it is rapidly absorbed by the exposed hydrophilic stroma in such cases (Fig. 15.19 (Plate 18)). It does not stain the lipidrich corneal epithelium or the acellular posterior basement membrane of the cornea (Descemet's membrane).

Fluorescein may also be used as a means of checking the patency of the nasolacrimal drainage apparatus (see below) and evaluating the stability of the tear film. In addition, it is highly recommended for detecting leakage of aqueous humour associated with penetrating corneal or scleral injury and melting ulcers, or microleaks from corneal sutures following corneal repair (Seidel's test).

Impregnated strips or single dose vials may be used, and in horses it is often simplest to place the strip or solution in the upper or lower conjunctival sac and allow the blink to distribute the fluorescein. To avoid false positives and provide sufficient moisture for adequate staining it is sometimes necessary to irrigate the eye with sterile saline or water. It is easier to detect subtle staining with a blue light source. Placing undiluted fluorescein dye in the eye to identify corneal ulcers should be routine in every clinical eye examination. Small corneal ulcers will stain that might otherwise be undetected.

Rose Bengal

Rose Bengal evaluates the integrity and stability of the mucin layer of the precorneal tear film. It is a red dye that stains damaged or devitalized epithelium, mucus and stroma following disruption or instability of the mucin layer of the tear film. It is indicated in horses with non-healing ulcers, keratoconjunctivitis sicca, herpes and/or keratomycosis and should be used after installation of fluorescein to identify the integrity of the tear film.

Schirmer tear production test

More and more disorders of the precorneal tear film have been described in horses and testing tear production is now regarded as a routine part of the equine ophthalmic examination. The Schirmer I tear test is the method most commonly employed for this purpose. Schirmer tear testing is a method to measure reflex tear production and should be used for chronic ulcers and eyes in which the cornea appears dry. The test must be undertaken prior to instillation of any medications into the eye. The test is easily performed using commercially available test strips that are up to 60 mm in length with a notch some 5 mm from the tip. The test strip is folded at the notch and the notched end is inserted

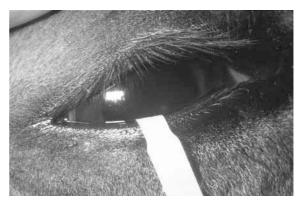


Figure 15.20 A Schirmer I tear test being performed on the conscious unsedated horse (sedation and anaesthesia will reduce tear production).

over the temporal lower lid margin (Fig. 15.20). The strip is removed after one minute and the length of the moist end is measured. Strips are frequently saturated in horses after 1 minute with values ranging from 14–34 mm wetting per minute considered normal. Values less than 10 mm wetting per minute are diagnostic of a tear deficiency state and values less than 5 mm are indicative of a lack of tear production, clinically manifest as keratoconjunctivitis sicca.

Investigation of nasolacrimal drainage

The upper and lower lacrimal puncta and the nasal ostium are readily visible in horses and this means that investigations can be performed from the proximal (puncta) and/or distal (ostium) parts of the system.

Visual inspection

Initial examination consists of visual inspection of the nasal ostium (Fig. 15.21) and the lacrimal puncta. The upper and lower lacrimal puncta are identified as fine, slit-like openings about 2 mm long situated close behind the free edge of their respective eyelids, about 8 mm from the medial canthus. Their presence, size and position should be checked.



Figure 15.21 Visual inspection of the (left) nasal ostium located medially on the floor of the nostril close to the mucocutaneous junction.



Figure 15.22 A nasolacrimal cannula has been inserted into the upper lacrimal punctum and canaliculus (right eye) after applying several drops of local anaesthetic to the upper punctum. The cannula is made of silver, a relatively soft and malleable metal, which is unlikely to damage the canaliculus.

Cannulation/catheterization

If samples are required for culture and sensitivity they may be obtained by irrigation with sterile water following topical local anaesthesia and cannulation/catheterization of the upper lacrimal punctum (Fig. 15.22). The upper lacrimal punctum is located and the upper eyelid is stabilized by tensing it upwards and everting it slightly, so as to move the canaliculus into a more vertical position. A silver cannula or plastic cannula/catheter can then be



Figure 15.23 A catheter has been inserted into the nasal ostium and nasolacrimal duct after the application of local anaesthetic drops to the upper or lower lacrimal puncta and local anaesthetic (usually as gel or spray) to the nasal ostium.

passed into the canaliculus via the punctum. Sedation may be required in addition to local anaesthesia. Alternatively, samples may be obtained by retrograde flushing with sterile water after cannulation/catheterization of the nasal ostium with a cannula or curved multipurpose syringe (Fig. 15.23). Again, local anaesthetic is applied to both the conjunctival sac and nasal ostium a few minutes before catheterization; sedation or application of a twitch may be necessary. These irrigation techniques will also indicate the patency of the duct.

Fluorescein drainage

If culture is not required, patency can be tested using fluorescein drops instilled into the lower conjunctival sac. These should appear at the ipsilateral nostril within 1–5 minutes of application (Fig. 15.24 (Plate 19)). Both sides should be tested.

Dacryocystorhinography

Dacryocystorhinography (contrast radiography of the nasolacrimal duct) is a useful technique for establishing the extent of congenital or acquired abnormalities of patency and, as the technique may confirm the necessity for surgical intervention, it is best performed under general anaesthesia. It is usual to cannulate/catheterize the upper lacrimal punctum and to inject approximately 5 ml of an iodine-based

Diagnostic techniques in equine medicine

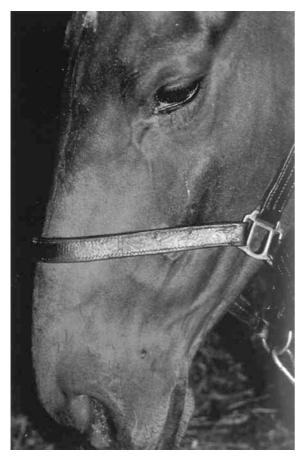


Figure 15.24 (Plate 19 in colour plate section) Fluorescein applied to the conjunctival sac should appear at the ipsilateral nostril within 1–5 minutes of application.

contrast agent. Lateral and oblique radiographs are taken (Fig. 15.25).

Tonometry

Tonometry to measure intraocular pressure (IOP) in identifying glaucoma in horses is most reliably performed with some form of electronic applanation tonometer (Fig. 15.26). Several models are available; the IOP measured in the horse with Tono-Pen (Reichert, Depew, NY) and TonoVet (Jorgensen Labs, Loveland, CO) applanation tonometers ranges from 7–37 mmHg, with a mean IOP of 23.3 ± 6.9 mmHg.

Glaucoma is an insidious disease in the aged horse, Appaloosas, warmbloods and horses

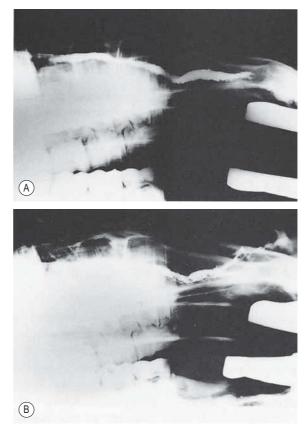


Figure 15.25 (A) Dacryocystorhinography with an iodinebased contrast agent has been used to confirm the results of clinical examination which showed that the nasal ostium was absent. The contrast agent delineates the extent of congenital atresia of the nasolacrimal drainage system prior to surgery. (B) The postoperative contrast radiograph demonstrating patency of the drainage system.

with uveitis, and applanation tonometry is critical to the identification and management of these cases.

Failure to use auriculopalpebral nerve blocks during tonometry may result in slight overestimates of IOP, but blocks are recommended in fractious horses. Horses that require sedation for ocular examination may show dramatic decreases in IOP, as illustrated by a study in which xylazine decreased IOP by 23–27%. Tonometry with the head positioned lower than the heart is 32% less than when measured with the head up.



Figure 15.26 Tonometry being performed on the conscious horse (sedation, anaesthesia and head position may affect intraocular pressure). Topical local anaesthetic drops are applied and palpebral nerve blocks are recommended prior to the procedure.

Diagnostic imaging

Radiography

Radiography is especially useful in fracture localization and diseases associated with bony destruction. Meticulous attention to detail in film handling and patient positioning, plus a thorough knowledge of normal equine orbital anatomy, is required for radiographic examination of the horse with signs of orbital disease. Good-quality films can be obtained with portable radiographic equipment and rare earth screens.

Radiographs should be taken using lateral, ventrodorsal, oblique and dorsoventral views in an attempt to differentiate ocular and orbital foreign bodies, tumours, fractures or the extent of soft tissue or bony lesions. Oblique views should be orientated to silhouette the area of greatest soft tissue swelling with a contralateral (normal) oblique view used for comparison. Special attention should be directed to examination of the nasal cavity, paranasal sinuses, tooth roots and calvaria. Dorsoventral views reveal nasal cavity or bony nasolacrimal duct problems. When evaluating unilateral lesions, the involved portion should be placed against the film cassette. Most lateral, dorsoventral and oblique views of the equine orbit can be obtained with the use of xylazine sedation. Ventrodorsal views of the orbit require general anaesthesia.

Increased soft tissue densities and lytic bone changes are associated with neoplasia and chronic inflammatory diseases. Calcification of soft tissues can occur in orbital cellulitis and tumours. Orbital aspirates and biopsies should be performed following radiographic study of the orbital region, so as not to introduce artefactual changes into the radiographic image.

The advent of high-resolution computed tomography (CT) and magnetic resonance imaging (MRI) has dramatically enhanced orbital evaluation in humans and small animals, and is the procedure of choice in these species. In the horse, CT and MRI techniques are often limited by the size of the animal, but foals can be evaluated easily by these methods.

Ocular ultrasonography

Ocular ultrasonography is a non-invasive diagnostic procedure that can qualitatively and quantitatively evaluate various globe and orbital abnormalities. Conditions identifiable using 7.5-10 MHz transducers include iris cysts, iris neoplasia, cataracts, vitreal opacities, retinal detachment, lens luxation, endophthalmitis, lens rupture, buphthalmos and retrobulbar masses. High-resolution ultrasonography (20 MHz transducer) has been used to evaluate corneal neoplastic and inflammatory lesions prior to surgical removal. Differentiation of solid soft orbital tissue masses versus cystic orbital masses, determination of the size of various globe or orbital components, and localization of orbital foreign bodies is possible. Since image resolution is directly related to frequency, a 10 MHz transducer will provide a superior image for discrimination of structures 300-400 µm. Real time B-scan ultrasound units emit focused sound waves to produce a twodimensional cross-section of orbital tissues, with ultrasound probes of 7.5 or 10 MHz providing the best resolution.



Figure 15.27 Ocular ultrasonography is easily performed in the horse.



Figure 15.28 Ultrasonography of the normal equine eye. In addition to showing normal detailed intraocular features of the globe, the technique is of value in demonstrating whether the retrobulbar tissues are also normal. C: cornea; CB: ciliary body; CN: corpora nigra; L: lens; ON: optic nerve; R: retina.

Technique

Sedation is recommended, but clipping over the evelids is not necessary in most instances. The ultrasonic transducer can be placed in contact with the eyelids via a methylcellulose coupling agent to evaluate the globe and anterior orbit (Fig. 15.27), or it can be placed in gentle contact with the cornea (following instillation of a topical anaesthetic) to evaluate the posterior orbit. When evaluating the orbit, the ultrasound beam is angled so that it enters the orbit between the globe and bony orbital wall. This bypasses the lens, which tends to attenuate the sound beam, and maximizes the echo intensity. Most orbital lesions exhibit lower reflectivity and less sound attenuation than the normal orbital contents. Foreign bodies are hyperechoic in many cases. The opposite globe and orbit provide a 'normal control' for comparison purposes.

The cornea appears uniformly echogenic (opaque/ white). The anterior chamber appears uniformly anechoic (black). The iris leaflets appear as echoic linear bands continuous with the ciliary body immediately posterior. The granula iridica are highly variable in structure and ultrasonographic appearance, but are often seen protruding into the anterior chamber on longitudinal images. Between the iris/ ciliary body and the posterior wall of the eye, the only echogenic structures are the portions of the anterior and posterior lens capsule that fall within the primary ultrasound beam. The vitreous is uniformly anechoic. The retina, choroid and sclera appear in combination as an echoic band defining the posterior aspect of the globe. The tissues posterior to the globe (muscle, fat, nervous tissue and vasculature) have varying echogenicities (Fig. 15.28).

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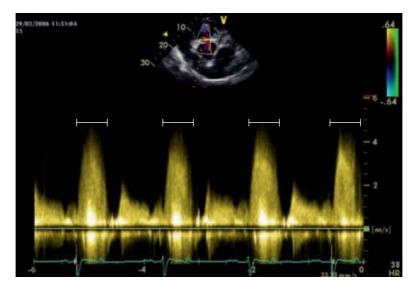


Plate 13 (Fig. 9.8) Continuous wave Doppler study of the abnormal flow through the ventricular septal defect of the horse in Figure 9.5. To perform this study, the transducer has been rotated from the position in Figure 9.5 to provide the best possible alignment with the abnormal flow through the defect. Blood flows at high velocity (4 m/s) through the defect from left to right ventricle (flow towards the transducer as shown by bars) during systole.



Plate 14 (Fig. 15.8) Closer naked eye examination of the eye and adnexa should note external details shown here such as the angle of the cilia on the upper eyelid, the dorsal and ventral orbital sulci (which divide the eyelids into tarsal and orbital portions), the position of the third eyelid and caruncle, and the amount of pigmentation present on the eyelids and conjunctiva. The limbus should be clearly defined; note the pigmented rim in this animal. The cornea should be transparent, allowing the fine structure of the iris to be clearly visualized. In this horse the grey line which marks the insertion of the pectinate ligament into Descemet's membrane and the cornea is very obvious laterally, less so medially. The pupil should be an almost symmetrical horizontal ellipse and granula iridica are usually obvious on the dorsal pupillary border, less so on the ventral pupillary border. In order to appreciate internal details beyond the pupil, examination must be continued in the dark.



Plate 15 (Fig. 15.9) This horse shows a normal variant of iris coloration known as heterochromia iridis, whereby different sectors of the iris are of different colours, reflecting the degree of iris pigmentation. When the deficiency of pigment is marked and generalized the heterochromic iris may be so hypoplastic that it is possible to view details of the underlying lens equator and zonule.

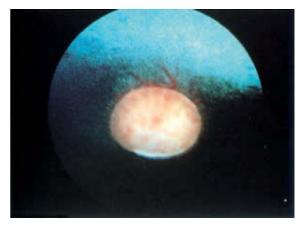


Plate 16 (Fig. 15.16) Normal fundus in a horse with a heavily pigmented iris. The optic disc (optic nerve head or papilla) is located within the non-tapetal fundus. Note the fine peripapillary retinal vessels which radiate from the optic disc; they are deficient in the 6 o'clock position (a normal variation which marks the site of the original fetal fissure). Choroidal vessels are visible dorsal to the optic disc because of suprapapillary hypopigmentation. The discrete black dots ('stars of Winslow') distributed throughout the green tapetal fundus represent choriocapillaris vessels viewed end on.



Plate 17 (Fig. 15.17) Normal fundus in a horse with a pale (subalbinotic) iris (china eye or wall eye). In this subalbinotic fundus there is no tapetum and very little pigment, hence both retinal and choroidal vessels are clearly visible against the creamy white of the sclera. The confluence of the choroidal vessels to form a vortex vein are very obvious in this animal.



Plate 18 (Fig. 15.19) Fluorescein has been used to stain the large ulcer which is present in this horse's cornea.

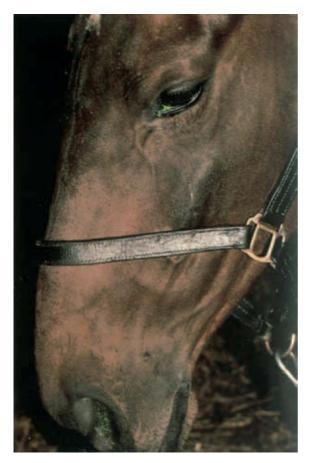




Plate 20 (Fig. 17.3) Photomicrograph showing the characteristic filamentous pattern of cocci in a *Dermatophilus* smear.

Plate 19 (Fig. 15.24) Fluorescein applied to the conjunctival sac should appear at the ipsilateral nostril within 1–5 minutes of application.

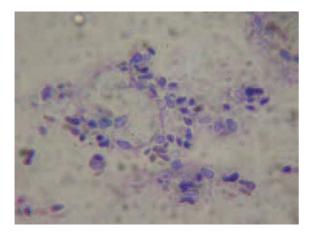


Plate 21 (Fig. 17.4) *Malassezia* organisms in an acetate tape preparation stained with Diff-Quik (100). (Courtesy of P J Forsythe.)

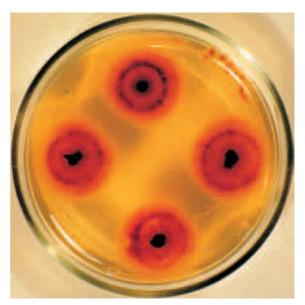


Plate 22 (Fig. 17.10) Typical dermatophyte colony on Sabouraud's medium.

CHAPTER 16

Fat diseases

Chapter contents	
Hyperlipaemia 323	Steatitis and fat necrosis 325
Diagnosis 324	Diagnosis 325
Fat tumours 324	
Diagnosis 325	Further reading 325

Fat diseases occur infrequently in equine practice. They include hyperlipaemia, fat tumours and steatitis/fat necrosis. Of these, the commonest is hyperlipaemia, a disturbance of fat metabolism to which ponies, miniature horses and donkeys are particularly susceptible. Tumours of the fat occur relatively commonly in the abdomen of older horses, but they do not invariably cause problems. Generalized steatitis and fat necrosis are recorded rarely and almost exclusively in foals. On very rare occasions a generalized or subcutaneous steatitis is seen in adults.

Hyperlipaemia

Hyperlipaemia is a disturbance of fat metabolism that is characterized by an abnormally high level of lipid in the circulation. This results in fatty infiltration of the tissues (primarily liver and kidney), circulatory failure and extensive vascular thrombosis. The disease is fatal unless the underlying cause is identified rapidly and treated successfully.

The disease is usually secondary to some form of stress or nutritional deprivation that is imposed upon susceptible animals. The most susceptible are ponies (especially Shetland ponies) and donkeys during late pregnancy or early lactation. It is less commonly seen in non-pregnant animals, but in this category obese ponies, miniature horses and donkeys are at most risk. Many factors can constitute a stress and typical examples include climatic extremes, poor husbandry, and intercurrent disease – particularly gastrointestinal disease.

The pivotal event in the development of this metabolic disease seems to be inhibition of insulin activity. Insulin normally promotes the deposition of fat, but interference with its activity leads to fat mobilization from adipose depots. Ponies and donkeys, and more especially obese ponies and donkeys, have an inherent physiological insensitivity to insulin. In addition, hormonal changes (associated with pregnancy and lactation) and increases in circulating cortisol levels (associated with disease or other stresses) also act to antagonize insulin sensitivity, thus compounding the inherent insensitivity in these animals. In summary, ponies, miniature horses and donkeys are metabolically predisposed to fat mobilization and conditions of pregnancy, obesity, disease, stress and/or reduced feed intake act to promote the tendency.

Diagnosis

The clinical signs are non-specific. Progressive hyperlipaemia is associated with drowsiness, depression, reluctance to move, dysphagia and circulatory congestion. Ventral oedema is common, but not pathognomonic. Signs of hepatic encephalopathy such as severe depression, blindness, abnormal movements, weakness and ataxia may follow fatty infiltration and subsequent failure of the liver. A pony in advanced pregnancy showing inappetence or depression should immediately be investigated for evidence of hyperlipaemia; early diagnosis is critical if there is to be any chance of a successful outcome.

In other cases, the clinical signs of hyperlipaemia may be masked by the more obvious signs of some primary disease that induces inappetence and/or a stress response. Any disease process that is associated with a reduced food intake has the potential to predispose to hyperlipaemia as a secondary complication. It should also be noted that healthy but overweight ponies, miniature horses and donkeys, with a history of being put on an abrupt diet, are prime candidates for hyperlipaemia.

In health, serum triglyceride concentrations are usually less than 1 mmol/l. In hyperlipaemic states they are greater than 5 mmol/l and can exceed 75 mmol/l in severe cases. Once the triglyceride concentration exceeds 5 mmol/l, the serum or plasma develops a visible opacity. Hyperlipaemia is easily demonstrated during clinical examination by taking a blood sample into anticoagulant. In a clear tube the light is reflected from the blood surface with a characteristic steel blue sheen. More obviously, when the tube has been standing for a few minutes to allow the red cells to settle out, the plasma reveals a cloudy, milk-like appearance.

Post-mortem examination shows extensive fatty change in tissues. There may also be evidence of some primary disease process that has predisposed to the hyperlipaemic state.

Comments

• Hyperlipaemia in horses is not accompanied by dramatic increases in blood cholesterol

concentration and its estimation is not diagnostically useful in this species.

