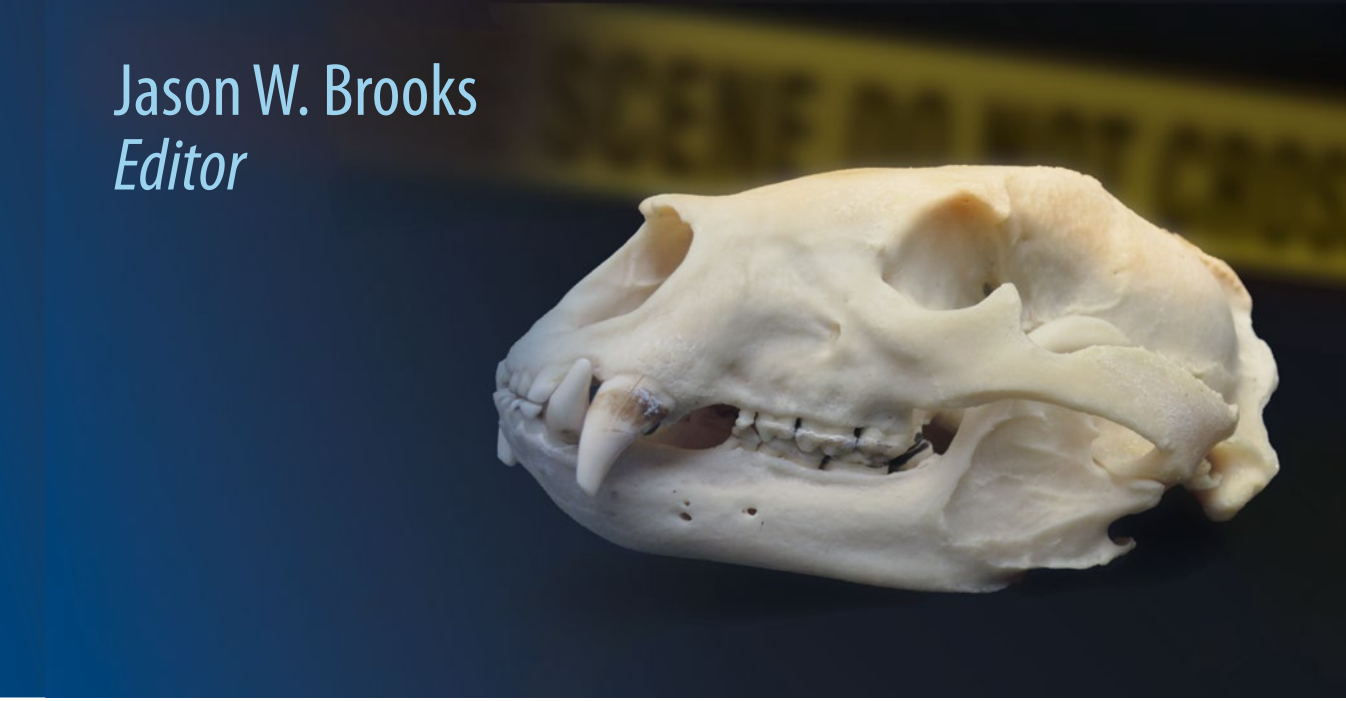
Jason W. Brooks
Editor



Veterinary Forensic Pathology

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For my Family, and for those who cannot speak for themselves
Speravit anima mea…exaudi vocem meam

Preface

What is truth? This fundamental concept has inspired me since childhood. The quest for truth has been a continual motivation through a lifelong journey in search of answers to questions that others would not ask. Truth, at times, can be humbling, unwelcome, and disconcerting to the unprepared mind. Truth can be foul and offensive, surprising and spectacular, dirty and horrific, but it remains truth nonetheless. Both liberating and incriminating, it cannot be defiled or adulterated. Truth is what is. This is its simple elegant beauty.

Forensic pathology is, at its core, a quest for the discovery of truth. The recognition and development of the subdiscipline of veterinary forensic pathology within the broader field of veterinary pathology seemed almost an impossibility only a decade ago. Yet today there is more interest and demand than ever for such expertise, and requests for forensic necropsy are increasing at an unprecedented rate. Yet, despite the apparent need and the interest of a small passionate segment of veterinary pathologists, there is a paucity of training courses and reference materials in the field. This textbook is an earnest effort to not only fill the void in reference materials but also pioneer a new era in veterinary medicine and pathology—an era in which the truth can be brought to light through science, and the voices of the victims may finally speak through those who understand.