- In advanced cases there is biochemical evidence of hepatic and renal failure. This takes the form of raised serum liver enzymes, bile acids and developing azotaemia. However, lipaemic serum or plasma is often unsuitable for biochemical analyses and these developments can be missed. In addition, lipaemia falsely increases the total protein (read on a refractometer), haemoglobin and MCHC. It also interferes with other biochemical estimations. However, the clinical pathology laboratory should be able to comment on the results that are affected by excessive amounts of lipids in the sample.
- Animals at risk, such as pregnant Shetland pony mares, can have their serum triglyceride levels monitored during late pregnancy to ensure that the energy intake is adequate and that stressors such as parasitism are held in check.
- Healthy nursing foals can have elevated triglyceride levels in their blood. This is due to normal chylomicron production after feeding.
- Hyperlipaemia is very rare in horses and when recognized is typically secondary to another disease process, usually renal disease.

NB: Short-term fasting in ponies (e.g. in transit) may produce a physiological lipaemia, which is reversible and without clinical sequel. This physiological state is sometimes referred to as *hyperlipidaemia*.

Fat tumours

Lipomas are benign tumours of the mesenteric adipocytes and are relatively common in older horses/ ponies. Those that develop on a lengthy pedicle have the potential to become entwined around the small intestine, thus causing an acute, often strangulating, obstruction resulting in colic. Much less commonly, the small colon may be obstructed. Lipomas are one of the commonest causes of strangulating obstruction in older horses, and ponies seem particularly predisposed.

Diagnosis

The clinical presentation is colic of acute onset, usually in the older horse or pony (>9 years), and examination suggests a small-intestinal obstruction. Definitive diagnosis requires laparotomy. Rectal examination will probably reveal turgid loops of small intestine within a few hours of onset, but it is most unlikely that a discrete lipoma will be palpable. Sometimes several loops of intestine are drawn together into a large palpable 'knot-like' mass.

Steatitis and fat necrosis

Steatitis and fat necrosis occur together as two extremes of an inflammatory condition but are extremely rare in the adult horse. The disease is characterized by widespread lesions within adipose tissues and is recognized externally by the appearance of firm, plaque-like swellings beneath the skin. The generalized condition is invariably fatal because progressively indurated fat lesions impinge on vital functions such as heart activity. Panniculitis, an unusual form of steatitis that is limited in distribution to the subcutaneous tissues, has also been described in the adult horse.

Diagnosis

Subcutaneous swellings of variable size, consistency and number are distributed over the body surface.

The firmer, plaque-like masses are immobile and may have a soft, liquefied centre. Clinical examination and haematology indicate a wasting, inflammatory condition. Diagnosis is easily achieved by biopsy of a solid subcutaneous swelling. A small wedge removed from beneath a skin incision reveals foci of fat necrosis and possibly mineralization. However, biopsy alone cannot indicate the extent of fat lesions within the body. Post-mortem examination shows widespread discoloration of body fat with patchy induration and focal areas of liquefaction.

Comment

 In other species, generalized steatitis and fat necrosis are associated with a vitamin E deficiency, which is part of the diagnostic criteria. In horses, vitamin E estimation is often normal and the aetiology, pathogenesis and treatment of the disease remain uncertain.

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CHAPTER 17

Skin diseases

Chapter contents

I. Assessing the problem 327 History 327 Clinical examination 328 Pruritus 328 Ectoparasites 329 Endoparasites 330 Contact irritant dermatitis and contact hypersensitivity 330 Atopic dermatitis and food allergy 330 Changes in hair growth 330 Alopecia 330 Hirsutism 331 Crusting and scaling 331 Nodules, papules and urticaria 333 Infectious nodules 333 Non-infectious nodules 334 Neoplastic nodules 334 Changes in pigmentation 335 Erosions and ulcerations 335

II. Practical techniques 337 Skin scraping 337 Skin surface cytology 337 Trichography 338 Fungal culture 338 Diascopy 339 Skin biopsy 339 Site preparation 339 Excisional biopsy 339 Punch biopsy 340 Elliptical biopsies 340 Biopsy for onchocerciasis 340 Tests to identify hypersensitivity reactions 340 Intradermal tests 340 Serum allergy testing 341 Elimination tests for irritants and allergens 341

Further reading 342

I. ASSESSING THE PROBLEM

Skin lesions are very common in horses, but few can be diagnosed on appearance alone and many have a similar appearance despite a variety of causes. Careful evaluation of the history followed by a thorough clinical examination should help to limit the differential diagnoses and simplify the choice of practical investigative techniques. The first part of this chapter is concerned with assessing the problem in order to select a diagnostic route and the second part gives details of the investigative techniques.

History

There are a number of helpful questions that must be included in the history:

- Are other horses affected? If so, what is the common link: direct contact; tack; grooming equipment; feedstuffs? It is also worth checking whether any of the handlers/riders are suffering from skin lesions
- Is the problem seasonal?
- Is it pruritic? Has it always been pruritic?
- Whereabouts on the body did it start; has it spread?
- What did it look like originally (i.e. the primary lesion); has it changed in appearance?
- Is there a recent history of using topical or systemic drugs?
- If therapy has been tried was there any beneficial response? Was there a resolution with subsequent relapse? Only a partial response, or did the condition worsen?

Look at the horse's environment as a source of potential irritants or allergens and consider the distribution of lesion(s) on the animal. For example:

- Bedding material contact areas are the lower limbs and belly
- Dust from rafters/loft falls over the head, neck and back
- Feed positions from above, food material falls over the face and neck; from below, it contacts the muzzle and lower limbs
- Rugs and/or blankets these will either protect the skin underneath from lesions or alternatively provoke lesions in that area.

Also note whether there are other animals or birds in the environment.

Clinical examination

This should include a general examination, quite apart from scrutiny of skin lesions, because some dermatoses are associated with systemic diseases. The principal features of the lesion(s) and their distribution are considered next. It is most useful to record details of the lesions and map their distribution on an annotated diagram.

Features of the lesion. The size, morphology and presenting characteristics are considered in an

attempt to establish differential diagnoses. The principal features of lesions may be described as follows:

- Pruritus
- Changes in hair growth: alopecia/hirsutism
- Crusting and scaling
- Nodules, papules and urticaria
- Changes in pigmentation
- Erosions and ulcerations.

NB: It must be emphasized that none of these features are mutually exclusive; for example, pruritus frequently results in secondary alopecia and crusting. In consequence, it is often impossible to define a lesion from the principal features alone. Nevertheless, it is useful to consider the potential causes of each principal feature in attempting to narrow the differential diagnoses. Guidelines to the differentials and selection of the appropriate diagnostic techniques are given below. Diseases are discussed under the primary lesion created during their pathogenesis.

Pruritus

Broadly, pruritus is most commonly associated with ectoparasitic infestations, infections or allergic skin disease. It may also be a feature of contact irritants, urticaria, early photosensitization and pemphigus foliaceous during the crusting phase of the disease.

Appropriate tests for investigating pruritus

- Skin scrapings and acetate tape preparations to rule out parasites.
- A therapeutic trial with a parasiticide may be indicated under some circumstances to rule out parasitosis.
- Skin surface cytology to identify infections.
- If the history and presentation are consistent with allergic skin disease then intradermal testing and/or dietary restriction are indicated.
- Skin biopsies are generally not helpful in the pruritic horse as a similar reaction pattern can be seen with multiple diseases.

Ectoparasites

Lice are a late winter/early spring problem associated with crowded housing conditions. In a good light they may be seen with the naked eye (or aided by a magnifying glass) at the base of the mane and tail. Biting lice (*Damalinia equi*) are fawn in colour and may be distributed in the dorsolateral trunk area. Sucking lice (*Haematopinus asini*) are a darker blue-black as a result of blood intake. The presence of copious scaling is likely, as are signs of rubbing. Shiny eggs ('nits') are seen attached to the hair.

Biting flies are probably the commonest ectoparasite causing pruritus. They are a summertime cause of annoyance and local skin eruption in the form of papules or weals. Lesions are exacerbated by individual animal hypersensitivities (allergies) to the bite. Horse flies (Tabanidae) inflict painful bites that can result in large wheals and focal crusting and ulceration. Stable flies (Stomoxys calcitrans) inflict severe bites to feed on blood/tissue fluids and can cause multiple wheals that develop into crusts along the neck, trunk and legs. Black flies (Simulium) attack the more sparsely haired areas such as the axillae, ventral midline and inguinal areas, producing a papular response. Horn flies (Haematobia irritans) are associated with a ventral midline dermatitis characterized by foci of hair loss, inflammation and scaling under the belly.

It is difficult to differentiate between these causes and a diagnosis of 'fly bite' may be achieved by the response to daytime stabling in a fly-proofed box. Biopsy of an early (primary) lesion will reveal a variety of tissue changes characteristic of an arthropod bite but it will not distinguish between them. In theory, hypersensitivity tests should identify specific allergies (see later).

Culicoides species are associated with the development of a common skin hypersensitivity known as 'sweet itch'. Predilection sites are the mane, croup and tail base. Horses rub the affected areas, resulting in alopecia, which is followed chronically by lichenification, hyperpigmentation, excoriations and crusts. A presumptive diagnosis is suggested by the history and clinical appearance. *Culicoides* hypersensitivity



Figure 17.1 Chorioptic mange. (Courtesy of P J Forsythe.)

can be confirmed by intradermal testing if the allergen is available.

Bees and wasps may attack as swarms producing multiple papules and plaques, which are painful rather than pruritic.

Acarid mites uncommonly produce infestations in horses, but should always be considered in the differential diagnosis of pruritus.

Chorioptic mange is caused by non-burrowing *Chorioptes equi* (Fig. 17.1). The condition is uncommon and tends to be seen in the winter months. Lesions are usually confined to the lower limbs and pruritus induces foot stamping. It may be more common in the 'feathered' draught horses. The mites are usually numerous and may be identified in a superficial skin scraping.

Psoroptic mange does not presently occur in the UK horse population. Psoroptic mites are occasionally found in the aural canal, but the absence of associated lesions makes their significance doubtful.

Sarcoptic mange (Sarcoptes scabei var. equi) is extremely rare in the UK horse population. Lesions

are associated with pruritus, alopecia, lichenification and crusting over the head, neck and ears, progressing caudally.

Harvest mites (Trombicula autumnalis and other species; 'chiggers') are an opportunist infection acquired in the late summer and autumn. They attach to areas of the horse in contact with herbage and weeds, so that the head and limbs are most commonly affected. The engorged red/orange mite is visible to the naked eye, assisted by a magnifying lens. There are no marked skin lesions, but often intense pruritus.

Endoparasites

Oxyuris equi is an uncommon gut parasite. The adult female causes intense perianal irritation by depositing eggs on the perineum. Large numbers of eggs may be seen at the anal sphincter ('anal rust'). Smaller numbers may be detected microscopically using an adhesive acetate tape sampling technique (see below).

Contact irritant dermatitis and contact hypersensitivity

Most contact dermatitis is caused by irritants damaging the skin as a result of persistent exposure to chemicals contained in tack treatments, drugs, certain plants, or body fluids such as urine. The distribution relates to the points of contact with the irritant, typically the head, limb extremities, ventral body surfaces and tack-associated areas. Lesions progress from erythema to crusting pruritus, lichenification and patchy alopecia. In some cases the initial contact response is urticaria. Contact dermatitis of the lower limbs may progress to the 'greasy heel' or 'mud fever' syndrome (see below).

Much less commonly, contact dermatitis has an immunological basis. In this situation the sensitizing agents act as haptens, forming allergens with skin proteins. A response may arise to an agent that has been present in the horse's environment, without harm, for years. Once sensitivity has developed, contact with the inciting agent produces a pruritic dermatitis within 1–3 days. The lesions produced are similar to those seen in contact dermatitis due

to irritants (see above). Diagnosis is by maintaining the horse in an 'allergen-free' area (see below under 'Elimination tests for irritants and allergens'). Biopsy of the affected area may aid diagnosis, but an accurate history and elimination of potential agents is most useful.

Atopic dermatitis and food allergy

Atopic dermatitis classically refers to an hereditary tendency to develop IgE-mediated hypersensitivity reactions to environmental allergens. The condition is not well characterized in the horse although a number of individuals develop seasonal or nonseasonal pruritus and positive reactions to environmental allergens can be demonstrated on intradermal testing. It is important to rule out other causes of pruritus, as this is essentially a diagnosis of exclusion. Most horses will present with pruritus, which variably affects the flanks, face, limbs, neck and tail base. Some will present with recurrent urticaria (see later). Concurrent recurrent airway obstruction is occasionally seen. A role for food allergens should be ruled out by performing an elimination diet. Intradermal testing is useful to formulate treatment with allergen specific immunotherapy.

Changes in hair growth

Alopecia

Alopecia can be a primary or secondary lesion. In this section we discuss diseases that result in primary alopecia. Alopecia occurs commonly secondary to pruritic skin disease.

Appropriate tests for investigating alopecia

- Skin surface cytology.
- Skin scrapings.
- Trichography and dermatophyte culture.
- In some cases a therapeutic trial with antibiotics is helpful.
- If this is a non-pruritic horse and the above tests are negative, then skin biopsies are indicated.

Bacterial folliculitis is quite common in the horse and results in areas of focal or multifocal alopecia,

which can be variably pruritic. There are numerous underlying causes including allergic skin diseases and tight saddle and tack. It can also be a component of the 'greasy heel' syndrome.

Equine demodicosis is rare and is usually associated with chronic glucocorticoid therapy. The horse hosts two mites, *Demodex equi* and *D. caballi*, which are considered part of the normal skin flora. Clinical disease presents as alopecia and scaling. Occasionally, papules or nodules may develop as a result of furunculosis. Mites can be demonstrated by deep skin scrapings or hair plucking (trichography).

Dermatophytosis or 'ringworm' is usually caused by *Trichophyton equinum* or *T. mentagrophytes*, and occasionally *Microsporum equinum*. Most cases occur in the autumn and winter in crowded housing conditions. The lesion appears as areas of alopecia, scaling and crusting. It is rarely pruritic and appears most commonly in areas rubbed by tack. Diagnosis is by fungal culture although direct microscopy is possible (see later). Wood's lamp examination is rarely positive in the horse.

Other causes of alopecia. Less common causes of alopecia include diseases such as alopecia areata, linear alopecia and sarcoidosis. Occasionally only the mane and tail might be affected, as seen in selenium toxicity. Patchy alopecia may be associated with anhydrosis. If common causes can be ruled out, then skin biopsies are usually helpful in determining the cause.

Hirsutism

A profuse, unkempt, curly coat is one of a number of clinical signs that are characteristic of *hyperadrenocorticism* in horses (pituitary pars intermedia dysfunction (PPID), often referred to as 'equine Cushing's disease'). Diagnosis is based on dynamic endocrinological tests, which demonstrate the presence of a functional pituitary tumour (see Ch. 5: 'Endocrine diseases'). Hirsutism may also result from chronic glucocorticoid therapy.

NB: Systemic infections are associated with the immunosuppressive effects of hyperadrenocorticism and these sometimes include dermatitis.

Crusting and scaling

Scaling refers to the excessive accumulation of stratum corneum that does not exfoliate in a normal fashion. The 'dandruff' that is seen clinically corresponds to clusters of corneocytes (cornified keratinocytes), or squames.

Crusts ('scabs') are composed of corneocytes, fibrin and blood cells. They are the external covers of underlying erosions or ulcerations.

Crusting and scaling are reasonably common skin lesions as they can follow any disruption to the epidermal barrier. Appropriate tests for crusting and scaling are:

- Skin scraping
- Skin surface cytology
- Dermatophyte culture
- Skin biopsy.

Bacterial folliculitis and superficial pyoderma present primarily as alopecia and pustules respectively, but crusting and scaling are common secondary lesions associated with these infections.

Dermatophilosis is a common form of bacterial folliculitis associated with wet weather in the autumn and winter months. It is an extensive, exudative infection caused by *Dermatophilus congolensis*. There is usually a symmetrical distribution of lesions over the hindquarters, commonly termed 'rain scald'. In muddy conditions the same lesions may occur in the lower limbs ('mud fever') and can affect the belly.

In appearance, the exudate mats the hairs, giving a tufted, 'paintbrush' appearance to crusts. When removed the crusts are large in diameter and the underside is coated with purulent material (Fig. 17.2). Diagnosis is by demonstrating the organism in a freshly lifted crust by impression smear (Fig. 17.3 (Plate 20)) or culture. NB: Old lesions may be overgrown by *Staphylococcus* spp.

Malassezia **species** are saprophytic yeasts that colonize intertriginous areas of skin. The affected areas are often greasy and scaly and variably pruritic. They may be found as a component of the 'greasy heel syndrome'. Identification is by skin surface cytology (Fig. 17.4 (Plate 21)).

The 'greasy heel' reaction (pastern dermatitis). The so-called 'greasy heel' or 'mud fever'



Figure 17.2 Crust typical of *Dermatophilus congolensis* infection.

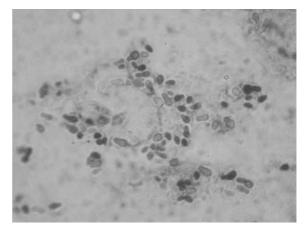


Figure 17.4 (Plate 21 in colour plate section) *Malassezia* organisms in an acetate tape preparation stained with Diff-Quik (100). (Courtesy of P J Forsythe.)



Figure 17.3 (Plate 20 in colour plate section) Photomicrograph showing the characteristic filamentous pattern of cocci in a *Dermatophilus* smear.

syndrome describes an intractable, painful, exudative dermatitis affecting the back of the pasterns (Fig. 17.5). There is exudation of a greasy, seborrhoeic material, which mats the hair and predisposes to secondary infection. It is a common sequel to a number of primary inflammatory causes already described above. Horses with feathering of the lower limbs are particularly prone. Among potential initiators are:

- Dermatophilosis
- Chorioptic mange
- Bacterial folliculitis

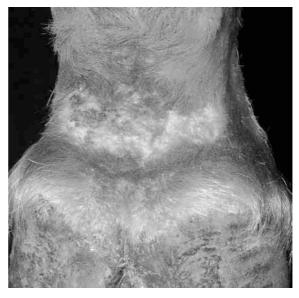


Figure 17.5 Lesions typical of 'greasy heel', 'mud fever' or pastern dermatitis.

- Dermatophytosis
- Contact dermatitis (e.g. grasses; bedding)
- Photosensitivity
- Malassezia dermatitis
- Leukocytoclastic vasculitis.

Greasy heel may be complicated by many of the above factors; therefore skin surface cytology, skin

scrapings and dermatophyte culture are the minimal tests required to investigate chronic lesions.

Generalized seborrhoea featuring dry scaling ('dandruff') or the production of large, greasy flakes is occasionally seen in horses. In this instance the body has a rancid odour and erythema and thickening of the skin is possible. Proposed aetiologies are confusing but, in the absence of a defined skin insult, internal disorders of digestion or endocrine function should be suspected and investigated.

Cannon keratosis is an uncommon dermatosis of obscure aetiology that affects the dorsal surface of the cannon region of both hindlimbs. The appearance is of a non-pruritic, non-painful scaling with matting of the hair in crusted plaques. Diagnosis is largely based on clinical appearance. *Linear keratosis* describes a visually distinctive line of hyperkeratotic skin extending vertically down the shoulder or lateral thorax. Bacterial and fungal infections should be eliminated in the diagnostic procedure and skin biopsies may be useful.

Pemphigus foliaceus is an uncommon autoimmune disease in which the autoantibodies are directed at adhesion proteins between the keratinocytes. The primary lesion is pustular, but these are very superficial and rupture easily, resulting in multifocal crusting (Fig. 17.6). Typically this will occur on the face and limbs but will often generalize. Ventral and limb oedema may be present and lethargy, inappetence and fever may be present. Multiple biopsies are required for diagnosis and should ideally include intact pustules and fresh crusts.

Nodules, papules and urticaria

As the term suggests, nodular diseases present as hard, circumscribed tissue masses of varying size. Their causative origins may be classified as inflammatory (infectious or sterile) or neoplastic. Small masses, less than 1 cm in diameter, are frequently referred to as papules. They are the early (primary) lesions associated with bacterial folliculitis and, in many instances, fly bites. Urticarial swellings are common in horse skin and appear as multiple elevated patches of varying diameter and may 'pit'

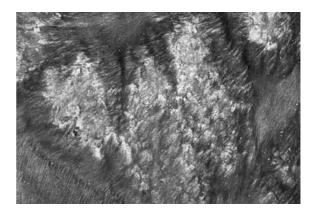


Figure 17.6 Multifocal crusting typical of pemphigus foliaceus.

on pressure. Appropriate tests for nodular skin disease are:

- Fine-needle aspiration cytology
- Skin biopsy for histopathology
- Skin biopsy for culture.

Infectious nodules

Equine viral papillomatosis is a common epithelial hyperplasia of yearlings and 2-year-old horses. They are distributed about the muzzle and head and occasionally spread further afield. They are pedunculated and verrucose and may achieve a size of 2 cm. The age incidence and appearance are diagnostic. Since they resolve spontaneously in 4–5 months, biopsy is unwarranted.