I am proud to be breaking new ground with the publication of the first textbook solely dedicated to veterinary forensic pathology. With expertise ranging from animal fighting and hoarding to ballistics, toxicology, thanatology, entomology, and a variety of traumatic injuries, this group of contributing authors represents a veritable force in the quest for truth through the investigation of animal crimes. These contributors are among the most talented and seasoned experts in the discipline, and I am honored and humbled to have each of them as a part of this team.

While no single resource alone can transform a professional into a competent veterinary forensic pathologist, this textbook will assist the properly trained veterinary pathologist, forensic veterinarian, or other veterinary professional as they attempt to interpret the tangled web of clues left among the dead. It is my sincere hope that this textbook will serve as a useful and practical reference for the forensic necropsy of the many unfortunate animal victims and that within its pages will be found some wisdom that will aid in the investigation and resolution of animal crimes.

The truth remains to be told to us if we are only wise enough to know how to listen.

Quid est veritas?

University Park, PA, USA Jason W. Brooks

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Medicolegal Investigation

Rachel Touroo, Jason W. Brooks,
Randall Lockwood, and Robert Reisman

1.1 Veterinary Forensic Medicine

Veterinary forensic medicine and pathology are rapidly evolving and flourishing fields of veterinary medicine, as evidenced by an increase in peer-reviewed publications, texts, graduate courses, international organizations, and inclusion of the topic in many veterinary medical conferences. The term "forensic" simply implies that information from a discipline or technique is being applied in a legal setting. The broad term "forensic medicine" is used to encompass all aspects of forensic work of a medical nature. In the past, this term was often used interchangeably with "forensic pathology." Forensic pathology, however, is currently understood to refer to the branch of forensic medicine that deals with death investigations. Veterinary forensic pathology is a subdivision of forensic pathology, and while there are well-defined criteria for the training and certification of human forensic pathologists, there is currently no formally recognized set of qualifications to identify a practitioner of veterinary forensic pathology. Because no such accepted definition exists, the term veterinary forensic pathologist is often avoided, although, in fact, the relevant work is undertaken by a group of interested individuals ranging from practicing clinical veterinarians to board-certified veterinary pathologists, with varying training and experience in forensic veterinary medicine and pathology. Similarly, veterinary forensic medicine is a subdivision of forensic medicine that also lacks formal recognition. Recently, the term "clinical forensic medicine" has been applied to the branch of forensic medicine involving the living.

The human medicolegal system in the United States is comprised of two parallel systems: the coroner system and the medical examiner system. Each state utilizes only one of these systems. The coroner system is the older of the two and relies upon an elected official who rules on the cause and manner of death in cases involving either violent deaths, sudden and/or unexpected deaths, and suspicious deaths or cases in which a physician is not in attendance at the time of death [1]. This indi-

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vidual is typically not a physician nor is he/she required to consult with a physician, although many coroners do rely heavily on the autopsy report provided by a medical pathologist [1]. In some areas of the country, the coroner must be a physician but not necessarily a pathologist [1].

The medical examiner system relies upon physicians experienced in the field of pathology, and in some newer systems a forensic pathologist, to determine the cause and manner of death in cases involving either violent deaths (accidents, suicides, homicides); suspicious deaths; sudden, unexpected deaths; or deaths occurring without the attendance of a physician [1]. Additionally, deaths in jails and penal institutions may fall under the medical examiners jurisdiction [1].

Though superficially the medical examiner (ME) system may be viewed as a foundation from which to build and develop the field of veterinary forensic pathology, it does not appear to be that simple. First and foremost, there is a lack of veterinary pathologists to fill this need. This shortage also exists on the human side, consequently leading to the continued used of the coroner system. Additionally, the current training and education of veterinary pathologists do not appear to adequately prepare veterinary pathologist for medicolegal casework [2]. Given the interest across the field of veterinary medicine, the industry may be better served by developing a formal educational program encompassing both clinical and pathological veterinary forensic medicine. While advances have been made toward the inclusion of veterinary forensic medicine into the veterinary curriculum with regard to electives, no such courses have been incorporated into the core curriculum, which serves as a barrier for recognizing the importance of animal abuse in society. Furthermore, fellowships have been and are being made available to fill this void within the veterinary curriculum.

The duties of a veterinarian during the course of a forensic necropsy are similar in some respects to those of a human medical examiner and include reviewing a medical history, reviewing witness statements, conducting scene investigation, scene and necropsy photography, necropsy of the deceased victim, necropsy report writing, and courtroom testimony [3]. Despite the similarities in duties and expectations, there is no unified system to support these activities on behalf of the veterinarian during the course of an animal crime investigation. Within the coroner system or the ME system, a human forensic pathologist typically operates within a well-defined network including law enforcement officers, emergency medical providers, hospital physicians, jurisdictional prosecutors and district attorneys, etc.