Habronemiasis is a granulomatous nodular condition caused by tissue reaction to the intradermal migration of the larvae of stomach worms (*Habronema* spp.). Larvae are deposited in areas of moisture, or in wounds, by stable or house flies, which are intermediate hosts for the parasite. Lesions are reported in the medial canthus of the eyes, in the prepuce and in wounds. The condition is rare in the UK. Diagnosis is by biopsy, which demonstrates the parasite in section, surrounded by eosinophils.

Other infectious nodules. Discrete nodules with draining tracts can be associated with infections such as botryomycosis (*Staphylococcus* spp.), abscesses (multiple bacteria) and occasionally fungi.

Aspirates or tissue samples should be submitted for culture and histopathology with special stains may identify more unusual organisms.

Non-infectious nodules

Nodular necrobiosis (synonyms: collagen necrosis; eosinophilic granuloma; collagenolytic granuloma) is probably the commonest nodular dermatosis of horses. Lesions occur as single or multiple nodules, 1–2 cm in diameter, over the withers and back, but sometimes extend beyond these regions. The underlying cause is unknown. Diagnosis is by biopsy, which demonstrates foci of degenerating collagen surrounded by an eosinophilic granulomatous reaction.

Aural plaques (aural hyperkeratosis) are small areas of raised, depigmented, papillomatous skin composed of hypertrophied epidermis. They are commonly seen in the ears of horses in the absence of any discomfort or irritation. They are of viral aetiology and are entirely benign. They do not usually warrant invasive diagnostic procedures.

Urticarial reactions are very common in horses and the lesions are often multifactorial in origin. They may be triggered by environmental or food allergens, drugs, pressure or physical activity. In some cases the inciting cause is never found. Pruritus may or not be a feature. Biopsies are usually unhelpful, except to rule out other skin pathologies, and diagnosis leans heavily on the associated history. Secondary serum exudation and crusting may develop, leading to hair loss. Occasionally, urticarial type lesions are a feature of purpura haemorrhagica, an immune-mediated vasculitis that is usually secondary to a previous systemic infection, particularly streptococcal infections. In this instance other clinical criteria such as mucosal petechiation are indicative of vasculitis.

Nodular panniculitis is a rare multifocal inflammatory condition of the subcutaneous fat (steatitis). The nodules assume a large size, several centimetres across, and eventually become cystic in their centre. At this stage they may ulcerate, discharging an oily material. Needle aspiration of a cystic centre yields a sanguinous fluid containing inflammatory cells. Cultures of this fluid are negative. Diagnosis is by biopsy of a solid nodule (see Ch. 16: 'Fat diseases'). **Amyloidosis** is a rare condition in which multiple, hard, painless plaques of amyloid are deposited in the skin over the head, neck and shoulders of horses. Diagnosis is by biopsy.

Warble flies (*Hypoderma* species). Cattle are the usual hosts of warble fly larvae and since their eradication from cattle warble flies are not currently seen in the UK horse population. The lesion is a hemispherical swelling of variable diameter, usually over the back. There may or may not be a central 'breathing hole'. Diagnosis is based on the appearance of the lesion and careful removal of the grub following fomentation. Biopsy should not be attempted, since this is likely to result in a severe local reaction.

Dermoid cysts are nodules occurring in the dorsal midline. Each consists of a fibrous wall lined with stratified epithelium and containing hair follicles, sweat glands and sebaceous glands.

Neoplastic nodules

Equine sarcoid. This is one of the commonest equine tumours. It is found around the eye, the paragenital region and the limbs. The clinical appearance is variable: verrucous (wart-like) tumours; fibroblastic tumours; mixed verrucous and fibroblastic tumours; and flat (occult) tumours characterized by hair loss, scaling and crust formation. Diagnosis is by biopsy, but it is preferable to submit a whole excised lesion. Occult and verrucous tumours, if quiescent, are best left undisturbed by biopsy, otherwise further activity may be provoked. **Melanomas** are common in aged grey horses. Pigmented lesions are seen under the tail, around the anus, on the ears and around the eyes. Diagnosis is usually based on appearance alone.

Squamous cell carcinoma. This is a relatively common tumour and is usually located in poorly pigmented, sparsely haired regions that are subjected to chronic ultraviolet light stimulation. In more temperate regions they may predominate in the penis and prepuce. Lesions appear as nonhealing ulcerated plaques with indistinct borders, or as cauliflower-like masses. Diagnosis is by biopsy. **Cutaneous mast cell tumour.** These tumours are uncommon and are probably a hyperplastic rather than a neoplastic process. They may be found on the head and limbs and are hairless, hyperpigmented and occasionally ulcerated. Diagnosis is by fineneedle aspiration or excision biopsy.

Cutaneous lymphosarcoma. Although lymphosarcoma is probably the commonest internal tumour of horses, the cutaneous form is very rare. Multiple nodules are distributed over the body surface and may achieve large diameters. Diagnosis is by biopsy.

Changes in pigmentation

Leukoderma describes a loss of pigment from focal areas of skin. In most cases it is the sequel to melanocyte destruction as a result of contact reactions, local trauma, surgery or cryosurgery. If the hair is affected this is termed *leukotrichia*. The associated history is often an important indicator of aetiology.

Melanoderma refers to hyperpigmentation of the skin. It may occur at previously inflamed sites and is often seen in chronic inflammatory conditions. If the patch of hair is also darker this is called *melanotrichia*.

Often biopsies taken from areas of pigmentary change will confirm the clinical diagnosis (i.e. loss of melanocytes), but they do not add information to the aetiopathogenesis of the disease. Knowledge of potential depigmenting diseases and their signalment is a clue to diagnosis of these conditions.

Onchocerciasis (Onchocerca cervicalis) is a widespread filarial nematode endoparasite of horses. Adults live in the ligamentum nuchae, producing microfilariae, which migrate out to connective tissues of the upper dermis. Many horses are infected, but few ever show evidence of clinical disease and it is rarely reported in the UK.

In clinical cases, hair loss, depigmentation, erythema and scaling are seen, particularly in the ventral midline region. Facial, cervical and proximal forelimb lesions are also described. Pruritus varies from mild to severe. Extraction of large numbers of microfilariae from finely chopped biopsy material provides a diagnosis, but it must be remembered that some microfilariae are to be expected in the skin of healthy horses. A more pragmatic diagnosis is the response to treatment with ivermectin, which resolves the inflammation within 3 weeks of treatment. However, the adult worm is unaffected and repeated anthelmintic treatment for microfilariae may be necessary

Genodermatoses. A number of genodermatoses are associated with lack of pigment such as albinism, Waardenburg–Klein syndrome, lethal white foal syndrome and lethal lavender foal syndrome. Diagnosis is usually based on the characteristic breed and the presence of other abnormalities. Arabian fading ('pinky') syndrome (vitiligo) is an acquired depigmentation of the skin, especially around the head, peculiar to Arab horses.

Erosions and ulcerations

Erosions describe partial epidermal damage above the level of the basement membrane, while ulceration is full-thickness epidermal damage in which the basement membrane integrity is compromised. They can be secondary lesions, most often associated with pruritus. Primary erosive or ulcerative diseases usually have a more discrete shape and margin.

Erosions and ulcerations readily become infected, so that skin surface cytology is indicated in all cases and it is prudent to treat any secondary infections present prior to further diagnostic testing, such as skin biopsies. Some of these diseases are associated with systemic signs, so that haematology, biochemistry and urinalysis may be additionally indicated.

Cutaneous vasculitis is characterized by purpura and oedema, which can progress to erosions and ulceration as a consequence of ischaemic damage to the skin. There are multiple causes of vasculitis, the most common being purpura haemorrhagica, which is most usually associated with streptococcal infections. However, other bacterial, viral and therapeutic agents can also be involved. Purpura can be differentiated from localized inflammation by a technique known as diascopy (Fig. 17.7).

Leukocytoclastic vasculitis (photoaggravated vasculitis). This is a relatively common inflammatory lesion of the unpigmented extremities, most



Figure 17.7 Diascopy: light pressure over an erythematous macule confirms vascular extravasation.

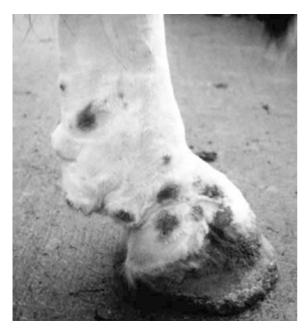


Figure 17.8 Leukocytoclastic vasculitis: typical circular ulcerations resulting from underlying vascular damage.

usually the pasterns, which occurs during the summer months (Fig. 17.8). This suggests that ultraviolet radiation has a role in the pathogenesis, but the condition is not a true photosensitization. It occurs in the absence of exposure to photosensitizing compounds and is not associated with liver disease. In the acute presentation the lesion is painful, with erythema, oozing and crusting. In more chronic cases it has a roughened, 'warty' appearance. Diagnosis involves ruling out primary and secondary forms of photosensitization (see above) and identifying the characteristic histopathological changes in a biopsy specimen.

Photosensitivity is recognized initially as erythema and oedema. A serous discharge then develops and the underlying skin becomes ulcerated. White, nonpigmented or flesh-coloured areas such as the star, muzzle and coronets are affected. Lesions are severe but localized. It is seen during the summer months and the initial erythema can be associated with pruritus. Sunburn has a similar appearance but is not a photosensitization.

Primary photosensitization occurs in conditions of high ultraviolet exposure and the grazing of plants that contain photodynamic agents (e.g. St John's wort; perennial rye grass). Several horses in a group may be affected. Secondary photosensitization is associated with liver failure where there is a decreased excretion of phylloerythrin, a product of bacterial activity on chlorophyll. This substance is photodynamic and provokes erythematous reactions in susceptible areas of skin. There is an extreme form of facial photosensitization termed 'blue-nose disease' in which the affected areas show a faint blue tinge of cyanosis. In all cases of suspected photosensitization, serum liver enzymes should be checked for evidence of liver-associated disease (see Ch. 4: 'Liver disease'). Otherwise, the diagnosis is supported by transferring the animal to shade, application of sun blocks and checking the pasture for implicated plant species.

Erythema multiforme. This describes an immunemediated cutaneous reaction pattern that can be triggered by drugs, infectious agents, neoplasia or food. In some cases an underlying aetiology is never identified. Clinically this presents as a symmetrical eruption characterized by early papules, plaques, vesicles or bullae progressing to erosions and ulceration. The haired skin is usually affected and in more severe cases the mucous membranes, particularly the oral mucosae, are involved. A characteristic interface dermatitis is seen on skin biopsies.

Epitheliogenesis imperfecta is the congenital absence of an area of skin, usually on a distal limb.

Junctional epidermolysis bullosa. This is a hereditary mechanobullous disease primarily affecting Belgian draught horses. They have a mutation in the gene coding for laminin-5, an important structural component of the epidermal basement membrane. Foals develop ulceration around the coronets, oral mucosae and pressure points in the first few days of life. There is no effective treatment.

Hereditary equine regional dermal asthenia (**HERDA**). This condition affects Quarter Horses and is thought to have an autosomal recessive mode of inheritance. Affected horses present as young adults with subcutaneous seromas and haematomas, skin ulceration and loose, easily tented skin over the dorsum and withers. A genetic mutation coding for cyclophilin B has been identified. A diagnosis can be made based on history and clinical presentation. Histopathology can be inconclusive in this condition. Genetic testing for this condition is commercially available in the USA.

II. PRACTICAL TECHNIQUES

Skin scraping

Skin scraping is used primarily for the demonstration of mites, of which *Chorioptes* spp. are the most important in horses. If the region is haired it should be lightly clipped before scraping, but no other preparation is necessary.

A scalpel blade is held at right angles to the skin and stroked swiftly to and fro under light pressure (Fig. 17.9). Multiple scrapings covering a large area of the affected region should be undertaken. Deep scraping is appropriate where demodicosis is a differential concern. In this instance the scraping must be deep enough to produce capillary ooze. A little mineral oil rubbed into the site prior to scraping produces a better sample for the examination of mites. Ideally scrapings are mounted on slides, a cover slip applied and transported in a slide case for examination in the laboratory.

The specimen is scanned under low power. Mites and their eggs can generally be identified at 100 and 400.



Figure 17.9 Skin scraping.

Skin surface cytology

Samples can be collected in a variety of different ways. For equine dermatoses swabs or adhesive acetate tape sampling are probably most useful. Direct impression smears from the underside of a crust would be indicated in suspected dermatophilosis, pyoderma or pemphigus foliaceus. In all cases the slide should be labelled with a case identifier and the body site from which it was harvested, particularly if immediate examination is not possible, or multiple samples from the same animal are being collected. The slides are then stained using a modified Wright's stain such as Diff-Quik (Dade Behring Inc., Deerfield, IL). For samples with a large lipid content, heat fixing is recommended prior to immersing in the alcohol fixative. In most other cases air-drying is sufficient.

Bacteriology swabs should be applied to the affected area of skin, and using moderate pressure a sample of the exudate is collected. This is then rolled on to the microscope slide, dried and stained as described above.

Acetate tape (clear Scotch pressure tape or Sellotape Diamond brand) is cut in 3–5 cm lengths and the central portion is pressed repeatedly on the affected skin using moderate pressure. This lifts organisms and cells off the immediate skin surface. After sample collection each end of the tape is lightly pressed on to a microscope slide, leaving the area on which the sample was harvested in a free loop in the centre of the slide. This allows the sample area to be more easily stained. When processing these samples the tape is stained directly. Do not dip in the alcohol fixative, as this will remove the gum and associated diagnostic sample from the tape. After staining the tape is blotted and flattened on to the slide, gum side down. The sample is viewed under the microscope through the tape. Microscope immersion oil can be directly applied to the tape for evaluation under high power.

In a suspected case of dermatophilosis, if a crust is lifted and there is suppuration beneath, an impression smear may be made on a glass slide. This is then heat-fixed for staining. The characteristic appearance under the microscope is of rows of cocci arranged in branching, filamentous tracks (Fig. 17.3 (Plate 20)). *Dermatophilus congolensis* stains well with Diff-Quik.

Acetate tape preparations are also used to diagnose *Oxyuris equi* infections. A piece of acetate tape is pressed over several areas in the anal and perianal region and then placed adhesive side down on to a glass slide coated with mineral oil. Under the microscope *Oxyuris* eggs are recognized as oval eggs with a cap (operculum) located at one end.

Acetate tape preparations may also be used to diagnose superficial mites such as *Chorioptes*, but skin scraping is more usual.

Trichography

This refers to the technique of plucking hairs and examining them under a microscope. It is primarily useful for the identification of arthroconidia and hyphae in dermatophyte infections. Hairs are plucked from the periphery of the lesion and mounted in mineral oil on a glass slide. Infected hairs have a 'fuzzy' appearance at the bulbar extremity due to disruption of normal growth. The fungal elements may be seen at high power. This technique takes practice and a negative finding should not be used to definitively rule out dermatophytosis.

Fungal culture

Dermatophyte culture is of greatest value in horses with focal or generalized hair loss, with or without crusting. Lesions should be wiped gently with 70% isopropyl alcohol to remove as many bacterial and saprophytic contaminants as possible and allowed to dry. Broken hairs, scales and lightly crusted lesions should be sampled from the periphery of lesions using sterile forceps and placed in a sterile container for transfer to the laboratory.

Culture kits for dermatophytes are now available to the practitioner as pre-poured culture plates. The medium is an amber-coloured Sabouraud's dextrose agar containing a pH indicator and antibiotic/ antimycotic agents to inhibit growth of contaminants. Samples are taken with sterile forceps and pressed on to (but not into) the medium. Colonies may develop after 2-10 days depending on ambient temperature, but should be allowed to incubate for 21 days before being declared negative. The dermatophyte colony is typically a white, powdery growth (Fig. 17.10 (Plate 22)). It produces alkaline metabolites that turn the medium indicator red. It is essential to check the plate on a daily basis (from 2-12 days if necessary), to ensure that the white colony growth and the colour change occur within a similar time course to one another. Contaminant colonies

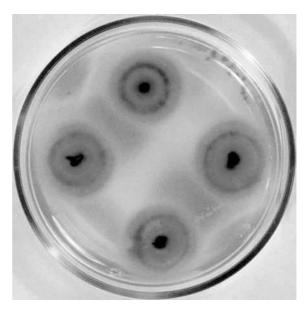


Figure 17.10 (Plate 22 in colour plate section) Typical dermatophyte colony on Sabouraud's medium.

17

are brown, grey or green, which at first do not alter the colour of the medium. Ultimately, they too will produce an alkaline colour change and for this reason red coloration after 12 days in association with a non-white colony should be regarded as contaminant growth. In doubtful cases the sample should be submitted to a diagnostic laboratory for specific identification.

Previous recommendations to incubate at room temperature may lead to false-negative results in some cases and in addition it is now recognized that some dermatophyte infections commonly affecting the horse may have more fastidious growth requirements than previously determined. Therefore 'in house' culture plates should be considered a useful screening tool, but where lesions persist submission of samples to a laboratory specializing in mycology is recommended.

Comments

- It is important to ensure sample collection from the periphery of lesions, where dermatophytes are actively expanding the infection.
- In difficult cases, biopsy sections submitted for periodic acid–Schiff staining may demonstrate dermatophytes.

Diascopy

This technique is employed to differentiate localized haemorrhage or vascular extravasation from inflammation. A glass slide is lightly pressed over the lesion. If local inflammation is present the underlying erythema will blanche whereas if there is loss of vascular integrity the erythema or purpura will remain.

Skin biopsy

The interpretation of skin histopathology requires considerable experience and it is advisable not to take biopsies before being sure that they can be examined by a competent dermatopathologist. A concise history, the distribution and features of the lesion, together with the site of sampling and the potential differential diagnoses should accompany the biopsy. This information enables the pathologist to make a better interpretation of the findings and consider the use of special stains, etc.

Biopsies should be undertaken in any condition that does not respond to appropriate treatment or where there is persistent ulceration, or suspected neoplasia. Exceptions to the latter are sarcoids, where it is preferable to submit a whole excised lesion for histopathology (see above under 'Neoplastic nodules').

Selected lesions should be fully developed primary lesions. Chronic lesions are not diagnostically useful. If possible, several biopsies should be taken to increase the chances of obtaining a diagnostic sample. Ulcerated lesions should be biopsied across the ulcerated margin encompassing normal and ulcerated tissue, so that the transition between diseased and normal skin can be examined. Care should be taken to avoid areas over superficial nerves, blood vessels, joint capsules or bony prominences.

Site preparation

Surgical preparation of the site is contraindicated. It may be soaked in 70% isopropyl alcohol but should not be scrubbed with antiseptics since this removes crusts and epithelial tissue that may be important in reaching a diagnosis.

Most biopsies can be obtained under local anaesthesia, with sedation if required. For punch biopsies, a 25G needle is inserted beneath the skin at the margin of the lesion until its bevel is buried in the subcutaneous tissues beneath the lesion. Adrenaline (epinephrine)-free lidocaine (0.5–1.0 ml) is then injected. For larger excisional or elliptical biopsies, a ring block is performed in the subcutaneous tissues around the periphery of the sample. Dermal or epidermal infiltration of local anaesthetic should be avoided, since this introduces artefactual changes into the sample. After infiltration, 5 minutes should be allowed before proceeding.

Excisional biopsy

If the lesion to be sampled is a single nodule, then excisional biopsy both removes the lesion and

provides a histopathological diagnosis. It is particularly useful for suspected vertucous sarcoids.

Punch biopsy

The disposable punch biopsy tool 6-8 mm (Stiefel Laboratories, UK) is useful for most skin biopsies and can usually be used to obtain two or three biopsies before the edge is dulled. The punch is placed over the lesion and rotated in a clockwise direction under light pressure until the blade enters the subcutaneous tissue. This is associated with a slight but palpable relief of resistance to pressure. On removal of the punch, the sample should be free of its adjacent dermis and remain loosely attached by connective tissue to the underlying subcutaneous tissue. The section is elevated by grasping the subcutaneous portion with fine rat-tooth forceps and it is cut free with fine, sharp scissors (Fig. 17.11). Care should be taken to avoid squeezing the sample with the forceps during this procedure. The sample is placed immediately in 10% buffered formalin for submission to the laboratory. The biopsy site may be closed with a single interrupted suture of 2-0 nylon, but this is not always necessary.

Elliptical biopsies

Elliptical biopsies are the method of choice for vesicular, bullous or pustular lesions, in which the biopsy can encompass the entire lesion; or for ulcer-



Figure 17.11 Releasing the punch biopsy sample.

ated lesions, in which the axis of the biopsy should include abnormal tissue, the edge of the lesion and the normal tissue beyond. The edge of an ulcerated lesion is the most rewarding in terms of histopathological diagnosis.