1.2 Fundamentals of Animal Abuse

During the recent evolution of veterinary medicine and pathology, there has been a concurrent evolution of animal law. Animal crime is gaining recognition by the criminal justice system (law enforcement, prosecutors, and judges) as an important area of the law. In the early 2000s, there were only a handful of student chapters of the Animal Legal Defense Fund in US law schools, whereas now there are hundreds of chapters. Animal law as a distinct area of the law is being taught in law schools across the country; however, animal law is still less well known by those in the criminal justice system than is necessary for successful investigation and prosecution of animal crime. For crimes in which there are both human and animal victims, the extent of the law enforcement focus on the animal crime is variable. It has been shown in New York City, where the New York City Police Department (NYPD) has since 2014 had the responsibility of investigating animal crime as well as the human crime, that adding the animal crime component has enhanced law enforcement in specific instances. In some cases, the prosecution of the animal crime is successful in the absence of successful prosecution of the human crime, and in other cases, the penalty for the animal crime has been greater than that for human crime. Additionally, investigation of complaints of animal crime may reveal human crime that has not previously been reported (this is discussed below).

Many different offenses are subsumed under state animal cruelty laws [4]. In 2016 the Federal Bureau of Investigation (FBI) initiated changes to incorporate tracking of animal cruelty incidents into the National Incident-Based Reporting System (NIBRS). Since the system is intended to gather data from all states, the FBI developed a working definition of "animal cruelty" that included elements common to all state laws. The FBI definition is:

> Intentionally, knowingly, or recklessly taking an action that mistreats or kills any animal without just cause, such as torturing, tormenting, mutilation, maiming, poisoning, or abandonment. Included are instances of duty to provide care, e.g., shelter, food, water, care if sick or injured; transporting or confining an animal in a manner likely to cause injury or death; causing an animal to fight with another; inflicting excessive or repeated unnecessary pain or suffering, e.g., uses objects to beat or injure an animal. This definition does not include proper maintenance of animals for show or sport; use of animals for food, lawful hunting, fishing or trapping.

For the purpose of inclusion of these incidents into NIBRS, the FBI breaks this definition down into four categories (NCOVAA, 2017):

A → simple/gross neglect: the failure of a person to provide for the needs of an animal (lack of food, water, shelter, grooming, or veterinary care)
I → intentional abuse and torture: the intentional act of harming an animal
F → organized abuse: dog fighting and cockfighting
S → animal sexual abuse

A major factor in initiating this process has been renewed interest in considering animal abuse not only as a crime against animals' welfare but also as a bellwether and a gateway to possible acts of interpersonal violence. Extensive research has identified acts of animal cruelty, abuse, and neglect as crimes that may be indicators and/or predictors of crimes of interpersonal violence and public health problems [5–10].

This has coincided with societal demand for increased prosecution and punishment of cruel acts against animals. All 50 states now have enacted statutes declaring the more aggravated forms of animal cruelty to be felony-level crimes, a process that accelerated rapidly since the early 1990s when only 5 states had such provisions. Now that many animal cruelty cases are no longer simple misdemeanors, increasing public, law enforcement, criminal justice, and veterinary resources are being brought to bear on the investigation and prosecution of cases. Veterinarians in general—and veterinary pathologists in particular—are being called upon regularly to offer expert testimony and diagnostic, clinical, and histopathological forensic evidence [11, 12].

1.3 The Role of the Veterinarian in Animal Cruelty Investigations

There may be considerable overlap in the scope of duties of the forensic veterinarian and the veterinary pathologist, as such their roles are often unclear when collaborating on an investigation. While it may be inferred that the forensic veterinarian examines live patients and the veterinary pathologist examines deceased victims, it is typically not this simple.

The primary role of the forensic veterinarian or pathologist is to conduct a separate, parallel, and independent investigation of a suspected animal cruelty. Working in conjunction, the forensic veterinarian and pathologist are a capable team, as many aspects of an investigation may land at the interface of clinical forensic medicine and forensic pathology. For example, injury patterns observed in live clinical patients are often very similar to those observed in dead animals at necropsy; thus, material presented in this volume is equally applicable to both forensic veterinarians and veterinary forensic pathologists.