The site is anaesthetized by a ring block and a full thickness of skin is cut with the scalpel. The ellipse of skin is then undermined with narrow, curved scissors (Metzenbaum scissors) and the wound closed with 2-0 nylon in an interrupted pattern. Elliptical biopsies should be mounted with the dermis side pressed lightly down on to a piece of card or wooden strip (e.g. a piece of tongue depressor) before immersion in 10% formalin, otherwise they curl during fixation.

Biopsy for onchocerciasis

Onchocerciasis is a rare clinical problem. In such cases biopsy may demonstrate foci of parasites surrounded by an inflammatory reaction. An alternative is to split the biopsy sample, submit one half in formalin for histopathology and the other in saline moistened gauze for live parasite examination. In the laboratory the section is placed on a glass slide and minced in a few drops of saline with a scalpel. It is then left for 30 minutes at room temperature and examined under the microscope. The slide is scanned at low power along the margins of tissue debris looking for indications of 'whiplash' movement. The microfilariae are then identified at high power. However, the simplest diagnosis is arguably the response to ivermectin (see above under 'Changes in pigmentation').

Tests to identify hypersensitivity reactions

Intradermal tests

Intradermal testing (IDT) is indicated when other causes of pruritus have been definitively ruled out and the client is prepared to try allergen specific immunotherapy as a means of controlling pruritus due to atopic dermatitis. This test is only reliable for the identification of environmental allergen hypersensitivity and should not be used for food allergens.

The intradermal test can be affected by various drugs such as corticosteroids, antihistamines and some sedatives, and drug withdrawal times should be checked prior to performing the test. Although not a difficult technique, the cost of maintaining an intradermal test kit can be considerable, which is why this procedure is usually performed at specialist centres.

Allergens to be used in veterinary IDT should be selected according to the regional location in which the testing is performed. Positive and negative controls are usually employed. Histamine phosphate 1:100000 and 0.9% buffered saline solution respectively are commonly used. The IDT is usually performed on the lateral neck. Sedation with an alpha-2 agonist is usually required. The site is lightly clipped with a number 40 blade and should not be scrubbed. Test sites are marked with an indelible marker and should be at least 3 cm apart. By convention, a volume of 0.05 ml of each solution is injected intradermally. Air bubbles should be expelled from the syringe prior to injection as these can confuse interpretation. A positive reaction is characterized by local oedema and erythema. These are read at 20-30 minutes post injection, and additionally at 4-6 hours and 24 hours to evaluate the late-phase response. There are no standardized criteria for evaluating IDT. Many clinicians record reactions on a scale of 0-4 where 0 is equal to the negative control and 4 the positive control. Reactions scored as 2+ or greater are considered significant (Fig. 17.12).

Serum allergy testing

Allergen-specific serum immunoglobulin (antibody) can be detected and measured in horses by an allergen-specific enzyme-linked immunosorbent assay (ELISA). The test may be used to identify serum IgE antibodies to environmental or insect allergens. Various laboratories offer this test worldwide. Because of inherent difficulties in the detection of equine IgE in serum this test is not currently (Courtesy of P J Forsythe.)

Figure 17.12 Positive reactions on an intradermal test.

recommended as a reliable indicator of hypersensitivity to allergens in the horse.

Elimination tests for irritants and allergens

Environmental irritants are the usual cause of contact dermatitis, with or without urticarial lesions. The pragmatic approach to diagnosis is by changing the horse's management or environment to eliminate the irritant. Consideration should be given to everything that comes into contact with the horse's skin – for example tack (including tack cleaners/ treatments); topical medications; bedding; timber treatments; foodstuffs; other livestock and their parasites (e.g. poultry fleas).

Less commonly, contact dermatitis is allergic in nature and allergic reactions to food, drugs or inhaled allergens are occasionally associated with the development of urticaria. If allergy is suspected, and the problem occurs indoors, then management at pasture may quickly resolve the problem. Alternatively, the horse should be stabled in an empty box on rubber mats devoid of bedding. Everything subsequently introduced into the stable environment should be considered for its potential irritant/allergic properties.

In relation to feed-associated allergies the diagnostic process is extended to elimination diets. If the

problem occurs at grazing, the horse should be brought indoors or transferred to an alternative pasture. If the problem occurs indoors, the diet should be changed to a grass hay not previously fed to the horse. Most commercial feeds have the same basic constituents and changing from one brand to another is unlikely to demonstrate a difference. It is therefore advisable to withhold concentrates and offer a bulk feed not previously used. Because food products can persist within the body for extended periods of time, feed trials should occupy a minimum of 4 weeks and arguably much longer. If the problem subsequently resolves, then individual parts of the old diet may be reintroduced at weekly intervals as a test challenge.

In respect of airborne allergens, the use of different bedding, spore-free prepacked 'haylage' feed products and the elimination of animal dander (e.g. roosting birds), should all be considered.

Comment

• The diagnosis of irritant or allergen exposure can be a frustrating and time-consuming process. The clinician should therefore be satisfied that all other potential causes of the lesion have been considered and tested before embarking on irritant and/or allergen elimination studies.

FURTHER READING

- Pascoe R R, Knottenbelt D C 1999 Manual of equine dermatology. W B Saunders, London
- Scott D W, Miller W H 2003 Equine dermatology. W B Saunders, St Louis, MO
- White S D 2003 Skin diseases. In: Robinson N E (ed) Current therapy in equine medicine, 5th edn. W B Saunders, St Louis, MO, p 174–220

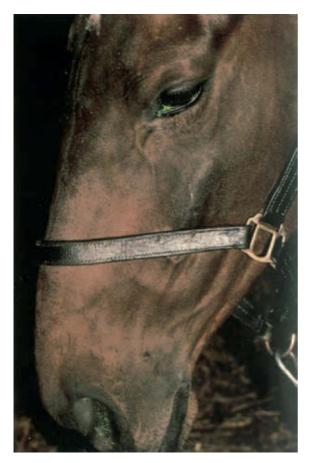




Plate 20 (Fig. 17.3) Photomicrograph showing the characteristic filamentous pattern of cocci in a *Dermatophilus* smear.

Plate 19 (Fig. 15.24) Fluorescein applied to the conjunctival sac should appear at the ipsilateral nostril within 1–5 minutes of application.

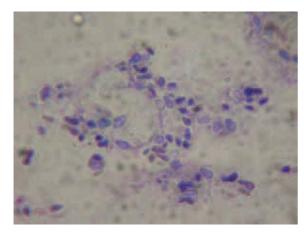


Plate 21 (Fig. 17.4) *Malassezia* organisms in an acetate tape preparation stained with Diff-Quik (100). (Courtesy of P J Forsythe.)

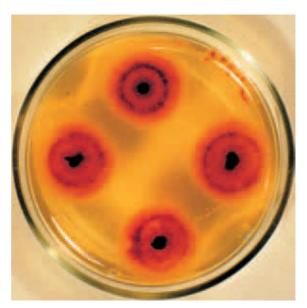


Plate 22 (Fig. 17.10) Typical dermatophyte colony on Sabouraud's medium.

CHAPTER 1

Post-mortem examination

Chapter contents		
Requirements 343	Inspection of the removed organs 348	
	Submission of samples 349	
Preliminary information 344	Examination of the locomotor	
External examination 344	system 350	
	Examination of the head and brain 350	
Opening the carcase 344	Examination of the spinal cord and peripheral nerves <i>352</i>	
Inspection and removal of abdominal viscera 345		
Inspection and removal of oral, cervical and thoracic viscera 347	Further reading 352	

It is invariably difficult to conduct a thorough postmortem examination under practice conditions because the time, facilities and practical experience are often lacking. In addition, there is the considerable and increasing problem of carcase disposal.

When it is known that the results of a postmortem examination will have implications for other horses in a group, or that an insurance claim or litigation are contemplated, the advisability of transporting the carcase to a referral centre should be considered. In this instance, however, the following points must be appreciated by the owner and all interested parties:

- The carcase must be submitted as soon as possible autolysis begins immediately after death
- The pathologist will require details of the animal's management, its clinical history and the time and circumstances of its death

- The procedure is expensive
- There is no certainty that the cause of illness or death will be determined.

Most usually, circumstances will dictate a compromise in which a partial examination is undertaken at the premises of a licensed slaughterer (knackerman) or a hunt kennels. This chapter describes a technique for a full post-mortem examination under practice conditions, which may be adapted to individual circumstances.

Requirements

The floor area to be used as an examination surface should be entirely washable, with suitable drainage, and there should be adequate lighting and washing facilities. The requirements for protective clothing includes overalls, gum boots, a rubber apron and gloves.



Figure 18.1 Basic kit for post-mortem examination.

The necessary investment in equipment is not prohibitive and includes: a hand saw; rib shears; a selection of knives with a sharpening steel; scissors; forceps; a scalpel; tissue containers with fixative, and various sterile equipment (syringes, needles, swabs and transport media) to sample fluid and other tissues for microbiology (Fig. 18.1). A supply of 10% buffered formalin with suitable sample pots should also be available for histopathology.

Preliminary information

The animal's management, its clinical history and the circumstances of its death should be considered carefully before post-mortem examination. If there is an insurance interest in the animal, the company should be informed since they may require their own agent to be present at the examination.

Throughout the examination, written or taped notes should be kept and ideally any lesion of significance should be photographed. Pathological changes in tissues should be recorded in terms of their location, colour, size, shape, consistency and the appearance of a cut surface.

A number of variations to the technique described below are possible, but the aim should always be to proceed in a systematic manner ensuring that no organ is overlooked, rather than seizing at the first potential lesion and being misled. The animal will probably be in lateral recumbency and for the technique described here should be rolled on to its right side, i.e. the examination proceeds from the left side of the horse.

External examination

Those distinguishing marks that would identify the animal in life should be recorded. This is especially important where there is an insurance interest or prospect of litigation. If possible, photographs of distinguishing features are an additional advantage.

External examination should include the skin, noting any abrasions that suggest either struggle or other trauma, or the presence of burns (e.g. lightning strike). The mucous membranes, sclerae, hooves and coronary bands should all be inspected for abnormalities.

Opening the carcase

A ventral midline skin incision is made from the mandibular symphysis to the region of the prepubic tendon. The skin overlying the upper half of the body is dissected free and reflected back as far as possible (Fig. 18.2). In the male, the skin incision is carried around the penis and prepuce and both are reflected caudally. In females, the



Figure 18.2 Reflecting the skin from the uppermost half of the body.



Figure 18.4 The abdominal wall reflected.

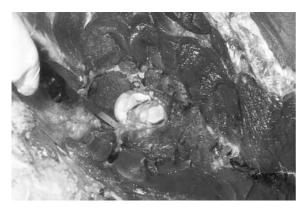


Figure 18.3 Cutting the round ligament at the head of the femur to enable the hindleg to be folded back.

mammary gland can be undermined and removed with the skin.

The foreleg is freed by cutting its medial muscular attachments, dissecting into subscapular tissues and folding it dorsally. The hindleg is treated similarly, but requires the capsule of the hip joint to be opened and the round ligament at the head of the femur to be cut (Fig. 18.3).

The abdominal wall is folded back in a ventral direction after incising along the costal arch and around the flank. Care must be taken not to cut into the underlying organs (Fig. 18.4).

The diaphragm is punctured near the sternum, at which time there should be an audible aspiration of



Figure 18.5 Opening the ribcage.

air as the lung collapses, indicating intact negative pleural pressure. The cut is continued dorsally to release the diaphragm from the costal arch.

Using shears, the ribs are cut at their dorsal and ventral attachments and the thoracic wall is removed (Fig. 18.5).

Inspection and removal of abdominal viscera

The abdominal viscera are first examined *in situ* for any gross abnormalities. Organs of the intestinal tract should be checked to ensure their correct anatomical position with respect to one another. The volume, colour and turbidity of peritoneal fluid is noted and, if appropriate, sampled for cytology



Figure 18.6 Removing the intestine. The pelvic flexure is lifted out first.

(EDTA), biochemistry (plain tube) or culture (sterile container).

The pelvic flexure is lifted out and placed ventrally (Fig. 18.6). The spleen is cut free and removed, followed by the left kidney. The kidney should be freed from its perirenal fat and its arterial supply cut close to the renal pelvis. It is then removed with its ureter intact.

Access to the coeliacomesenteric ganglion

The adrenal gland, which is adjacent to the kidney, should still be attached by its vasculature, fat and fascia to the aorta at the point where the renal artery was cut. Between the adrenal gland and the aorta lies the coeliacomesenteric ganglion, which is required for definitive histopathological diagnosis of grass sickness. This fusiform structure is about the width of a pencil, some 4-5 cm long in the adult horse and has fibrous nerve attachments at each end. However, being soft and white it is often extremely difficult to differentiate this structure from the surrounding fat. Consequently, the adrenal gland, together with its adjacent segment of aorta, vena cava and the attached fat, should be removed en bloc and fixed in formalin. After fixing for 24 hours, the ganglion becomes harder and is more easily distinguished once the overlying adrenal gland has been dissected away (Fig. 18.7). Its crosssectional appearance has the typical creamy white colour of nerve tissue. Other sympathetic ganglia

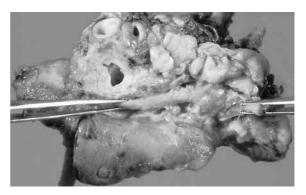


Figure 18.7 Dissection of the coeliacomesenteric ganglion from fixed (formolized) fat between the reflected adrenal gland (bottom of the picture) and the aorta (hidden underneath). The renal artery is seen at bottom left.



Figure 18.8 Removal of the whole intestine.

will provide evidence for the diagnosis of grass sickness, but none will provide such large amounts of readily accessible tissue.

The small colon is cut free as far within the pelvic region as possible. At the other end of the gut the small intestine is stripped from its mesenteric attachment. The stomach is then cut free and exteriorized with the small intestine. The caecum and colon are freed of their attachments, thus enabling the whole intestine to be removed intact (Fig. 18.8). Depending upon the available facilities, it may be more convenient to ligate sections of the alimentary tract with string and remove them for further examination as more manageable units.

The opposite kidney is then removed as described above. If necessary, a second attempt to retrieve a coeliacomesenteric ganglion can be made on this side. The bladder is drawn over the pelvic brim and opened *in situ*. In the mare, the uterus and ovaries are removed by drawing the tract forward and sectioning behind the cervix. If necessary, the pelvic organs may be removed, but this requires cutting the bones of the pelvic cavity using the hand saw, or splitting the pelvis with an orthopaedic chisel.

The liver is removed by cutting its attachment to the diaphragmatic crura.

Inspection and removal of oral, cervical and thoracic viscera

The contents of the thoracic cavity, including the volume, colour and turbidity of pleural fluid, are examined. The tongue, pharynx, larynx (including adjacent thyroids), trachea, lungs, heart and oesophagus are then removed as one unit. This is achieved by freeing the tongue from its attachments with the oral cavity. Working from the underside of the jaw, a handsaw is used to cut across the rami of the mandible just behind the incisor region. The rami are prised apart and the tongue's attachments are cut on either side, parallel to each mandibular ramus (Fig. 18.9). Its base will also need to be freed from the stylohyoid apparatus by cutting through the cartilaginous attachment on either side. The whole is then pulled through the intermandibular space and further cuts medial to the stylohyoid apparatus will free the larynx, cranial trachea and oesophagus. The trachea and oesophagus are then dissected free from the neck as far as the thoracic inlet (Fig. 18.10). The pericardium is released from its sternal attachment and the dorsal mediastinum is cut along its length. This enables the entire thoracic contents to be removed once the aorta and oesophagus are severed from the diaphragm (Fig. 18.11).

The parietal pleura is checked for evidence of inflammation or adhesion. The aorta can be exam-



Figure 18.9 Cutting the tongue free of its attachments through the intermandibular space.



Figure 18.10 Separation of the trachea and oesophagus from the neck.

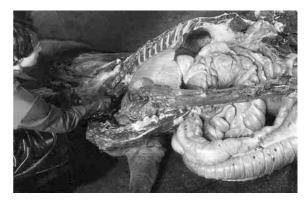


Figure 18.11 Removal of the thoracic contents.



Figure 18.12 Opening the stomach and small intestine.

ined by opening along its length and into its tributaries with scissors, starting at the thoracic end. If the examination procedure has been followed as described, the arterial system within the mesentery should still be intact for inspection.

The appearance of each individual organ is then assessed and recorded separately.

Inspection of the removed organs

Abdominal contents

The stomach is opened along its greater curvature and the small intestine is opened along its length using scissors (Fig. 18.12). The volume and nature of the gastrointestinal contents are noted and the mucosa is examined after rinsing with water. The large intestine is examined in like fashion. The size and colour of the colonic and mesenteric lymph nodes should be noted.

The liver, spleen, kidneys and adrenal glands should be sectioned and the cut surfaces examined for any abnormality of colour, content or shape. The kidneys should be cut lengthwise from the outer surface towards the pelvis and the capsule should be



Figure 18.13 Peeling back the kidney capsule.

peeled back to check for any pathological change at the cortical surface (Fig. 18.13).

Oral, cervical and thoracic contents

The tongue is examined and sectioned, the oesophagus is opened along its length for inspection and then removed from the trachea. The thyroid glands are identified and examined in cut section. The cervical parathyroids are difficult to identify because of their small size, variable location and similar appearance to cervical lymph nodes. A larger pair of caudal parathyroid glands is located on the ventrolateral aspect of the trachea, close to the level of the first rib.

The pharynx, epiglottis, larynx and retropharyngeal lymph nodes are examined and the trachea is opened along its length to the bronchial tree (Fig. 18.14). This is facilitated by prior removal of the heart from the pericardium and its vascular attachments. An excess of pericardial fluid or other pathological change should be noted. The lungs are then palpated to detect any changes in consistency and are sectioned at several sites to check for small internal lesions and the presence of abnormal fluid or exudate (Fig. 18.15).

In cases of sudden death, particular attention should be paid to examination of the heart and its associated vessels. Ideally, the heart should be opened in such a way that the valves and their



Figure 18.14 Opening the trachea.

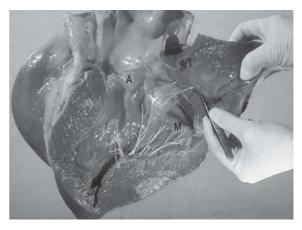


Figure 18.16 The left side of the heart opened for gross examination. The aortic (A) and mitral (M) valves are clearly exposed.



Figure 18.15 Sectioning the lungs to detect gross lesions and assess fluid content.

attachments can be examined intact before pathological evidence is lost by dissection. This can be accomplished using the following procedure.

• *Left side of the heart*. The aorta is separated from the overlying pulmonary artery and is cut open so that the aortic valve can be inspected from above. A downward incision is then made through the valve into the left ventricular wall, opening up the aortic vestibule. This enables a limited view of the mitral valve and its attached chordae from below. The left atrium is then opened by extending a cut through the pulmonary vein to expose the dorsal surface of the intact mitral valve for inspection. A downward incision is then made through the

valve and into the left ventricular wall as far as the apex. A third cut to join the two incisions in the ventricular wall allows the left chamber to be opened to full view (Fig. 18.16)

• *Right side of the heart.* The right atrium is opened by joining incisions into the anterior and posterior vena cavae. The intact tricuspid valve is then inspected from above. The pulmonary artery is cut open to expose the pulmonary valve from above. An incision is then made down through the valve, opening the wall of the right ventricle as far as the apex. This provides a limited view of the tricuspid valve from below. A final cut is made through the tricuspid valve to open up the front of the right ventricle and join the pulmonary artery incision at the apex, thus exposing the right chamber to full view (Fig. 18.17).

Submission of samples

At this stage appropriate samples should be considered for histopathology, culture, serology, biochemistry and toxicology. Tissue samples of major organs and all observed lesions can be collected for routine histopathology in 10% buffered formalin. The fixative to tissue ratio should be at least 10:1 by volume to ensure adequate penetration. Serology and serum



Figure 18.17 The right side of the heart opened for gross examination. The pulmonary (P) and tricuspid (T) valves are clearly exposed.

biochemistry may be achieved by retrieving heart blood from the right ventricle if ante-mortem samples are not available. In cases of suspected poisoning, liver, kidney, fat, stomach or intestinal contents, urine and heart blood should be frozen down as soon as possible (see 'Causes of fatal poisoning' in Ch. 19: 'Sudden and unexpected death'). In ideal circumstances, sufficient material should be retained to enable duplication of tests, or alternative testing if required.

Examination of the locomotor system

Joints should be examined if indicated by the clinical history. Prior to opening a joint it may be necessary to aspirate synovial fluid for cytology, biochemistry or culture. Contaminant-free culture is facilitated if the joint is first skinned. Normal fluid is scant, straw-coloured and viscous.

Cases of laminitis should be examined by cutting off the foot above the coronary band and cutting the whole in longitudinal section (Fig. 18.18).

Skeletal muscle should be examined in cut section and submitted for histopathology if appropriate.

Examination of the head and brain

The head is removed from the neck at the atlantooccipital joint (Fig. 18.19).

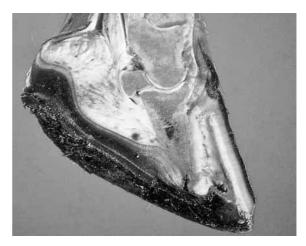


Figure 18.18 Longitudinal section of a laminitic foot. Note the displacement of the pedal bone and the accumulation of exudate between its dorsal edge and the hoof wall.



Figure 18.19 Removal of the head by section of the atlanto-occipital joint.

Access to the brain is obtained by stripping back the skin over the poll and removing the temporal muscles overlying the dome of the skull. An oscillating circular saw is ideal to open the bony cranium, but a handsaw can be used. The head must be held tightly for this procedure, preferably in a vice. A cut is made across the head through the frontal bones at a point just caudal to the zygomatic arches. Two further cuts are then made at right angles, with the line of the blade passing just medial to the occipital condyles (Fig. 18.20). The bony plate is then carefully prised off in a caudal direction while



Figure 18.20 Dorsal view of saw cuts in the cranium prior to removal of the bony plate.



Figure 18.21 Separating meningeal attachments as the cranial plate is prised open.

separating the meningeal attachments beneath (Fig. 18.21).

For removal, the brain is supported in one hand and the cranial nerves, meningeal and vascular attachments are cut while an assistant tilts the head



Figure 18.22 Dorsal view of the pituitary in situ (arrowed).

back. Once the brain is removed, the pituitary gland is seen from above (Fig. 18.22) and can be lifted up by forceps and cut free of its fossa. In cases of pituitary adenoma (pituitary pars intermedia dysfunction; see Ch. 5: 'Endocrine diseases'), the optic chiasma should be checked for evidence of distortion and the adrenals should be examined for cortical hypertrophy.

If necessary, the whole brain may be immersed in 10% formalin, but a week is then required for complete penetration of the fixative.

The eyes are removed by cutting through the periorbital skin and dissecting through the tissues of the orbit with curved scissors, using much the same procedure as for surgical enucleation.

The mandibles can be separated from the upper jaw by cutting through the soft tissues of the cheeks in a line towards the temporomandibular joints. The mandible is held steady and the upper jaw is pulled up and away to disarticulate the joint. The head can then be sawn longitudinally to reveal the nasal passages and paranasal sinuses.

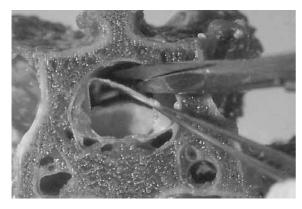


Figure 18.23 Removal of the spinal cord from a cut segment of the vertebral column.

Examination of the spinal cord and peripheral nerves

The removal of an intact spinal cord is both difficult and extremely time-consuming (several hours). The exercise demonstrates how well the organ is protected under ordinary circumstances. It is usually acceptable, and certainly faster, to remove short sections of the cord from a cut-down vertebral column. If appropriate, post-mortem radiographs can be used to locate a site of cord compression.

The spinal column is freed from the limbs, ribs and its superficial muscle attachments. It is then transected into several convenient segments by sawing through vertebral bodies. The siting of the cuts must be chosen to avoid the direct area of interest. Further cuts are then made through the arches and bodies of the vertebrae adjacent to the supposed clinical location of the lesion; these cuts should avoid the intervertebral joints.

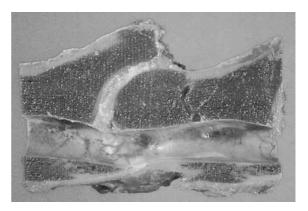


Figure 18.24 Longitudinal section of a vertebral segment exposing the spinal canal and intervertebral articulation.

In each short segment the spinal cord is removed by grasping the dura with forceps and cutting through the spinal nerve roots with a pair of scissors (Fig. 18.23). Appropriate cord sections can then be cut into short lengths (1 cm) for immersion in fixative. The associated sections of vertebrae can then be cut again in longitudinal section to examine the spinal canal and the intervertebral articulation (Fig. 18.24).

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CHAPTER 19

Sudden and unexpected death

Chapter contents

History 353

Examination of the environment 354 Potential sources of poison 354

Post-mortem examination 355

Causes of sudden death (death observed) 355

Causes of unexpected death (found dead) 356

Investigating causes of fatal poisoning 357

Feedstuffs 357 Industrial and agrochemical pollutants 358

Further reading 358

When a horse is seen to die suddenly it is usually an unexpected event. When a horse is found dead it may also be unexpected, but in that instance death may or not have been sudden. The distinction is important because the implicated causes are often different. Sudden death is frequently associated with exertion, but horses found dead may have suffered a disease process of several hours' duration. However, in either case the approach to investigation is the same.

A detailed history is required and the animal's environment must be examined carefully. These parts of the investigation assume paramount importance because there will be few, if any, clinical signs to indicate a cause. Detailed notes recorded at the time of the examination are essential and a photographic record is preferable in cases of potential litigation or insurance claim.

History

The previous veterinary history is required, together with details of the horse's management. Any changes in the feeding or exercise practice should be noted and the current health of any other horses in a group is relevant. The clinician should be aware that in some circumstances the handler might withhold vital information for fear of exposing negligence.

Recent drug administration should be considered with respect to an adverse response or overdosage. All drugs have the potential to provoke an adverse response. Fatal responses often take the form of a systemic hypersensitivity (anaphylactic response) within a short time of intravascular injection. Penicillin preparations are often incriminated. Where a reaction has followed a supposed intravenous injection into the jugular vein, the possibility of accidental intracarotid injection should also be considered.

The horse has a marked susceptibility to overdosage with certain drugs such as warfarin and phenylbutazone. Current therapy with either should be checked. Warfarin is used on rare occasions for the therapeutic treatment of navicular disease. Unless carefully monitored, the rate of blood coagulation can become critically extended and minor traumas can produce fatal haemorrhages. In the case of phenylbutazone, prolonged use at the upper therapeutic limits results in ulceration of the alimentary tract and a protein-losing enteropathy. Most usually, depression, inappetence, weight loss and ventral oedema are associated with low-grade colic. However, some cases may result in intractable colic and shock may be precipitated by extensive submucosal oedema of the large intestine.

Potential sources of poison should be considered within the history and the examination of the environment.

Examination of the environment

If possible this should be undertaken with the carcase in situ. There may be signs of recumbency and struggling before death, or a significant amount of haemorrhage may be seen. Following a recent storm, a horse found dead under a tree suggests lightning strike. Alternatively, the animal may be found near power lines, suggesting electrocution. Signs of struggling indicate a protracted death, as in a gastrointestinal catastrophe, whereas electrocution causes sudden death without struggle.

Owners often seize on poisoning as the explanation of sudden and unexpected death, but in reality it is an extremely uncommon cause. Nevertheless, the possibility should be investigated by considering the animal's feed and the possibility of industrial or agrochemical pollution.

Potential sources of poison

Feedstuffs

Poisonous plants. A huge number of native plants are potentially poisonous, but they are seldom

eaten by grazing horses unless driven by hunger or, significantly, they are trimmed, cut down or treated with herbicides. On these occasions, horses seem positively attracted to the wilting plant. Hedge, ditch, shrub, tree or weed material that is cut and dumped within the grazing area should be viewed with great suspicion. Samples should be held over and frozen for identification and further analysis.

In horses, plant poisoning most usually follows the chronic intake of plants whose toxic principles survive the haymaking process (e.g. ragwort, horsetail, bracken, St John's wort). Consequently, there are usually signs of toxicity long before death supervenes and sudden unexpected death is very unlikely. However, those plants that may be associated with rapid death include: black nightshade (*Solanum nigrum*); blue-green algae (*Microcystis* spp.); castor bean (*Ricinus communis*); oleander (*Nerium oleander*); water hemlock (*Cicuta* spp.) and Japanese yew (*Taxus* sp.).

Adulterated feed. Horses are exquisitely susceptible to ionophore toxicity. Ionophore antibiotics (e.g. monensin, salinomycin) are included in compounded livestock feeds as growth promoters or coccidiostats. The finished material has the appearance of a horse or pony cube and may be fed accidentally, or maliciously, resulting in a generalized myopathy, which includes cardiac myodegeneration with consequent dysrhythmia and failure. The clinical course is usually chronic, but sudden death may occur within 24 hours of intoxication. Reports occur from time to time of horse feeds that have been inadvertently contaminated at the feed mill.

Forage poisoning. Horses are particularly susceptible to botulism and the most usual source is big bale silage. Ingestion of the preformed toxin results in a generalized flaccid paralysis, the severity of which is directly proportional to the amount of toxin consumed. Characteristically, the horse is unable to retract its tongue once exteriorized at the interdental space. Death follows respiratory failure, but the clinical course can be extended over several days. Access to big bale silage on the premises should alert suspicion.

Industrial and agrochemical pollutants

Industrial emissions. For the most part, factory emissions are controlled by modern legislation, but the proximity of industry should be noted. Old industrial processes such as lead mining or smelting may have left dangerous amounts of residue in the topsoil. The course of lead poisoning in horses is usually chronic. Nevertheless, asphyxiation associated with laryngopharyngeal paralysis continues to be recorded in horses following chronic lead poisoning. On enquiry, it is likely that the locality is known to be a source of lead poisoning in horses.

Agrochemicals. Herbicides, fungicides, pesticides and fertilizers may either be ingested from the grazing and/or contaminated watercourses, or inhaled as a drifting aerosol spray. The use of agrochemicals within the grazing area should be checked and the possibility of rain 'wash-off' from land into adjacent watercourses should be considered.

Post-mortem examination

Once the history and examination of the environment are completed, the need for a post-mortem examination is considered. If other animals are at risk, or there is an insurance interest and/or prospect of litigation, the advisability of referring the carcase to a specialist diagnostic centre should be considered. However, the potential costs of post-mortem investigation must be kept in perspective, together with the realization that most surveys of sudden and unexpected death in adult horses show that 30% or more are unexplained, despite careful and extensive post-mortem study.

If post-mortem examination is elected but is not to be undertaken at a specialist centre, then the following notes on potential causes of sudden and unexpected death may prove helpful during the examination. However, the cause should not be prejudged by lists of possibilities such as these, and the clinician should resist the temptation to seize upon a convenient diagnosis. In all cases a systematic examination should be undertaken as outlined in Chapter 18: 'Post-mortem examination'.

Causes of sudden death (death observed)

During extreme exertion, as in racing, sudden death is often associated with haemorrhage into the lungs, thorax, abdomen or brain. That said, many instances of death during exertion are unexplained and in the particular instance of racehorses a toxicological examination is required, emphasizing again the need for specialist facilities and experience. Sudden unexpected death at rest is less common. It may be associated with an iatrogenic cause, e.g. an anaphylactic response to the administration of a drug. Alternatively, lightning strike, electrocution or poisoning is possible.

Some of the causes of observed sudden death are considered below by organ system.

Cardiovascular system

Massive internal haemorrhage. This is the commonest cardiovascular lesion causing sudden death in horses and usually follows the rupture of a major vessel at exercise. The pulmonary vessels are commonly involved and the usual result is a profuse nasal haemorrhage, although a vessel will occasionally rupture into the pleural space. Less commonly, increased intra-aortic pressure predisposes to tears in the aorta and the resultant haemorrhage into the pericardium can produce extreme pressure on the heart (cardiac tamponade). Haemorrhage from an aortic rupture may enter the thoracic cavity or dissect along the aorta into the abdominal cavity. Older breeding stallions occasionally succumb to rupture of the aorta during sexual activity. Haemorrhage may also be associated with bone fracture and laceration of an adjacent major vessel, or rupture of a verminous mesenteric arterial aneurysm. Occasional fatal haemabdomen may occur due to spontaneous or traumatic vascular rupture or haemorrhage from a vascular neoplasm such as phaeochromocytoma or renal carcinoma.

Rupture of the mitral chordae tendineae. In this instance, the obvious post-mortem finding is extensive pulmonary oedema. Examination of the heart at post-mortem requires careful dissection, otherwise the primary lesion is easily overlooked (see Ch. 18: 'Post-mortem examination').

Fatal dysrhythmia. This is not detectable at postmortem examination and is therefore a speculative conclusion, but it is often assumed to be the cause of sudden death at maximal exercise when postmortem abnormalities are undetected. Visible abnormalities of the myocardium warrant histopathology.

Respiratory system

Exercise-induced pulmonary haemorrhage. Severe engorgement of pulmonary vessels with massive haemorrhage into the alveoli, airways, interstitium and subpleural tissues are obvious post-mortem findings, but the aetiology remains somewhat controversial. The condition may be predisposed by chronic lung disease.

Pneumothorax. This is rare and is usually associated with trauma or penetrating wounds. It is easily missed at post-mortem examination since the normal lung will collapse as soon as the diaphragm is opened.

Central nervous system

Trauma. Running into solid objects, rearing over backwards or kicks to the head in foals may be associated with trauma and intracranial haemorrhage; with or without fracture of the skull.

Adverse drug responses

Acute respiratory distress as a result of pulmonary oedema or bronchospasm is often a feature of adverse drug reactions in horses. The gross postmortem findings can be unremarkable, but there may be froth in the airways and histopathological evidence of acute pulmonary oedema.

The accidental injection of a medication into the common carotid artery, rather than the jugular vein, is likely to cause sudden severe signs or death. These circumstances may then be mistaken for an anaphylactic response. In such cases a haematoma usually forms rapidly at the arterial puncture site. The site itself may be low in the neck, because the carotid is more superficial there and the accident is therefore more likely to occur at that point.

Poisoning

See below under 'Investigating causes of fatal poisoning'.

Causes of unexpected death (found dead)

Horses unexpectedly found dead may have suffered a more protracted death. In these circumstances it might be anticipated that a post-mortem examination will be more revealing, but this is not necessarily the case. The causes of unexpected death (found dead) can be the same as those of observed sudden death, but with additional possibilities. In this group, gastrointestinal lesions tend to be the commonest finding. It should be emphasized that 'unexpected death' assumes that the owner's opinion of previous good health is accurate and that some prior disease process has not been overlooked.

Gastrointestinal tract

Gut rupture. Rupture and peracute peritonitis usually follow an abdominal catastrophe. This is usually a sequel to colic, but on rare occasions the caecum may be ruptured during foaling. It should also be remembered that rupture could be a post-mortem artefact; haemorrhage or fibrin release at the margins of the tear are evidence of ante-mortem rupture.

Torsion and strangulation of the hindgut. This produces an overwhelming toxaemia, which is rapidly fatal.

Gross tympany of the hindgut. This may or may not be associated with torsion and strangulation (above), but the extensive tympany causes dyspnoea and circulatory failure.

Peracute enteritis with endotoxic shock. On rare occasions peracute colitis associated with virulent salmonellosis or *Clostridium* spp. may be rapidly fatal. There may be oedema and petechiation of the large bowel wall. Fresh caecal and colonic tissue and their contents should be submitted for culture and toxin assay.

Cardiovascular system

Slow exsanguination. This may be the result of damage to a medium-sized vessel following rupture or trauma. An example of the latter is rupture of the middle uterine artery during parturition in older brood mares.

Rupture of the internal carotid artery. The horse is found dead in a pool of blood discharged from

its nostrils. The lesion is associated with guttural pouch mycosis.

Venoms

Bee and wasp stings produce a local reaction, but repeated stings can produce a systemic response resulting in collapse and death.

Blister beetles can induce irritation of the alimentary tract (cantharidin toxicosis), sufficient to induce shock.

Snake bites can produce massive tissue reactions. Since the head region is likely to be attacked during contact at grazing, the reaction may be sufficient to interfere with respiration.

Other poisons

See below.

Investigating causes of fatal poisoning

The post-mortem examination in cases of suspected poisoning is usually non-specific and selection of tissues for histopathology and/or toxin analysis relies heavily upon conclusions drawn from the history and environmental examination. *If possible, it is extremely useful to obtain guidance from a veterinary toxicologist before undertaking the post-mortem examination.* In a potentially litigious case the clinician may prefer, depending upon experience, to refer the carcase to a specialist diagnostic laboratory.

It is a wise precaution to hold over samples suitable for toxicological analysis for as long as the investigation is pursued. Samples collected for toxin analysis should include: liver, kidney and fat (at least 200 g of each); stomach or intestinal contents (400 g); urine (100 ml), and serum from heart blood (20 ml). Consideration should also be given to storing samples from suspected sources of poison: feed; baits; soil or crop dressings; water and plants. Clean glass or plastic containers that can be tightly sealed are ideal. Each should be labelled with the owner and animal identification, the date, and the type of tissue or specimen contained. The samples are then frozen. If they are eventually to be submitted to a specialist laboratory, every attempt should be made to deliver the material quickly and in a frozen condition.

The laboratory analysis of tissues for specific toxins is highly specialized. The concept of 'screening' for poisons is unrealistic. If pursued, it would be prohibitively expensive and in all probability unrewarding. The most practical approach is to discuss the history, environmental and post-mortem findings with a veterinary toxicologist and then decide upon the most worthwhile tests to pursue. Before submitting material it is of the utmost importance to discuss the case with the toxicologist as follows:

- Report fully the history, environmental and post-mortem findings
- Decide in discussion the specific toxin test(s) to be undertaken
- Check which tissues/specimens are required, in what bulk, and how they should be packaged and dispatched
- Always warn the laboratory of any possible litigation, since the handling of samples and recording of results will need to follow a formal process of scrutiny.

NB: The isolation of a potentially poisonous substance in tissues is proof of exposure but not necessarily proof of poisoning, unless the amounts found are consistent with toxicity. For example, all horses grazing areas known to contain lead will have lead in their tissues, but not necessarily in toxic amounts. The interpretation of laboratory results must therefore be undertaken with the full guidance of the referral laboratory.

Feedstuffs

Poisonous plants

The post-mortem findings are often non-specific and the stomach contents should be checked for evidence of recent ingestion. However, plant material reaches the horse's stomach in a well masticated condition and it will be difficult to identify. A specimen of contents should be stored in case further investigation is required.

Yew is exceptionally poisonous; so much so that leaves may still be found in the mouth. As little as 100–200 g is fatal in horses.

Adulterated feed

Sudden death from the ingestion of ionophorecontaining feeds is likely to produce non-specific signs (heart failure), unlike the chronic situation where myopathy would become recognizable. If suspicious, samples of gut contents and a specimen of feed should be collected.

Forage poisoning

Post-mortem findings in cases of botulism are nonspecific and diagnosis is based on the identification of toxin in the serum, feed, liver or faeces. The toxin is extremely labile and samples should be frozen and submitted for assay as soon as possible; a negative result does not disprove botulism in horses. Evidence of secondary fermentation in big bale silage (ammoniacal smell and/or alkaline pH) indicates conditions favourable to the growth of *Clostridium botulinum*.

Industrial and agrochemical pollutants

Lead

Post-mortem findings are likely to be non-specific. Food debris in the trachea, or aspiration pneumonia, suggest ante-mortem dysphagia. In known areas of lead contamination, liver and kidney samples should be submitted for analysis; concentrations greater than 15 ppm support a diagnosis.

Agrochemicals

If a particular product has been implicated during the history and environmental investigation, it is worth contacting a hospital-based regional poison centre. In the UK, there is the Veterinary Poisons Information Service (subscription required; email: vpis@gstt.nhs.uk; tel: +44 207 635 9195 (London), +44 113 245 0530 (Leeds)). These centres are an excellent source of up-to-date information concerning the likely effects of poisoning with commercially available products. This enables a more focused post-mortem examination and helps to define the tests that should be undertaken by the veterinary toxicologist.