When law enforcement has at their disposal both a forensic veterinarian and a veterinary pathologist, these roles can be more clearly distinguished. Often, however, only one of these experts is available, necessitating one individual to assume both roles. In this case, it is not unusual for veterinary pathologists to be asked to conduct scene investigations or for forensic veterinarians to perform necropsies.

1.4 Conducting the Investigation

1.4.1 Initial Investigation: Reviewing the Medical History, Witness Statements, and Scene Investigation

A postmortem exam should not be conducted until the circumstances of the death are known [1, 13]. Although there is an opinion that providing key information concerning the deceased, including crime scene information, may bias the interpretation of postmortem findings, this is preferable as such information may be necessary for the veterinarian to accurately interpret the evidence at hand [14–16].

Often times with animal victims, a medical history is unavailable. However, all available medical records as well as any owner or witness statements and law enforcement reports, photos, and videos should be reviewed prior to the necropsy [1]. Ideally the forensic veterinarian or pathologist is present on scene in order to observe and document findings, for just as in human death investigations, the scene is pertinent in every case [17]. It is important to note that if the deceased animal is owned, it is necessary for the owner to give permission for the necropsy. If that permission is not forthcoming, law enforcement must get a search warrant in order for the necropsy to take place.

1.4.2 Forensic Photography

Forensic photography is a fundamental component of any necropsy and crime scene investigation. Oftentimes in the case of a forensic veterinarian or pathologist, the veterinarian must also function as the photographer. The opinion of the forensic veterinarian or pathologist should remain unbiased; therefore, it is important that the photographs be a clear and accurate representation of what was observed [18]. For this reason it is important to understand the basics of forensic photography and how to properly utilize the camera available, ideally a digital single-lens reflex (DSLR). If the veterinarian is not the photographer, they should actively direct the photographer in order to obtain the necessary images. As with any investigation, it is best to follow the same routine for all forensic photographic investigations. For example, regardless of whether there are abnormal findings visible, for all forensic necropsies, photographs should be created of the complete external surface of the animal's body as well as the subcutaneous space and body cavities. Additionally, as bleeding from cut blood vessels is impossible to avoid during a necropsy, all blood that is present in the visual field as a result of the necropsy procedure should be cleaned before photography as not to introduce this confusing artifact. Stray instruments should also not be included in the visual field.

1.4.3 Forensic Necropsy

The objective of a forensic necropsy, akin to a human medicolegal death investigation, is not only to establish the cause and manner of death but also to recognize, identify, collect, preserve, and examine physical evidence associated with the death [19]. Physical evidence encompasses any tangible object that can establish whether or not a crime has been committed or can provide or disprove a link between a crime and its victim or a crime and a suspect. Physical evidence may be located at the crime scene or on a body.

The collection of evidence proceeds over the course of the necropsy and may include diagnostic imaging such as radiography, examination of packaging materials in which the body was found or submitted, examination of both the external aspects of the body and the internal tissues, microscopic evaluation of major body organs and lesions, and possibly ancillary diagnostic testing such as parasitology, microbiology, or toxicology. In some cases, the forensic veterinarian or pathologist may be required to collaborate with experts from other forensic disciplines such as those traditionally offered in crime labs such as ballistics or DNA analysis.

All evidence collected during a forensic necropsy must be maintained in a manner that ensures its integrity. Chain of custody refers to the chronological documentation of every person who has had contact with the item from the time it was seized or collected until it is disposed of. This includes how the item was packaged, the persons involved, dates, times, and purposes of all transfers. In pursuance of the maintenance of chain of custody, evidence must be preserved from the time it is collected until a conviction is final, which does not occur until the conviction is affirmed on appeal or the case is otherwise dismissed. In order to demonstrate that evidence has remained intact, the forensic veterinarian or pathologist may be asked in court if the evidence presented was what they collected or received, the time and date that evidence was received or transferred, and/or verify that the evidence was not tampered with while in their custody.

1.5 Report Writing

The forensic pathology report is critical to cases involving deceased animals. The purpose of the forensic pathology report is to educate the investigator, prosecutor, defense attorney, judge, and jury. Accordingly it must be easily understood by this lay audience for which it is intended. Medical terms should be defined. Reports should be clear, thorough, reasonable, and objective [20]. The forensic veterinarian or pathologist should discuss with the submitting agency the relevant questions at hand, specific to their area of expertise, in order to adequately address these areas within their report. Such questions will likely be determined by the applicable laws. The opinion of the forensic veterinarian or pathologist should be based on the facts of the case. Such facts will come from any available history, medical examination findings, crime scene findings, diagnostic and forensic test results, as well as potentially other sources [21]. The forensic veterinarian or pathologist must remain impartial and only draw conclusions based on what the evidence shows [21]. Additionally, they must understand the gaps in veterinary science and not extend beyond what the current science allows for them to state.