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INDEX

Abaxial sesamoid nerve block, 261 Abdomen auscultation, 35-36 post-mortem examination, 345-346, 347 ultrasonography, 54-55 Abdominal neoplasia, 45, 75 Abdominal paracentesis (peritoneal tap), 45-50 chronic wasting, 75, 76, 80 colic, 45, 47, 58 lymphosarcoma, 200 Abducens nerve (cranial nerve VI), 289, 291 Abortion, 132-133 Acarid mites, 329 Acer rubrum, 154 Acetate tape preparations, 337-338 Acid-base balance, 63, 203, 213-214 Acidosis, 211, 212 metabolic, 63, 113, 212, 214 respiratory, 214 Actinobacillus equuli, 234 Activated partial thromboplastin time see Partial thromboplastin time Acute phase proteins, 14, 16 Adenocarcinoma, 76 Adenovirus, 242 Adnexa, ocular, 309-310, 315 Adrenal glands, 346 Adrenaline (epinephrine), 94 Adrenocortical insufficiency, 93-94 Adrenocorticotrophic hormone, 89-90, 91, 93 stimulation test, 93 Adulterated feed, 354, 358 Adverse drug reaction, 356 Adynamic ileus, 38 Aflatoxins, 82 Agrochemical pollutants, 355, 358 Airflow sounds, 232 abnormalities, 231 adventitious, 233 Albinism, 335 Albumin: globulin ratios, 15-16 alimentary disease, 61 Albumin, 14, 15, 84, 208 alimentary disease, 61 measurement, 209 Alimentary disease, 27-69 causes of unexpected death, 356

Alimentary tract per rectum examination, 36-45 post-mortem examination, 345-347, 348 ultrasonography, 54-55 Alkaline phosphatase, 17, 62, 80, 82-83, 200, 202 peritoneal fluid, 49 Alkalosis, 211, 212 Allergen elimination tests, 341-342 Allergic skin disease, 328, 329, 330 Alopecia, 328, 330-331 Alopecia areata, 331 Alpha-2 globulin, 15, 16 Alpha-melanocyte stimulating hormone, 90 Amies charcoal transport medium, 7 Amylase, 17, 62 peritoneal fluid, 49 Amyloidosis, 334 Anaemia, 146-158, 169 blood loss, 146, 147-148 chronic, 63, 68 chronic wasting, 79 diagnostic approach, 167 dyserythropoiesis (decreased blood cell production), 146, 154-155 haemolytic, 146, 148-154 liver disease, 85 non-regenerative, 146 regenerative/responsive, 146, 147 'Anal rust', 330 Anaphylactic drug reaction, 353 Androgens, 99 Anoplocephala, 24, 67 Anorexia, 19, 84, 93, 149 Antebrachiocarpal joint, intrasynovial analgesia, 268 Anterior enteritis (proximal duodenojejunitis), 38, 57 Antibiotic sensitivity tests, 7-8 Anticoagulants, blood samples, 2, 3, 4 Antidiuretic hormone (arginine vasopressin), 95 Anti-erythrocyte antibodies, 152 Anthelmintic resistance, 66-67 Anuria, 114 Anus, sunken, 119 Aortic regurgitation, 173, 184-185 Aortic rupture, 194 Aortic thromboembolism, 192-193

Aortoiliac thrombosis, 255 Apex beat palpation, 174 Aplastic anaemia, 155 Aqueous flare, 313 Aqueous humour, 313, 315 Arabian fading syndrome, 335 Arboviruses, 294 Arginase, 83 Arrhythmias, 175, 178, 185-189 exercise tests, 182 sudden death, 356 Arterial blood collection, 241–242 Arterial blood gas analysis, 241, 244 Arterial blood pressure, 207-208 Arterial pulse, 172-173 Arteriovenous fistula, 193 Arthroscopy, 276 Artificial vagina, 139, 140 Arytenoid cartilage collapse, 222 range of movement, 230-231 Arytenoid depression test, 229-230 Ascarids, 24 Aspartate aminotransferase (AST; AAT), 17, 80, 83 musculoskeletal disease, 280, 281, 283 Ataxia, 94, 197, 292, 293, 296, 299 Atelectasis, 239 Atlanto-occipital space, cerebrospinal fluid collection, 302-303 Atopic dermatitis, 330, 340 Atrial fibrillation, 175, 184, 187-188 paroxysmal, 188 Atrial premature complexes, 186, 187 Atrioventricular block second-degree, 175, 178, 182, 185 third-degree, 186 Atypical myoglobinuria, 281-282 Aural plaques/hyperkeratosis, 334 Auriculopalpebral nerve block, 306-307, 320 Auscultation abdominal, 35-36 airflow sounds, 232 cardiac arrhythmias, 185, 186, 187, 188, 189 cardiac murmurs, 175-176, 183 cardiovascular system evaluation, 174-176, 182 equipment, 174

heart failure, 190 lower respiratory tract, 231 lung sounds, 231 proximal limb fracture, 254 thoracic, 231-232 Australian flatweed, 301 Avian tuberculosis, 65, 77 Azotaemia, 19, 93, 94, 109-110, 115 chronic wasting, 75, 80 Azoturia see Exertional rhabdomyolysis Babesia caballi, 149 Babesia equi, 149 Babesiosis, 149, 150 Back, 255-256 Back pain, chronic, 255 Bacteraemia, 165-166 Bacterial culture see Microbial culture Bacterial endocarditis, 76, 172, 191 Bacterial folliculitis, 330-331, 332 Base deficit, 214 Basophils, 14 Bee sting, 329, 357 Benzimidazole anthelmintic resistance, 66 - 67Beta-1 globulin, 15, 16, 61, 78 Beta-2 globulin, 15, 16 Beta-endorphin, 90 Bicarbonate, 210, 211-212 acid-base balance assessment, 214 alimentary diseases, 63 Bile acids, 19 chronic wasting, 80 enterohepatic cyle, 83 see also Total serum bile acids (TSBAs) Bile salt deficiency/dysfunction, 78 Biliary enzymes, 80, 82-83 Bilirubin, 19, 84, 148, 149 Biochemistry adrenocortical insufficiency, 93 alimentary disease, 60-62 chronic wasting, 79-80 interpretation of results, 14-21 liver disease, 82-85 parathyroid disorders, 99 peritoneal fluid, 49 phaeochromocytoma, 94 pleural fluid, 243 reference ranges, 25-26 samples, 4, 5 urinary tract disease, 108-113 see also Serum biochemistry profiles

Biopsy bone marrow aspiration, 155, 156-158 chronic wasting, 75 endometrial, 126-128, 136, 137 eyelids, 316-317 liver, 85, 86-88 lung, 237-238 lymph node, 195-197, 200 lymphosarcoma, 200 malabsorption, 78 myopathies, 281, 282 rectal, 53-54 renal, 107-108 skin, 330, 331, 333, 334, 339-340 specimens, 8 Biopsy needles, 86-87 Black flies (Simulium), 329 Black nightshade, 354 Bladder catheterization, 102-104 per rectum examination, 38, 102 ultrasonography, 107 Bladder pressure, 114 Bladder rupture, 45 abdominal paracentesis, 49 Bleeding time, 163 Blepharospasm, 306 Blood ammonia, 84 Blood culture, 165-166 samples/appropriate containers, 6, 7 Blood disorders, 145-166, 169-170 Blood glucose, 19, 84 equine metabolic syndrome, 93 Blood loss anaemia, 146, 147-148 Blood products, 209 Blood samples, 6, 7, 19, 146 blood ammonia, 84 electrolytes, 5, 210, 211 immune-mediated haemagglutination, 152 oral glucose absorption test, 64 platelet clumping, 161 Blood sampling systems, 2 Blood smear preparation, 3-4 Blood tumours, 164-165 Blood urea and creatinine, 19 Blue-green algae, 354 Blue-nose disease, 336 Body fluid samples, 6 'Bog spavin', 254 Bone marrow aspiration/biopsy, 146-147, 155, 156-158 'Bone spavin', 254 Borborygmi, 35

Borna virus, 294 Borrelia burgdorferi, 284, 285 Borreliosis (Lyme disease), 284-285 Botryomycosis, 333 Botulism, 296, 299-300, 354 Bracken, 354 Brain disorders, 293-297 post-mortem examination, 350-351 Brain abscess, 293 Breeding soundness mares, 118-129 semen, 139-142 stallions, 138-142 Bronchi, endoscopy, 221-222 Bronchoalveolar lavage, 233, 236-237 fluid cytology, 243 Bronchopneumonia, 233, 243 Brucella spp., 76 Brucellosis, 80, 284 Buphthalmos, 321 Burns, 208 Caecal intussusception, 41 Caecal obstruction/impaction, 40-41 Caecal torsion, 42 Caecum, 40-42 Calcium, 20-21, 98, 212 renal disease, 113 Calcium metabolism disorders, 97-98 Callipers, testicular measurement, 138 Cannon keratosis, 333 Capillary refill time, 57, 172 hypovolaemia, 206 Cardiac murmurs, 175-176, 179, 183-185 diastolic, 184-185 during exercise, 184-185 exercise tests, 182-183 point of maximum intensity, 176 presvstolic, 185 systolic, 183-184 Cardiac rhythm, 175, 177 during exercise, 178 Cardiac tamponade, 192, 355 Cardiac troponin I, 19, 191 Cardiovascular disease, 171-194 chronic wasting, 75-76, 79 sudden death, 355-356 unexpected death, 356-357 Cardiovascular system, 172-183 auscultation, 174-176, 182, 183 echocardiography, 179-182 electrocardiography, 176-179 exercise tests, 182-183

fluid balance (dehydration) assessment, 207-208 radiography, 182 Carotid artery, blood collection, 241 Carpus ('knee'), 253-254 radiographic projections, 273 Cartilage degeneration, 285 Castor bean, 354 Cataplexy, 294-295 Cataract, 313, 321 Catheter urine samples, 102, 103, 104, 108 Catheterization bladder, 102-104 female, 103-104 male, 102-103 guttural pouches, 229 nasolacrimal drainage apparatus, 319 ureter, 105 Central venous pressure, 173, 208 Cerebrospinal fluid analysis, 293, 294, 295, 297, 298, 304 Cerebrospinal fluid collection, 301 - 304atlanto-occipital puncture, 302-303 lumbosacral puncture, 303-304 Cervical diseases, 137 Cervical laceration, 137 Cervical tone, 121 Cervical vertebral malformation/ stenosis, 297 Cervicitis, 137 Cervix cyclical changes, 127 digital examination, 126 per rectum examination, 121, 129 pregnancy diagnosis, 129 Chest auscultation, 231-232 percussion, 233 post-mortem examination, 347, 348-349 radiography, 237 ultrasonography, 238-239 Chifney bit, 121 'Chiggers', 330 Chloride, 20, 208, 210, 211-212 alimentary diseases, 63 fractional excretion, 112 renal disease, 113 'Choke', 296 Cholangiohepatitis, 75 Cholelithiasis, 75 Cholesterol granuloma, 293

Chordae tendinae rupture, 184, 355 Chorioptes spp., 329, 337, 338 Chorioptic mange, 329, 332 Chromosome analysis, 128, 134 Chyloabdomen, 45 Chylous effusions, 243 Cicuta spp., 354 Clearance studies, 110-111 Clitoral swabbing, 120-121 Clostridial myositis (malignant oedema), 281 Clostridium botulinum, 299 Clostridium perfringens, 68 Clostridium sp., 68, 149, 281, 356 Clostridium tetani, 300 Clot retraction time, 163 Coagulation cascade, 162 Coagulation disorders coagulopathies, 161-162 liver failure, 85 Coagulation tests, 162-164 Coagulopathies, 158-164, 168 coagulation disorders, 161-162 thrombocytopenia, 160-161 vascular disorders, 159-160 Cobalt deficiency anaemia, 155 Coeliacomesenteric ganglion, 346-347 'Coffin joint' see Distal interphalangeal joint Cold ambient temperature, nutritional demands, 78 Colic, 35, 39, 55-60, 62, 63, 75, 93, 295, 298 abdominal paracentesis, 45, 47, 58 acute, 60, 62 chronic/recurrent, 59 differential diagnosis of behaviour, 56 fluid/electrolyte/acid-base disturbance, 215 gut sounds, 35, 36, 57 rectal examination, 36, 44, 57 stomach intubation, 57-58 Colitis, 53 Collagen necrosis, 334 Collagenolytic granuloma, 334 Colloid fluid replacement, 204, 208, 209, 210, 215 Colloid osmotic (oncotic) pressure, 208, 209 Colon per rectum examination, 37, 42-44 ultrasonography, 54-55

Colonic left dorsal displacement (nephrosplenic entrapment), 42-43, 55 Colonic obstruction, 42-43, 44, 55 Colonic right dorsal displacement, 43 Colonic torsion, 43-44 Colostrum, 17 Complement fixation test, 284 Computed tomography (CT) head trauma, 293 orbit, 321 paranasal sinuses, 226 seizures, 295 Conformation, 251 Congenital heart defects, 190 Congenital hypothyroidism, 97 Congenital soft palate lesions, 220 Conjunctiva, 310 biopsy, 316-317 Contact dermatitis, 330, 332, 341 Contact hypersensitivity, 330 Contagious equine metritis, 120 see also Taylorella equigenitalis Containers, sample, 5-6 Coombs antiglobulin test, 152, 153, 284 Copper deficiency anaemia, 155 Cornea, 310, 311, 312, 313, 322 sampling of lesions, 316 Corneal scrapings, 316 Corneal ulcer, 306, 316, 317, 318 Corpus haemorrhagicum, 122, 124 Corpus luteum, 122, 124, 129 Corticotropin-like intermediary peptide, 90 Cortisol, 91, 93, 323 Corvnebacterium pseudotuberculosis, 198 Coughing, 245, 248 Coumarins, 162 Crackles ('rales'), 233 Cranial nerves, 288-299 disorders, 293-297 Creatine phosphokinase (CPK; CK), 17, 80, 83, 191 musculoskeletal disease, 280, 281, 283 Creatinine clearance, 110, 111 Creatinine concentration, 62 chronic wasting, 80 dehydration, 207 Cruciate ligaments, 254 Crusting, 328, 331-333 Cryptorchidism, 22-23, 99-100, 142-143

Crystalloid fluid replacement, 204, 208, 215, 216 Culicoides, 329 'Curb' (plantar ligament inflammation), 254 Curschmann's spirals, 243 Cutaneous glanders ('farcy'), 198 Cutaneous trunci (panniculus) reflex, 292 Cutaneous vasculitis, 335 Cvanosis, 172 Cyathostominosis, 61 chronic wasting, 78, 80 Cyathostomins, anthelmintic resistance, 66 Cyclophilin B, 337 Cystitis, 114 Cystoscopy, 104 Cytology endometrial, 125, 126, 136 peritoneal fluid, 48-49 synovial fluid, 285 tracheal aspirates, 243 Cytopathology samples, 8 Dacryocystorhinography, 319-320 Damalinia equi, 329 Deficiency anaemias, 155 Dehydration, 20, 58, 62, 63, 77, 146, 204, 205, 209, 214-215 blood parameters, 206-207 cardiovascular system measurements, 207-208 clinical signs, 206 exertional, 216 serum biochemistry, 60 Demodex caballi, 331 Demodex equi, 331 Dental problems, 74, 77, 244, 296 Dental radiography, 31 Dental speculum, 30 Dependent oedema, 173 Dermatophilosis, 331, 332 impression smears, 338 Dermatophyte culture, 330, 331, 338-339 Dermatophytosis, 331, 332 Dermoid cysts, 334 Dexamethasone suppression test, 91 Diabetes insidius, 95 Diabetes mellitus, 96 Diarrhoea, 77, 211, 213, 214, 295 acute, 71 chronic, 53, 65, 71 cyathostominosis, 61

fluid/electrolyte losses, 62, 215-216 metabolic acidosis, 63 severe (fluid faeces), 68 Diascopy, 339 Diastolic murmurs, 184-185 Dictyocaulus arnfieldi, 24, 243 Diffuse cerebral disease, 295 Digital imaging systems, 271 Digital tendon sheath, intrasynovial analgesia, 267 Direct ophthalmoscopy, 313-315 Disseminated intravascular coagulation, 14, 161, 205, 210 Distal interphalangeal ('coffin') joint, 252, 253 intrasynovial analgesia, 266 Distal intertarsal joint, intrasynovial analgesia, 269 Digital pulse, 252 Diverticulitis, 229 Domperidone challenge test, 91 Doppler echocardiography, 180-181, 184, 190 Doppler ultrasound, vascular disease, 192, 193 Dorsal metacarpal periostitis, 253 'Dropped elbow', 301 Drug hypersensitivity, sudden death, 353-354 Drug-induced anaemia, 151, 155 Ductus deferens, sample culture, 139 Dyserythropoiesis, 146, 154-155, 169 Dysphagia, 32, 33, 70 chronic wasting, 76-77 neurological disease, 296-297 Eastern equine encephalitis virus, 294 Ecchymotic haemorrhages, 158, 170 Echocardiography, 179-182, 184 bacterial endocarditis, 191 heart failure, 190 Holter monitoring, 191 major vessel rupture, 193, 194 pericarditis, 192 ventricular septal defect, 190 Ectoparasites, 328, 329-330 Ectopic ventricular beats, 185

161

Effusions, 49-50

178

Ehrlichia equi, 149, 150

Electrolytes, 19-21, 203, 210-213 blood samples, 5 deficit calculation, 212-213 fractional excretion, 111-112 Elliptical skin biopsy, 340 Embryo, ultrasonography, 130-131 Endocarditis, 76, 172, 191 Endocrine diseases, 89-100 Endocrine tests blood samples, 5 cryptorchidism, 142 pregnancy diagnosis, 131-132 result interpretation, 21-23 Endometrial biopsy, 126-128, 137 acute endometritis, 136 Endometrial cyclical changes, 128 Endometrial cysts, 136-137 Endometrial cytology, 125, 126 acute endometritis, 136 Endometrial swabbing, 124-125, 126 Endometrial transluminal adhesions, 137 Endometriosis, 137 Endometritis, acute, 135-136 Endoparasites, 66-67, 75, 78, 330 Endophthalmitis, 321 Endoscopy bronchoalveolar lavage, 236 guttural pouches, 228-229, 297 larynx, 230-231 nasopharynx, 33-34 oesophagus, 34-35 paranasal sinuses, 223, 226-227 tracheal fluid aspiration, 235-236 upper alimentary tract, 33-35 upper respiratory tract, 218-222 EDTA (ethylenediamine tetra-acetic uterus (hysteroscopy), 128-129, acid), 3, 7, 8, 14, 46, 146, 152, 137 Endotoxaemia, 172, 205 Enophthalmos, 310 Enteritis, peracute, 356 Einthoven limb lead system, 176, 177, Enterolithiasis, 44, 75 Eosinophilia, 63-64

Elbow, 254

189

heart failure, 190

renal disease, 113

intrasynovial analgesia, 268

Electrocardiography, 176-179

during exercise, 178, 185

Holter monitoring, 179, 186

Electrolyte disturbances, 214-216

alimentary disease, 62-63

radiographic projections, 273

arrhythmias, 185, 186, 187, 188,

Eosinophilic enterocolitis, 77 Eosinophilic gastroenteritis, 65 Eosinophilic granuloma, 334 Eosinophils, 13 peritoneal fluid, 49 Epididymis, 138 infection, 139 Epiglottic entrapment, 221, 222 Epiglottic hypoplasia, 221 Epiglottic retroversion, 222 Epiglottis, 221 Epiphora, 310 Epitheliogenesis imperfecta, 336 Epizootic lymphangitis, 198 Equine chorionic gonadotrophin, 21, 132 Equine Cushing's syndrome (pituitary pars intermedia dysfunction), 19, 23, 89-91, 94, 277, 331 Equine degenerative myeloencephalopathy, 299 Equine demodicosis, 331 Equine ehrlichiosis, 149, 150 Equine granulomatous disease (equine sarcoidosis), 77, 334 Equine herpesvirus type 1, 242 myeloencephalopathy, 298, 304 Equine herpesvirus type 4, 242 Equine infectious anaemia, 76, 80, 149-150, 152, 160 Equine influenza virus, 242 Equine metabolic syndrome, 91-93 Equine motor neuron disease, 78-79, 299 Equine protozoal myeloencephalitis, 297-298 Equine recurrent uveitis, 313 Equine sarcoid see Equine granulomatous disease Equine viral arteritis, 133, 159-160, 242 Equine viral papillomatosis, 333 Equine viral rhinopneumonitis, 132-133 Erosions, 328, 331, 335-337 Erythema multiforme, 336 Erythrocyte parameters, 11-12, 146 alimentary disease, 63 respiratory disease, 245 Erythrocyte-bound immunoglobulins, 151, 152, 153 Erythrocytosis (polycythaemia), 164 Escherichia coli, 136 Ethmoidal haematoma, 219 Excisional biopsy, 339-340

Exercise cardiac murmurs, 184-185 electrocardiography, 178, 185 intolerance, 93 nutritional demands, 78 Exercise tests, 182-183 Exercise-induced pulmonary haemorrhage, 222, 356 Exertion-related sudden death, 355 Exertional dehydration, 216 Exertional rhabdomyolysis, 255, 283 acute, 280-281 recurrent, 281 Exophthalmos, 310 Extension tests, lameness, 258 Extracellular fluid, 208, 211 osmolality calculation, 210 Extravascular haemolysis, 148, 149 immune-mediated, 152-153 Extravascular space, 208 Exudates, 50, 238, 243 Eye, 309-310, 311, 315 anterior segment, 311, 312, 322 post-mortem examination, 351 surface, 310 see also Ocular examination Evelids, 310 biopsy, 316-317

Facial artery blood collection, 242 pulse, 173 Facial nerve (cranial nerve VII), 290, 291 Faecal analysis, 66-69 Faecal blood, 68 Faecal egg count (FEC), 23-24, 148 chronic wasting, 80 intestinal parasites, 66, 80 response to anthelmintic, 66 Faecal egg count reduction (FECR), 66-67 Faecal larvae, 24, 67 Faecal leukocytes, 68 Faecal samples, 6, 7 Faecal sand, 68-69 Faecal tapeworm segments, 24 Farcy (cutaneous glanders), 198 Fasciola hepatica, 85 Fat diseases, 323-325 Fat necrosis, 323, 325 Fat tumours, 323, 324-325 Fatal dysrhythmia, 356 Fatty liver disease, 82, 85, 324