The responsibility to “prove” or “disprove” a case does not lie on the forensic veterinarian or pathologist. The case investigation is a multidisciplinary approach, and the veterinary evidence is only part of the case. Ultimately, it is the prosecutor’s duty to prove the case and the judge or juror’s duty to decide guilt or innocence. The forensic veterinarian or pathologist should simply present the facts and their interpretation or conclusions drawn from these facts [21].

1.6 Expert Witness Testimony

If unfamiliar, the courtroom and legal procedures can be intimidating. A basic understanding of courtroom procedures will help to prepare the forensic veterinarian or pathologist for their role in court. In most cases the veterinarian will be asked to testify voluntarily either by the state or the defense. However, a veterinarian may also become involuntarily involved as a witness and subpoenaed to appear before the court if they have assisted with or have firsthand knowledge of the individual animal or the incidence.

In general there are two types of witnesses: a factual witness and an expert witness. A factual witness can testify only to what they have seen, heard, felt, smelt, tasted, or did in association with the event in question. An expert witness, however, can render an opinion on evidence that falls within their area of expertise. A forensic veterinarian or pathologist will often testify as both a fact witness and an expert witness as they will have direct and firsthand knowledge based on personal examination and observations and are typically qualified as an expert based on their education and experience [20].

Preparation for court should begin as soon as the veterinarian becomes involved in a case. Since one cannot anticipate which cases will end up in the courtroom, every case should be documented, from the time of initial involvement, as though you will be going to court.

Prior to appearing in court, it is vital that the veterinarian meet with the attorney retaining their services. This allows for the veterinarian to explain their testimony, practice direct examination, become familiar with exhibits that will be introduced through their testimony, prepare for anticipated areas of cross-examination, and become acquainted with the likely sequence of events [20].

The expert witness and the attorney have far different roles. It is the attorneys' duty to advocate the position of their client. The expert witnesses' duty is to the court, acting as a teacher, not an advocate [22]. The expert must be unbiased and objective. According to the *Federal Rules of Evidence* (Rule 702), the role of the expert witness is to assist the trier of fact in understanding the evidence or to determine fact in an issue. Therefore, the expert witness assists either the judge or the jury in reaching a verdict by rendering an opinion on a fact in contention.

1.7 Criminal Proceedings

There are a variety of criminal proceedings that the forensic veterinarian or pathologist may be involved with, including a grand jury or preliminary hearing, a Frye or Daubert hearing, jury trial, bench trial, sentencing hearing, or post-conviction proceeding. Additionally, a veterinarian may also become involved in a civil disposition hearing.

A grand jury is a process by which the prosecution presents evidence to a jury in order to determine if there is probable cause to proceed to a trial. The purpose of a grand jury is not to determine guilt or innocence, and it does not resemble a trial. The prosecutor runs the proceedings, and a judge is not present. A preliminary hearing has the same purpose but is heard and decided by a judge rather than a jury.

In some cases, the admissibility of testimony based on novel application of forensic techniques to veterinary medicine or pathology may be subject to the court's review. This could include a Frye, Daubert, or other hearing depending on the state. The Frye test determines whether the expert's methods are generally accepted as reliable by the relevant scientific community. In contrast, during a Daubert test, the judge must decide whether the underlying reasoning of the opinion in question is scientifically valid and whether it can be properly applied to the facts of the issue [22]. In order to make this determination, the judge will consider whether the hypothesis can be tested, if the methodology has been subject to peer review and publication, rate of error, existence and maintenance of standards and controls, and general acceptance by the relevant scientific community [23]. Therefore, testimony must be based on sufficient facts and data as well as reliable principles and methods, not speculation or theories that cannot be tested. While some states do not adhere to either the Frye or Daubert test, they will nonetheless weigh similar consideration with respect to reliability.

Criminal proceedings are either heard before a judge or a jury at the defense's request. A jury trial is heard in front of a judge and a jury, where the jury decides guilt or innocence and makes recommendations concerning sentencing but ultimately the judge decides the punishment. A bench trial is heard and decided solely by a judge.