Femoropatellar joint effusion, 254 intrasynovial analgesia, 269 Fertility, 118-143 Fetlock, 253 Fibrin degradation products, 162 Fibrinogen, 14, 85, 208, 209, 283 alimentary disease, 63, 64 chronic wasting, 76, 79 lymphosarcoma, 201 'Fistulous withers', 284 Flaviviridae, 294 Flexion tests, lameness, 257 Flies, biting, 329 Fluid balance, 203, 204-208 calculation of losses, 212-213 osmotic equilibrium, 208 plasma protein status, 208-210 Fluid balance disorders, 214-216 alimentary disease, 62 Fluid replacement therapy, 204, 208, 209, 213, 215 Fluorescein, 317-318, 319 Fly-borne disease, 149 Foals, sickness in newborn, 132-133 Folate deficiency anaemia, 155 Food allergy, 78, 330 elimination diets, 341-342 Foot, 251-253 radiographic projections, 273 Foot flight patterns, 257 Forage poisoning, 354, 358 Forelimb, 251-254 intrasynovial analgesia, 266-268 sites of nerve block, 261-264 Founder, 277 Four-point nerve block, 261-263, 264 Fresh frozen plasma, 209 Fumonisin mycotoxin, 295 Functional cardiac murmurs, 183, 184 Fundus examination, 313, 314 Fungal (dermatophyte) culture, 338-339 Fungal infection, 76, 133 Gait, 256-257, 292-293 'Gallop rhythm', 175, 178 Gamma globulin, 15, 16 Gamma glutamyl transferase (GGT), 18, 80, 82, 112 Gastric fluid aspiration, 57 Gastric reflux, 52 Gastric tumours, 54 Gastric ulceration, 75

Gastrointestinal bleeding, occult, 68

Genital disease, 118-143 differential diagnosis in mare, 133-138 Genital tract, mares cyclical changes, 122 per rectum examination, 121-122 ultrasonography, 122-124 Genodermatoses, 335 Glaucoma, 320 Globulins, 14, 15, 16, 208 alimentary disease, 61 chronic wasting, 79 measurement, 209 Glomerular filtration rate, 109-111 Glomerulonephropathy, 61 Glossopharyngeal nerve (cranial nerve IX), 290, 292 Glucosuria, 23 Glutamate dehydrogenase (GLDH), 18, 80, 83 Glutathione peroxidase, 18, 284 Glycogen branching enzyme deficiency, 282 Glycogen synthase mutation (GYS1), 281 Goitre, 96-97 Granulomatous enteritis, 65, 77 Granulosa cell tumour, 99, 134, 135 Grass sickness, 33, 34, 35, 56, 75, 296, 300 'Greasy heel' reaction (pastern dermatitis), 330, 331-333 Gut rupture, 44, 356 Gut sounds colic, 57 see also Abdomen, auscultation Gut tap, accidental, 46, 47 Guttural pouch disease, 229, 296 Guttural pouches, 228-229 endoscopy, 219-220, 297 samples, 242 Habronemiasis, 333 Haemangiosarcoma, 76 Haematobia irritans, 329 Haematology alimentary diseases, 63-64 blood smear preparation, 3-4 chronic wasting, 79 dehydration, 206-207 interpretation of results, 11-14 liver disease, 85 lymphosarcoma, 201 musculoskeletal disease, 283

respiratory disease, 244-245 samples, 3, 5 urinary tract disease, 113 Haematoma brain, 293 ethmoidal, 219 Haematopinus asini, 329 Haematuria, 280 Haemoglobin, 12, 147, 148, 149 immune-mediated haemolytic anaemia, 152, 153 Haemoglobinaemia, 148, 153 Haemoglobinuria, 23, 148, 280 Haemolysis, 146, 148, 149, 169 Haemolytic anaemia, 148-154 infectious causes, 149-151 Haemorrhage, 158, 169, 205, 208 acute, 63, 147 chronic, 147-148 major vessel rupture, 193-194 slow exsanguination, 356 sudden death, 355 Haemosiderin deposition, 82 Hair growth changes, 90, 93, 328, 330-331 Harvest mite, 330 Head neurological examination, 288 post-mortem examination, 350-351 trauma, 293 Head shaking, 296 Hearing loss, 291 Heart failure, congestive, 76, 172, 173, 174, 184, 189-190, 233 Heart, post-mortem examination, 348-349 Heart rate, 56-57, 175 exercise tests, 183 Heart sounds, 174-175 Heinz body anaemias, 154 Hepatic encephalopathy, 81, 82, 84, 295, 297, 324 Hepatic portal vein, 86 Hepatic veins, 86 Hepatitis, chronic active, 75, 82 Hepatocellular enzymes, 80, 83 Hereditary equine regional dermal asthenia (HERDA), 337 Hetastarch, 209, 210, 215 Hindgut torsion/strangulation, 43-44, 356 Hindlimb, 254-255 intrasynovial analgesia, 268-270 sites of nerve block, 264-265

Hip, 255 radiographic projections, 274 Hirsutism, 328, 331 Histoplasma farciminosum, 198 Histoplasmosis, 77 Hock, 254 radiographic projections, 274 Holter monitoring, 179, 186, 191 Hoof, 251-252 Hoof testers, 252 Hoof-pastern axis, 251 Horn flies, 329 Horner's syndrome, 296 Horse flies (Tabanidae), 329 Horsetail poisoning, 354 Human choronic gonadotrophin stimulation test, 142 Hymen, persistent, 119, 138 Hyperadrenocorticism, 19, 331 Hyperammonaemia, 84, 295 Hyperbilirubinaemia, 149, 153, 304 Hypercalcaemia, 20, 99, 113 Hyperchloraemia, 20 Hyperdynamic shock, 205 Hyperfibrinogenaemia, 201, 208, 209 Hyperglobulinaemia, 208, 209 liver failure, 84 lymphosarcoma, 202 Hyperglycaemia, 19, 84 Hyperkalaemia, 20, 93, 94, 113, 211 Hyperkalaemic periodic paralysis, 282 Hyperlipaemia, 21, 85, 323-324 Hyperlipidaemia, 324 Hypernatraemia, 20, 210, 211 Hyperparathyroidism, 98-99 Hyperphosphataemia, 94, 99 Hyperplastic lymphoid nodules, 219 Hyperproteinaemia, 209 lymphosarcoma, 202 Hypersensitivty reactions, 340-342 Hyperthyroidism, 96, 97 Hyphaema, 313 Hypoalbuminaemia, 60, 61, 84, 199, 202, 208, 209 chronic wasting, 75, 78, 79 Hypocalcaemia, 20, 77, 94, 98, 99, 113, 212 tetany, 98 Hypochloraemia, 20, 93, 113, 212, 214 Hypoderma spp., 334 Hypodynamic shock, 205 Hypofibrinogenaemia, 208, 209 Hypoglossal nerve (cranial nerve XII), 290, 292 Hypoglycaemia, 19, 84, 93

reference ranges, 25-26

Hypokalaemia, 20, 113, 211 Hypomagnesaemia, 99, 212 Hyponatraemia, 20, 93, 94, 113, 210, 211 Hypoparathyroidism, 98-99 Hypophosphataemia, 99 Hypoproteinaemia, 173, 208-209 Hypopyon, 313 Hypothyroidism, 23, 96, 97 congenital, 97 Hypovolaemia, 63, 204, 205, 206, 209, 215 Hypovolaemic shock, 147 Hypoxaemia, 244 Hypoxia, 205 Hypoxic ischaemic encephalopathy (neonates), 297 Hysteroscopy, 128-129, 137 Idiopathic hyperammonaemia, 295 Idiopathic (immune-mediated) thrombocytopenia, 160, 161 Ileal impaction, 38 Ileocaecal intussusception, 35, 38-39 Ileocaecal valve activity, 35 Iliac thromboembolism, 192-193, 255 Immune-mediated haemolytic anaemia, 148, 151-153 Immune-mediated (idiopathic) thrombocytopenia, 160, 161 Immune-mediated myositis, 283 Immunoglobulin G (IgG), 17 Indirect ophthalmoscopy, 313 Industrial pollutants, 355, 358 Infection abortion/stillbirth, 132-133 chronic wasting, 76 haemolytic anaemia, 149-151 lymphangitis, 198 nodular skin disorders, 333-334 Inflammatory bowel disease, 61, 75, 77,78 Inflammatory disease, anaemia, 154-155 Inguinal canals, 38, 39 Inguinal hernia, 39 Inhibin, 135 Insulin resistance, 84, 91-93, 323 Intensifying screens, 271 Internal carotid artery rupture, 356-357 Intestinal alkaline phosphatase (IAP), 17 Intestinal neoplasia, 55

Intestinal parasites, 66-67 Intracellular fluid, 208, 211 Intracranial haemorrhage, 356 Intradermal testing, 330, 340-341 Intraocular pressure, 320 Intrasynovial analgesia, 259, 265-270 forelimb, 266-268 hindlimb, 268-270 Intravascular haemolysis, 148, 149 immune-mediated, 151-152 Intravascular space, 208 Iodine imbalance, 96-97 Ionizing radiation regulations, 31 Ionophore toxicity, 354 Iris, 311, 312, 315, 322 Iris cyst, 321 Iris neoplasia, 321 Iron deficiency anaemia, 155 Ischaemic-reperfusion injury, 212

Japanese encephalitis virus, 294 Japanese yew, 354 Jaundice, 19, 84, 170 haemolysis, 148, 149 Jugular pulse, 173 Jugular vein distension, 173 Junctional epidermolysis bullosa, 337

Keratoconjunctivitis, 159, 160 Keratoconjunctivitis sicca, 318 Kidnevs per rectum examination, 37, 102 post-mortem examination, 346, 347, 348 ultrasonography, 55, 105, 106-107 Klebsiella pneumoniae, 136, 139 Labelling, samples for transport, 9 Laboratory reference ranges, 10, 14 Lacrimal apparatus, 310 Lacrimal puncta, 318, 319 Lactate dehydration, 207 pleural fluid, 244 Lactate dehydrogenase (LDH), 18, 83, 191 Lactation, nutritional demands, 78 Lactation tetany, 98 Lameness, 250-258, 284, 292 nerve blocks, 259 provocation tests, 257-258 Laminin-5, 337 Laminitis, 75, 90, 91, 92, 252, 277-280, 350 Laparotomy, 58

Laryngeal hemiplegia/paralysis, 33-34, 229, 230, 231, 292 Laryngopalatal dislocation, 220, 221 Larvnx, 229-231 endoscopy, 221 Lateral femorotibial joint, intrasynovial analgesia, 270 Lateral palmar nerve block, 263 Lawsonia intracellularis, 77, 80 Lead poisoning, 155, 296, 355, 358 Lens, 312, 313, 315, 322 luxation, 321 rupture, 321 Leptospira, 76, 149, 150 Leptospirosis, 80, 149, 150-151 Lethal lavender foal syndrome, 335 Lethal white foal syndrome, 335 Leukocyte parameters, 12-14 alimentary diseases, 63-64 Leukocytoclastic (photoaggravated) vasculitis, 332, 335-336 Leukocytosis, 13 alimentary diseases, 63 chronic wasting, 79 Leukoderma, 335 Leukoencephalomalacia, 295 Leukopenia, 12-13 alimentary disease, 63 Leukotrichia, 335 Lice, 329 Lidocaine, 259 Limbs neurological examination, 292 pain, chronic wasting, 75 Linear alopecia, 331 Lipase, 17, 62 Lipomas, 324, 325 Lithium heparin anticoagulant, 4, 210 Liver biopsy, 85, 86-88 post-mortem examination, 348 ultrasonography, 85-86 Liver disease, 17, 18, 19, 81-88, 295 acute, 81 chronic, 81-82 chronic wasting, 75, 79 coagulation disorders, 162 Liver enzymes, 80, 83 chronic wasting, 75, 80 liver disease, 82-83 Liver failure, 61, 81, 84, 85, 148, 295, 324 Liver fluke (fascioliasis), 82, 85 Liver function tests, 83-85 chronic wasting, 75

Liver tumours, 82 Local anaesthetic solutions, 259 Local analgesia, 259-270 ocular examination, 306-308 'Lockjaw', 300 Lolitrem, 299 Lower respiratory tract, 231-242 auscultation, 231 collection of samples, 233 Lumbosacral space, cerebrospinal fluid collection, 303-304 Lung biopsy, 237-238 Lung consolidation, 231, 238, 239 Lung sounds, 231 Lungworm, 24, 243 Lyme disease, 284-285 Lymph node biopsy, 195-197, 200 Lymphadenitis, 195 Lymphadenopathy, 195 Lymphangitis, 197-198 epizootic, 198 infectious, 198 sporadic, 197-198 ulcerative, 198 Lymphatic disease, 195-202 Lymphocytes, 13 peritoneal fluid, 49 Lymphocytic-plasmacytic enteritis, 77 Lymphoid hyperplasia, 33 Lymphoplasmacytic hepatitis, 82 Lymphosarcoma, 16, 65, 151, 164, 195, 198-202 abdominal (alimentary form), 199-200 chronic wasting, 76, 77, 79 cutaneous, 201, 335 multicentric (generalized form), 200 thoracic (mediastinal form), 200 Magnesium, 21, 212 Magnetic resonance imaging head trauma, 293 musculoskeletal system, 276 orbit, 321 paranasal sinuses, 226 seizures, 295 Major vessel rupture, 193-194 Malabsorption, 53, 64-66, 199 chronic wasting, 77-78 Malassezia dermatitis, 331, 332 Maldigestion, chronic wasting, 77-78 Malignant hyperthermia, 283 Malignant oedema (clostridial myositis), 281

Manipulation of limb joints, 251 Massive exudative effusion, 61 Massive internal haemorrhage, 355 Mast cell tumour, cutaneous, 335 Mean corpuscular haemoglobin concentration (MCHC), 12, 149 Mean corpuscular haemoglobin (MCH), 12, 149 Mean corpuscular volume (MCV), 12, 147 Medial collateral ligament, 254 Medial femorotibial joint, intrasynovial analgesia, 270 Medial palmar nerve block, 263 Median artery pulse, 173 Median nerve block, 263 'Medullary washout', 95 Mega-oesophagus, 32, 33 Melaena, 68 Melanoderma, 335 Melanoma, 76, 334 Melanotrichia, 335 Menace reponse, 309 Meningitis, 293, 295, 297 Mepivacaine, 259 Mesenteric abscess, 44, 75 Mesenteric tumour, 44 Mesentery, root, 37 Mesothelioma, 76 Metabolic acidosis, 63, 212 bicarbonate therapy, 214 renal failure, 113 Metabolic alkalosis, 212-213 Metacarpal stress fracture, 253 Metacarpophalangeal (fetlock) joint intrasynovial analgesia, 267 radiographic projections, 273 Metacarpus, 253 radiographic projections, 273 Metatarsophalangeal joint, 273 Metatarsus, radiographic projections, 273 Methaemoglobin, 154 Microangiopathic haemolytic anaemia, 148, 153-154 Microbial culture abortion/stillbirth, 132, 133 acute endometritis, 135-136 bacteraemia, 165-166 faecal samples, 67-68 nasal/nasopharyngeal swabs, 242 respiratory disease, 245 samples, 6, 7-8 synovial fluid, 285 tracheal aspirates, 234, 235

Microcystis spp., 354 Microsporum equinum, 331 Midcarpal joint, intrasynovial analgesia, 267 Middle uterine artery rupture, 356 Midstream urine samples, 6, 108 Mites, 337 Mitochondrial myopathy, 283 Mitral regurgitation, 172-173, 180, 182, 184 Monday morning disease see Exertional rhabdomyolysis Monensin toxicity, 191 Monoclonal gammopathy, 202 Monocytes, 13 Mononuclear cells chronic wasting, 79 peritoneal fluid, 49 Mouldy corn poisoning, 295 Mouth examination, 28-30 post-mortem, 347, 348-349 Mouth odour, 30 Mouth wedge, 29-30 Mucous membranes, 57, 172 'Mud fever' syndrome, 330, 331-332 Multiple myeloma, 165 Multiple organ dysfunction syndrome (MODS), 205 Multisystemic eosinophilic epitheliotropic disease, 77 Muscle biopsy, 281, 282 Muscle enzymes, 280, 281, 282, 283 Muscle wasting, 254, 255 Musculoskeletal diseases, 249-285 chronic wasting, 75 Musculoskeletal system imaging techniques, 270-276 post-mortem examination, 350 Mycobacterium spp., 76 Mycoplasma spp., 234 Mydriatics, 312, 313 Myelogenous leukaemias, 164-165 Myeloid:erythroid cell ratio, 158 Mvelophthisis, 155 Myeloproliferative disease, 155 Myocardial disease, 174, 190-191 Myocarditis, 190 Myoglobinuria, 23, 280, 281 atypical, 281-282 Myopathy, 17, 75, 77, 280-283 chronic wasting, 80 Myotonic dystrophy, 282

Narcolepsy, 294–295 Nasal chambers, 218–219 Nasal discharge, 245, 246-247, 310 Nasal ostium, 318 Nasal swabbing, 242 Nasogastric intubation, 50-53 Nasogastric tubes, 50 Nasolacrimal drainage apparatus, 317, 318-320 Nasopharyngeal collapse, 222 Nasopharynx endoscopy, 33-34, 219 swabbing, 242 Navicular bursa, intrasynovial analgesia, 266 Navicular disease, 75 Neck, neurological examination, 292 Necrotizing pancreatitis, acute, 49, 62 Needle core biopsy, 196-197 Neoplastic disease abdominal, 45, 55, 75 anaemia, 155 chronic wasting, 76 evelids, 310 nodular skin disease, 334-335 ocular/orbital, 321 Neospora hughesi, 297 Nephrosplenic entrapment, 42-43, 55 Nephrosplenic ligament, 37 Nerium oleander, 354 Nerve block, 259-270 forelimb, 261-264 hindlimb, 264-265 ocular examination, 306-308 Neurological disease, 287-304 Neurological examination, 288-293 Neuropathies, 299-301 Neutropenia, 13 Neutrophilia, 13, 63 chronic wasting, 79 Neutrophils, 13 peritoneal fluid, 48-49 'Nits', 329 Nodular necrobiosis, 334 Nodular panniculitis, 334 Nodular skin disease, 328, 333-335 Noradrenaline (norepinephrine), 94 Nuclear scintigraphy musculoskeletal system, 275-276 paranasal sinuses, 228 Nutrient intake, inadequate, 73-78 hypokalaemia, 211 Nutritional muscular dystrophy (white muscle disease), 282, 284 Nystagmus, 291, 292

Obesity, 91, 92, 323 Obstructive gastrointestinal disease, 38, 39-40, 213, 214 gut sounds, 35 per rectum examination, 38 Ocular discharge, 310 Ocular disease, 305-322 Ocular examination, 305-315 biopsy, 316-317 in the dark, 311-315 diagnostic imaging, 321-322 direct ophthalmoscopy, 313-315 indirect ophthalmoscopy, 313 in the light, 308-311 pen light examination, 311-312 slit lamp biomicroscopy, 312-313 smears, 316 swabs/scrapes, 316 topical ophthalmic stains, 317-318 Ocular foreign body, 311, 321, 322 Oculomotor nerve (cranial nerve III), 288, 289 Oedema, 209 malignant (clostridial myositis), 281 Oesophageal diverticulum, 32 Oesophageal stricture/obstruction, 32-33, 34, 35, 296 Oesophageal transit time, 33 Oesophagitis, 32, 34 Oesophagus endoscopy, 34-35 radiography, 32 Oestrogens, 99 Oestrone sulphate cryptorchidism, 22, 142 pregnancy diagnosis, 21, 131-132 Oestrus cycle cervical changes, 127 endometrial changes, 128 endometrial swabbing, 125-126 genital tract per rectum examination, 122 progesterone assay, 128 ultrasonography, 123 Oleander, 354 Olfactory nerve (cranial nerve I), 288, 289 Oliguria, 114 Onchocerciasis, 335, 340 One-stage prothrombin time see Prothrombin time Optic nerve (cranial nerve II), 288, 289, 313, 315