There is a formal process to criminal proceedings, and the trial will progress in the following order. Initially there will be jury selection, if the trial is by jury. Then typically the witnesses are sworn in and excluded, meaning they are sequestered from the courtroom and unable to hear the testimony of others. The trial begins with opening statements by both parties; then the prosecution presents their evidence. Each individual providing testimony will go through the following process. Initially, if the individual is testifying as an expert witness, they must be qualified by the court. The lawyer retaining the individual must convince the judge regarding what medical opinions can be admitted into evidence. This is done through a series of questions regarding education, training, and experience, referred to as "voir dire," in order to establish expertise within a given area. If qualified, the prosecution will proceed with direct examination, followed by cross-examination by the defense. The prosecution will then have an opportunity to question the expert again; this is referred to as "redirect." Finally, the defense will have the opportunity to recross-examine the expert. Once the prosecution rests, the defense will present their evidence in the same fashion. Once the defense rests, the prosecution can then call rebuttal witnesses who are again subject to cross-examination by the defense. This is followed by closing remarks by both sides. Following the trial the judge or jury will deliberate and then rule. Sentencing will typically occur at a later date.

A sentencing hearing is conducted to determine punishment. In order to effectively make such a determination the judge should be informed about the nature of the crime committed and how the crime affected not only the victim(s) but others as well [24]. Testimony of the veterinarian may be solicited to assist the judge in understanding the impact of the crime on the victim. However, following sentencing there is the possibility of a retrial or post-conviction proceeding, at which time expert witness testimony and physical evidence may need to be presented again.

A disposition hearing is a civil proceeding which must occur, often within a set time frame, following the seizure of an animal by law enforcement. A disposition hearing has the sole purpose of determining if the seizure of the animal or animals was appropriate and just according to the law. Correspondingly, such a preceding does not determine if a crime has been committed, though that may play a role in the seizure of the animal(s) and the veterinarian may be asked to testify to their findings and opinions.

1.8 The Future of Veterinary Forensic Pathology

The disciplines of clinical forensic medicine and pathology, as applied to human patients and victims, have been developing and improving for centuries. Comparatively, the application of such knowledge to matters involving veterinary patients and victims, however, is a much more recent development. While currently developing, veterinary forensic medicine lags behind the well-developed medicolegal system that is currently in place for human death investigations. In part, this deficiency is due to inadequate training of veterinary medical professionals, and in part it is due to the absence of a cohesive medicolegal structure for animal crimes.

In order to advance the discipline, it will become necessary to substantially enhance the training opportunities for interested candidates. Currently no board certification, formal residency training programs, or inclusion in the core veterinary curriculum are offered in veterinary clinical forensic medicine or pathology, and only a small handful of graduate programs or certificate programs are offered in the field of veterinary forensic sciences. Enhanced educational opportunities may be coupled with the formal recognition of the discipline of veterinary forensic medicine, perhaps with eventual board certification for qualified practitioners.

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Crime Scene Findings and the Identification, Collection, and Preservation of Evidence

2

Rachel Touroo and Amanda Fitch

Though it may seem logical, a forensic necropsy does not commence on the necropsy table; rather it begins at the crime scene [1–4]. Unfortunately, veterinarians conducting forensic necropsies are frequently unable to be present during the investigation of the scene [5–7]. However, whether they are able to visit the crime scene location or not, it is important for them to recognize the significance of the crime scene findings and how they may be useful in rendering an opinion [3, 5, 8].

In addition to comprehending the value of crime scene findings, the forensic veterinarian and pathologist must also have a thorough understanding of how to identify, collect, and preserve veterinary forensic evidence [3]. This is not to say the veterinarian should be an expert in multiple forensic disciplines as is portrayed in fictitious television shows and movies. In actuality, most veterinary forensic cases will require a multidisciplinary approach—including assistance from other specialists such as toxicologists, entomologists, and osteologists. One of the most important concepts of forensic science work has nothing to do with actual science—it is knowing one's own limitations and recognizing when to utilize the expertise of other forensic professionals [3].

2.1 Evidence

Evidence, generally speaking, is defined as anything that can prove or disprove a fact in contention [9]. Such facts may be anticipated based on the applicable laws as well as prior experiences. More specifically, evidence is used to prove guilt or innocence, identify victims, and identify suspects. The body presented for a forensic necropsy is evidence, as is anything removed from the body. Additionally, evidence may be present on the scene(s) as well as on the perpetrator(s). The evidence linkage diagram is used to illustrate how each piece of evidence is a means of linking the scene, physical evidence, the victim, and the suspect (Fig. 2.1) [9, 10].

It should be understood that the desire to collect an item does not equate to permission to collect the item. For an item to be considered legally obtainable as a piece of evidence, it must be included in the search warrant obtained by law enforcement. This is often another area in which forensic veterinarians can be of assistance to the lead agency. For example, there have been many cases in which bodies excavated from the ground

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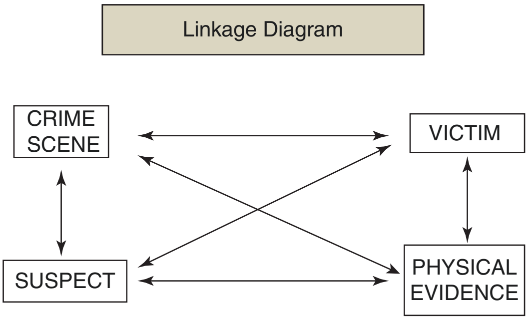


Fig. 2.1 Evidence linkage diagram [3]

were determined to be inadmissible in court because the search warrant did not include areas "below ground." In these situations, any evidence gleaned from the body would also be considered inadmissible as the result of a legal doctrine called "fruit of the poisonous tree." This doctrine holds that evidence gathered from illegally obtained information will also be excluded from trial [3].

In addition to legal collection, items must also fall within other criteria in order to be accepted as evidence into a court of law. In 1975, the US Congress created the *Federal Rules of Evidence*. The use of these rules is only mandatory for federal courts; however, many states have also adopted them in an effort to establish consistency with the federal courts. There are two basic criteria the forensic veterinarian and pathologist should be aware of: first, an item must be relevant to the case (Rule 402). This is generally covered by the search warrant as a judge will not typically sign a warrant including irrelevant items. Second, an item must meet conditions of authentication (Rule 901) to be accepted as evidence in a court of law. This simply means that some form of proof must be given to show that the item in question is in fact what it is claimed to be. This is typically obtained through testimony of a witness with knowledge. Therefore anyone who comes into contact with an item of evidence, whether it is a body submitted for a forensic necropsy or a fecal sample collected from said body, may be called to testify regarding their knowledge of and interaction with the evidence. A chain of custody (discussed later in more detail) is the documentation that must be maintained to record all who have come into contact with the item [3].

All evidence, from the crime scene or necropsy, can be divided into two basic categories: testimonial and physical. Testimonial evidence is based on the statements made by witnesses and suspects. Because it is provided by humans, who are open to error, testimonial evidence cannot always be trusted. Stories can be altered through intentional deceit or forgetfulness, and people often interpret the same information differently. Testimonial evidence should not be ignored, but it should be supported by tangible facts rather than left to stand on its own [9]. It is not uncommon for information collected from a postmortem examination to be used in proving or disproving testimonial evidence, rather than the testimonial evidence being used in proving or disproving other facts.

Physical evidence encompasses any object, or observation of an object, that can be used to establish whether or not a crime has been committed or can provide or disprove a link between a crime and its victim or a crime and a suspect. Physical evidence can be found in many forms, some of which are tangible and capable of being collected and others which are not collectible, but rather need to be documented photographically. For example, if paint flecks are found adhering to fur near a patterned contusion on the exposed skin, then both the fur and contusion are of evidentiary value. It is possible to physically collect the paint flecks and a sample of the contused tissue, but the *pattern* of the contusion can only be "collected" through documentation.

There are two characteristics of physical evidence that should be understood by the forensic veterinarian and pathologist: transience and transference. When present, these characteristics can be very important in the analysis of physical evidence. Transient evidence includes elements of the scene or findings on the body that are temporary and can change. The wetness of a bloodstain, odors, condensation on a glass, and temperature are all examples of transient scene evidence. Rigor mortis, algor mortis, and a wet hair coat are likewise all examples of transient evidence on the body. Transient evidence will offer situational information particular to the moment in time at which the scene or body is first encountered, but such evidence will change with time. Transient evidence, because of this characteristic, must be documented expeditiously. Documentation should, ideally, occur as soon as possible following arrival on scene or after the acceptance of the body into your custody. Failure to promptly document transient evidence may result in its loss. This type of evidence will likely never solve a crime; however, it may help to clarify details. For example, consider the case in which an individual calls law enforcement because he found his dog deceased in the house with its throat incised. The owner states he was only gone from the home for 30 min and found the dog upon his return, but the dog is in full rigor mortis. Documenting the stage of rigor mortis as well as the ambient temperature of the house is very important. Waiting to document the stage of rigor mortis until the body is ready for necropsy could result in the rigor passing and a misinterpretation of the timeline. This information is important not necessarily to prove the owner guilty, but to indicate that the evidence does not match his story.