Oral glucose absorption test, 64-65, 200-201 chronic wasting, 75, 78, 79, 80 Orbit, radiography, 321 Orbital foreign body, 311, 321, 322 Orbital lesions, 321, 322 Oropharynx, radiography, 31-32 Osmotic equilibrium, 208 Osmotic fragility test, 152 Osteoarthritis, 75 Osteodystrophia fibrosa, 99 Otitis interna/media, 297 Ovarian abscess, 135 Ovaries enlarged, 134 per rectum examination, 122, 129, 134 pregnancy diagnosis, 129 small, 133-134 ultrasonography, 123-124, 134 Ovulation, 122, 123, 124 Oxyuris equi (pinworm), 67, 330, 338 Packaging, samples for transport, 8-9 Packed cell volume (PCV), 12, 58, 60, 62, 63 anaemia, 146, 147, 149, 152, 153, 155 dehydration, 206-207 fluid/electrolyte/acid-base disturbance, 206-207, 215 Pain, chronic wasting, 75 Palatal arch, 220-221 Palmar metacarpus nerve block, 261, 262-263 ultrasonography, 274, 275 Palmar nerve block, 261, 262 Palpebral nerve block, 307 Pancreatic enzymes, 62 Pancreatic necrosis, 17 Pancreatitis, 45, 62 acute necrotizing, 49, 62 Panniculitis, 325 nodular, 334 Papules, 328, 329, 333 Paranasal sinuses, 222-228 computed tomography, 226 endoscopy, 223, 226-227 magnetic resonance imaging, 226 nuclear scintigraphy, 228 percussion, 222-223 radiography, 225 sinoscopy, 226-227 sinus centesis, 223-225

trephination, 227-228 trephine hole sites, 226 Paraovarian cyst, 134 Parasitic disease eosinophilia, 63-64 test result interpretation, 23-24 see also Ectoparasites; Endoparasites Parathyroid disorders, 98-99 Parathyroid hormone, 98, 99 Paresis, 293 Partial thromboplastin time, 162, 163 Pastern, 253 radiographic projections, 273 Patellar intermittent upward fixation, 254 Pelvic flexure impaction, 42 Pelvic fracturs, 255 Pelvis, 255 Pemphigus foliaceus, 333 Pen light, ocular examination, 311-312 Penicillin hypersensitivity, 353 immune-mediated haemolytic anaemia, 151 Penis, 138-139 Pentastarch, 210 Percussion, paranasal sinuses, 222-223 Perennial rye grass, 336 Pericardial effusion, 174, 192 Pericardiocentesis, 192 Pericarditis, 76, 192 Perineal examination, mares, 118-119 Perineal reflex, 292 Perineural infiltration analgesia, 259-261 needle placement, 260-261 Peristalsis, gut sounds, 35 Peritoneal fluid, 47, 48-50, 86 samples, 345-346 Peritoneal tap see Abdominal paracentesis Peritonitis, 35, 57, 75 abdominal paracentesis, 45, 47, 48 chronic wasting, 76 Peroneal nerve block, 264-265 Peroneal nerve injury, 301 Peroneus tertius rupture, 254 Petechial haemorrhages, 158, 159, 160, 170 Phaeochromocytoma, 94 Pharyngeal cysts, 221 Pharyngeal paralysis, 32, 34, 219, 292

Phenothiazine idiosyncratic reaction, 154 Phenylbutazone, 55 immune-mediated haemolytic anaemia, 151 overdose, 354 Phosphorus, inorganic, 21 fractional excretion, 112 Photoaggravated vasculitis, 335-336 Photosensitivity, 332, 336 liver disease, 82 Phylloerythrin, 336 Physostigmine salicylate provocation test, 295 Pigmentation changes, 328, 335 Pinworm (Oxyuris equi), 67, 330, 338 Pituitary function, 23 Pituitary pars intermedia dysfunction see Equine Cushing's syndrome Placental examination, abortion/ stillbirth, 132, 133 Placental insufficiency, 133 Placental premature separation, 133 Plant toxins, 82, 354, 357 Plantar ligament inflammation ('curb'), 254 Plantar metatarsus nerve block, 264 ultrasonography, 274, 275 Plantar nerve block, 264 Plasma bicarbonate, 214 Plasma cell myeloma, 16, 76, 165 Plasma fibrinogen see Fibrinogen Plasma proteins, 209-210 Plasma samples, 4 Plasma volume, 209 Platelet counts, 161 thrombocytopenia, 160 Platelets, 14 Pleural abscess, 231 Pleural effusions, 200, 231, 233, 237, 238, 239, 243 Pleural fluid, 243-244, 347 sampling, 240 Pleural friction sounds, 233 Pleuritis, 233, 239 Pleuropneumonia, 245 Pleuroscopy (thoracoscopy), 239 - 240Pneumonia, 232, 237, 243, 245 Pneumothorax, 231, 239, 356 Pneumouterus, 120 Pneumovagina ('windsucking'), 118, 119-120, 137-138

Poisoning, 354-355, 357-358 dyserythropoiesis, 155 post-mortem sample collection, 350 'Poll evil', 284 Polycythaemia, 164 Polydipsia/polyuria, 90, 94-96, 111, 114-115 Polymerase chain reaction, 281, 284, 298 Polyneuritis equi, 296, 297, 298 Polysaccharide storage myopathy, 281 Post-anaesthetic myopathy, 281 Post-mortem examination, 343-352, 356, 357, 358 sample collection, 349-350 sudden death, 355 Postoperative ileus, 35 Potassium, 20, 208, 210, 211 alimentary diseases, 63 fractional excretion, 112 renal disease, 113 supplements, 211 Pregnancy, 118-143, 121 diagnosis, 21-22, 129-132 hyperlipaemia, 323, 324 nutritional demands, 78 Pressure tests, lameness, 258 Presystolic cardiac murmurs, 185 Prilocaine, 259 Progesterone, 99, 128 Progestogens, 21-22 Proliferative enteropathy, 77, 80 Pro-opiomelanocortin-derived peptide hormones, 89, 90 Prostate swabs, 139 Protein cerebrospinal fluid analysis, 304 see also Total plasma protein; Total protein Protein-losing enteropathy, 61, 75, 78, 199, 202, 208 Proteinuria, 23 Prothrombin time, 161, 162, 163 Provocation tests, lameness, 257-258 Proximal duodenojejunitis, 38, 57 Proximal interphalangeal (pastern) joint, 253 intrasynovial analgesia, 267 Pruritus, 328-330, 340 Pseudomonas aeruginosa, 136, 139, 198 Pseudomonas mallei, 198 Psoroptic mange, 329 Psychogenic polydipsia, 94-95 Pulmonary abscess, 231, 238 Pulmonary artery rupture, 193

Pulmonary oedema, 173, 174, 190, 233 Pulmonary regurgitation, 184 Pulse rate, 56-57 hypovolaemia, 206 Punch biopsy, 340 Pupillary light reflex, 288, 308-309, 311-312 Pupils, 311, 312 Purkinje-Sanson images, 312 Purpura haemorrhagica, 159, 335 Pyelonephritis, 114 Pyloric stricture, 40 Rabies, 294 Radial nerve injury, 301 Radiographic film, 271 Radiographic projections, 272 carpus, 273 elbow, 273 foot, 273 hip, 274 hock, 274 metacarpo/metatarso-phalangeal (fetlock) joint, 273 metacarpus/metatarsus, 273 pastern, 273 shoulder, 274 stifle, 274 terminology, 272-273 Radiography back, 255 cardiovascular system, 182 guttural pouches, 229 interpretation, 274 laminitis, 278-279 larvnx, 231 musculoskeletal system, 271-274 ocular examination, 321 oesophagus, 32 oropharynx, 31-32 paranasal sinuses, 225 safety of personnel, 272 technique/equipment, 271-272 teeth, 31 thoracic, 237 upper alimentary tract, 31-33 Ragwort (pyrrolizidine alkaloid) poisoning, 18, 75, 86, 295, 354 Rebreathing bag, 173, 231–232 Rectal biopsv, 53-54 Rectal examination, 36-37, 292 alimentary tract, 36-45 female genital tract, 121-122, 134, 137

ovaries, 134 pregnancy diagnosis, 129-130 urinary tract, 102 Rectal tear, 37 Rectal temperature, 57, 76 Rectal ultrasound transducers, 54 Recurrent airway obstruction, 232, 233, 243 Recurrent exertional rhabdomyolysis, 281 Red blood cell count, 12, 147, 149, 152, 153 Red maple toxicity, 154 Reference ranges, 10, 14 haematological/biochemical, 25-26 Reflux oesophagitis, 34 Renal biopsy, 107-108 Renal disease, 19, 99, 109, 114, 115, 213. 324 chronic wasting, 75, 79, 80 metabolic acidosis, 113 plasma electrolyte concentrations, 113 polydipsia/polyuria, 95 prerenal (hypovolaemic), 62 Renal tubular function assessment, 111-113 Reproduction, nutritional demands, 78 Reproductive tract endocrinopathies, 99-100 Respiratory acidosis, 214 Respiratory alkalosis, 214 Respiratory disease, 217-245 causes of sudden death, 356 Respiratory pattern, 173-174 Respiratory sounds, 173 Respiratory viral infection, 242, 245 serology, 244 Restraint female genital tract examination, 121 mouth examination, 28 nasogastric intubation, 50 ocular examination, 306 per rectum examination, 36, 121 perineural infiltration analgesia, 259-260 radiography, 272 ultrasonography, 275 upper respiratory tract endoscopy, 218 Retina, 312, 313, 315, 322 Retinal detachment, 315, 321 Retrobulbar mass, 321

Retropharyngeal abscess, 296 Rhabdomyolysis, 17 see also Exertional rhabdomyolysis Rhinovirus, 242 Rib aspiration, bone marrow biopsy, 157-158 Ricinus communis, 354 Ringworm, 331 Rose Bengal, 318 Ryegrass staggers, 299 St John's wort, 336, 354 Salmonellosis, 53, 57, 63, 67, 68, 356 Samples, 1-10 accompanying information, 8 appropriate containers, 5-6 biochemistry, 4 cytopathology, 8 faeces, 6, 7 guttural pouch, 242 haematology, 3 histopathology, 8 microbiology, 6, 7-8 packaging for transport, 8-9 peritoneal fluid, 46 post-mortem collection, 349-350 respiratory virus isolation, 242 serology, 4, 6 suitability for intended test, 2-8 synovial fluid, 265, 285 toxicology, 357 urine, 6 Sand colic, 44, 75 Sand in gastrointestinal tract, 35, 44 Sarcocystis neurona, 297 Sarcoidosis, 331 Sarcoptes scabei var. equi, 329-330 Sarcoptic mange, 329-330 Scaling, 328, 331-333 Schirmer tear production test, 310, 318 Seborrhoea, generalized, 333 Sedation, ocular examination, 306 Seidel's test, 318 Seizures, 93, 295 Selenium, 283-284 deficiency, 282, 284 toxicity, 331 Semen, 139-142 Sepsis, 205 Septic inflammation, 14, 16, 64 Septic shock, 205 Serology musculoskeletal disease, 284-285 respiratory viral infection, 244 samples, 4, 6

Serum samples, 4 separation technique, 4, 6 Serum alkaline phosphatase (SAP; ALP), 17, 80, 82-83, 200, 202 alimentary diseases, 62 Serum allergy testing, 341 Serum amyloid A, 14, 16-17, 85, 283 chronic wasting, 76, 79 Serum bilirubin, 19, 84 Serum biochemistry profiles, 21, 22 lymphosarcoma, 199-200, 201, 202 Serum enzymes, 17 Serum hepatitis (Theiler's disease), 82 Serum protein electrophoresis, 16 alimentary disease, 61 chronic wasting, 79 lymphosarcoma, 202 Serum proteins, 14-15, 84 Set fast see Exertional rhabdomyolysis Severe sepsis, 205 Shivering (shivers), 301 Shock, 205, 215 clinical signs, 206 Shoulder, 254 intrasynovial analgesia, 268 radiographic projections, 274 Sick euthyroid syndrome, 96 Simulium, 329 Sinker, 277 Sinoatrial block/arrest (sinus block), 182, 186 Sinoscopy, 226-227 Sinus arrhythmia, 178, 185-186 Sinus aspirates, 244 Sinus centesis, 223-225, 244 Sinusitis, 244 Skin biopsy, 330, 331, 333, 334, 339-340 Skin disease, 327-342 Skin scrapings, 330, 331, 337 Skin surface cytology, 337-338 Slap test, 230 Slit lamp biomicroscopy, 312-313 Small intestine obstruction, 38, 39-40 per rectum palpation, 38-40 ultrasonography, 54 Snake bite, 357 Sodium, 20, 208, 210-211 alimentary disease, 63 fractional excretion, 112 renal disease, 113 Sodium bicarbonate therapy, 214 Sodium citrate, 3

Sodium sulphanilate clearance, 110 - 111Solanum nigrum, 354 Sorbitol dehydrogenase (SDH), 18, 83 Space-occupying lesions, brain, 293 Sperm motility/morphology, 141-142 Spinal accessory nerve (cranial nerve XI), 290, 292 Spinal cord disorders, 297-299 post-mortem examination, 352 Spleen per rectum palpation, 37 post-mortem examination, 346, 348 Splenic abscess, 44 Splenic tumour, 44 Splints, 253 Squamous cell carcinoma, 76, 310, 334 Stable flies, 329 Staphylococcus, 149 Steatitis, 323, 325 Sternal aspiration, bone marrow biopsy, 156-157 'Steroid letdown', 93 Stethoscope, 174 Stifle, 254-255 radiographic projections, 274 Stillbirth, 132-133 Stomach per rectum palpation, 38, 40 ultrasonography, 54 Stomoxys calcitrans, 329 Strangles, 242, 245 Streptococcus equi subsp. equi, 151, 159, 195, 242, 245, 283, 293, 296 Streptococcus pneumoniae, 234 Stress fracture, 253, 276 Stringhalt, 257, 301 Strongyle parasitism, 16, 61 chronic blood loss, 148 chronic wasting, 78 faecal analysis, 66 faecal egg counts, 24 see also Cyathostominosis Strongyloides, 24 Strongylus vulgaris, 16 Subarachnoid haemorrhage, 304 Subcarpal block, 263 Sudden death, 353-358 post-mortem examination, 355 Superficial pyoderma, 331 Supraorbital nerve block, 307-308 Suprascapular nerve injury, 300

Supraventricular arrhythmias, 185-188 Swabs, 7 clitoral, 120-121 endometrial, 124-125, 126 nasal, 242 nasopharyngeal, 242 ocular, 316 skin surface cytology, 337 stallion genital tract, 138-139 Swallowing, 32, 33, 34 'Sweeney', 300 'Sweet itch', 329 Synchronous diaphragmatic flutter ('thumps'), 98 Synovial effusion, 253 Synovial fluid, 285 sample collection, 265, 285 Systemic inflammatory response syndrome (SIRS), 205, 208 Systolic murmurs, 183-184 Tail tone, 292 Tapeworm, 24, 67 Tarsal sheath distension ('thoroughpin'), 254 Tarsocrural joint effusion (bog spavin'), 254 intrasynovial analgesia, 269 Tarsometatarsal joint, intrasynovial analgesia, 268-269 Taxus sp., 354 Taylorella equigenitalis, 120, 136, 139 Tear film, 310, 318 Temporohyoid osteoarthropathy, 297 Testes, 138, 139 Testosterone, 135, 142 stimulation test, 22-23 Tests interpretation of results, 10-24 selection, 2 Tetanus, 296, 300 Tetany, hypocalcaemia, 98 Theiler's disease (serum hepatitis), 82 Thoracic auscultation, 231-232 Thoracic radiography, 237 Thoracocentesis, 240-241 lymphosarcoma, 200 'Thoroughpin' (tarsal sheath distension), 254 Thrombocytopenia, 14, 170 coagulopathies, 160-161 Thrombosthenin, 163 Thyroid adenoma, 96 Thyroid disorders, 96-97

Thyroid function tests, 23 Thyroid gland enlargement, 96-97 Thyroid stimulating hormone stimulation test, 96 Thyrotropin releasing hormone (TRH) stimulation test, 96, 97 Thyroxine (T4), 23, 96, 97 Tibial nerve block, 264 Tick-borne disease, 149, 150 Tissue thromboplastin (factor III), 162 Togaviridae, 294 Tongue, use as gag for mouth examination, 29 Tonometry, 320 Topical ophthalmic anaesthesia, 306 Topical ophthalmic stains, 317–318 Total plasma protein, 49, 58, 60, 62, 209-210, 215 dehydration, 207 Total protein pleural fluid, 244 synovial fluid, 285 Total serum bile acids (TSBAs), 83 Trachea auscultation, 231 endoscopy, 221-222 Tracheal aspirates, 243, 245 Tracheal fluid aspiration, 233-236 Transbronchial lung biopsy, 238 Transit tetany, 98 Transport, sample packaging, 8-9 Transtracheal aspiration, 233-235 Transudates, 49, 238, 243 modified, 243 Transverse facial artery, blood collection, 241-242 Trauma plasma protein status/fluid balance, 208 sudden death, 356 Treadmill endoscopy larynx, 230, 231 upper respiratory tract, 222 Treadmill exercise tests, 183 Tremorgen, 299 Trephination, paranasal sinuses, 227-228 Trichography, 330, 331, 338 Trichophyton equinum, 331 Trichophyton mentagrophytes, 331 Tricuspid regurgitation, 184 Trigeminal nerve (cranial nerve V), 289, 291 Triglycerides, 21, 85, 324 Tri-iodothyronine (T3), 23, 96, 97

289, 291 Trombicula autumnalis, 330 Trunk, neurological examination, 292 Twinning, 131, 133 Tying up see Exertional rhabdomyolysis Tympany, 35 hindgut, 356 Typhlitis, 42 Ulcerations, 328, 331, 335-337 Ulcerative lymphangitis, 198 Ulnar nerve block, 263-264 Ultrasonography abdominal mass, 55 alimentary tract, 54-55 biopsy guidance, 87, 88, 107 bladder, 107 chest, 238-239 cryptorchidism, 142 genital tract, mares (transrectal), 122-124 larynx, 231 liver, 85-86 lymphosarcoma, 199, 200 major vessel rupture, 193 musculoskeletal system, 274-275 ocular/orbital, 321-322 ovaries, 134 pericardiocentesis guidance, 192 pregnancy diagnosis, 130-131 testicular measurement, 138 urinary tract, 105-107 uterus, 135, 136, 137 vagina, 138 vascular diseases, 192-193 see also Echocardiography Ultrasound transducers, 54, 122-123, 131 Umbilical cord damage, 133 Umbilical hernia, 58 Unexpected death (found dead), 353-358 causes, 356-357 Upper alimentary tract endoscopy, 33-35 radiography, 31-33 Upper respiratory tract endoscopy, 218-222 post-mortem examination, 347, 348 Urea concentration, 62 chronic wasting, 80 dehydration, 207

Trochlear nerve (cranial nerve IV),

Ureters catheterization, 105 per rectum examination, 102 Urethra per rectum examination, 102 post-ejaculatory swabs, 139 Urethroscopy, 105 Urinary diseases, 101-113 Urinary enzymes, 112 Urinary fractional clearance ratio, 19 - 20Urinary protein:creatinine ratio, 113 Urinary retention, 114 Urinary tract disease, 114-115 examination per rectum, 102 ultrasonography, 105-107 Urine analysis, 23, 108-109, 111 Urine output, 94, 206 Urine samples, 6, 102, 103, 104, 108 Urine sedimentary characteristics, 110 Urine specific gravity, 108, 111 dehydration, 207 Urolithiasis, 114 Urovagina, 138 Urticaria, 328, 333, 334, 341 Uterine body pregnancy, 133 Uterine disease, 135-137 Uterus endoscopy (hysteroscopy), 128-129, 137 per rectum examination, 121-122, 129 pregnancy diagnosis, 129 ultrasonography, 123, 135, 136, 137 vaginal speculum examination, 135 Vagina per rectum examination, 137 ultrasonography, 138 Vaginal disorders, 137-138 Vaginal examination, 126, 138 uterus, 135 Vaginal speculum, 126, 138 Vaginitis, 137 Vaginovestibular fold, 119 Vagus nerve (cranial nerve X), 290, 292 Valvular heart disease, 173, 180, 181, 183-185 diastolic murmurs, 184-185

systolic murmurs, 183–184 Vascular disorders, 192–194 coagulopathies, 159–160

Venezuelan equine encephalitis virus, 294 Venoms, unexpected death (found dead), 357 Venous oxygen saturation, dehydration, 207 Venous thrombosis, 193 Ventricular arrhythmias, 188-189 Ventricular fibrillation, 189 Ventricular premature beats, 182, 188 Ventricular septal defect, 180, 181, 183, 190 Ventricular tachycardia, 189 Vesicular gland swabs, 139 Vestibular disease, 291, 292 Vestibular syndrome, 297 Vestibule examination, 119 Vestibulocochlear nerve (cranial nerve VIII), 290, 291-292 Viral encephalitis, 294, 297

Viral serology, 244 Vision assessment, 308–309 Vitamin E, 283–284, 325 deficiency, 282, 299 Vitamin K deficiency, 162 Vitiligo, 335 Vitreous, 312, 313, 315, 322 Vocal fold collapse, 222 Vulvar examination, 118–120 Waardenburg–Klein syndrome,

335 Warble flies, 334 Warfarin overdose, 162, 354 Wasp sting, 329, 357 Wasting, chronic, **73–80** Water deprivation test, 95, 111 Water hemlock, 354 Water intake, 94, 204 reduced, 214–215 Waterhammer pulse, 173, 184 Wedge section, lymph node biopsy, 196
West Nile virus, 294
Western equine encephalitis virus, 294
Wheezes ('rhonchi'), 233
White blood cell count, 12–13, 283 cerebrospinal fluid analysis, 304 respiratory disease, 245
White muscle disease (nutritional muscular dystrophy), 282
Wild onion toxicity, 154
'Windsucker test', 119
'Windsucking' (pneumovagina), 118, 119–120, 137–138
'Wobbler syndrome', 297

X-ray machines, 271 Xanthochromia, 298, 304 D-Xylose absorption test, 65–66

Yew poisoning, 354, 357