Transfer evidence is produced when physical contact between two objects results in the exchange of matter. This concept is based on the Locard exchange principle which, when stated simply, equates to "every contact leaves a trace." [9] Transfer evidence is typically thought of as being small in size (e.g., hair or fibers) or "trace evidence" which is discussed later. However, it is entirely possible sometimes that transfer evidence is large enough to be clearly seen with the naked eye. If a suspect suffered a dog bite wound and then grabbed the dog's collar before killing it, any blood deposited on the collar would be considered transfer evidence.

Evidence can also be labeled in terms of how reliable it is in proof of fact. The terms *circumstantial evidence* and *direct evidence* are commonly used in the court of law. However, whether an item of evidence is considered circumstantial or direct should be of no concern to the person collecting it, and in fact, this categorization will most likely be unknown at the time of collection. Circumstantial evidence (also known as indirect evidence) is that which requires inference to prove a fact [11]. In contrast, direct evidence "clearly and directly relates the proof of an existence of a fact" without any inference or presumption required [12]. If a suspect has had no known contact with an animal, yet fibers from his carpet are found on the animal, this could be considered direct evidence. In contrast, consider the case in which the owner of an animal is suspected of its injuries and the owner's hair is found on the animal. This would be considered circumstantial evidence because the fact that the owner's hair is on the animal could be a result of having caused the injury or by normal contact with the animal. For both situations, at the time of collection, the forensic veterinarian or pathologist would not know from whom the questioned hair originated. Therefore, it would be necessary to collect the hair, regardless of its later usability by the court.

Despite all of the information that can be gleaned from evidence, the best characteristic of physical evidence is that it cannot lie [9]. It may be misinterpreted or the analyst may be dishonest about test results, but the physical evidence will always tell the truth. Physical evidence can be large or minute and may require processing at the scene or packaging for processing later at a laboratory. Depending on the type of physical evidence, it may not always be necessary to collect an item. In some situations documentation with photographs and sketches may be sufficient [3, 9].

Most items of evidence will have characteristics that will define their class or individualization. Class characteristics are properties that can only be associated with a group and never a single source. While it may not be possible to individualize the item of evidence, class characteristics can be helpful in eliminating possible suspects. For an item to be compared to and identified to a specific source, individual characteristics are necessary. These characteristics are ones that can be attributed to a common source with an extremely high degree of certainty. One of the most familiar types of evidence with both class and individual characteristics would be blood. While the blood type is the class characteristic that could eliminate potential donors, the DNA would be the individual characteristic that allows the blood to be identified to a specific individual. Although it is not always possible for an item of evidence to be identified as having come from a single source, this does not diminish the importance of the information it can offer. It is important to recognize the needs of the case and how the information that is available can be utilized [3].

2.2 Importance of the Crime Scene Investigation

A forensic necropsy begins at the crime scene [1–4]. Knowledge of the scene may be crucial to interpreting findings at necropsy and determining the cause of death, making it preferred for a forensic veterinarian or pathologist to examine the scene for every case in which they assist [8]. In many cases, however, this may not be possible. They may not be available or the investigating agency may not be aware that they should be present or is willing to travel to the crime scene. Although reviewing crime scene photographs and law enforcement reports is beneficial, the information that can be gleaned from examining the scene in person is irreplaceable [1, 2, 13]. Examining the body at the scene is analogous to taking a medical history, thereby requiring practice and skill [8]. Many law enforcement officers do not possess the specialized training required to investigate an animal abuse scene, making it possible for information to not be noted or simply overlooked. The same issues can occur at the scene of a human death. This is why, in many localities, the medical examiner (or coroner) or their investigators are routinely present on scene with the sole responsibility of examining the body and the conditions that may be related to the death. Unfortunately, due to lack of personnel or funding, the forensic pathologists will often only examine the scenes of deaths that appear to be complicated or unusual. Not examining the body at a death scene has been regarded as one of the classical mistakes in forensic pathology [1–3].

It is not uncommon for a forensic pathologist to rely on evidence from the scene to determine the cause and manner of death in situations where the autopsy only rules out possible alternate causes, but does not lead to a firm determination. In these situations, the crime scene findings are just as important as the autopsy [8]. If a forensic veterinarian or pathologist is not present on scene, they should work closely with those who were, ideally another veterinarian, to ensure that they are provided with all pertinent information regarding the case. The provided information should focus particularly on how, when, and where the body was found, by whom, and under what circumstances (Table 2.1) [3].

Table 2.1 Information that should be obtained prior to submission for a forensic necropsy [3]

• Submitting agency and case lead, contact names and numbers
• Submitting agencies case number
• Unique animal identification number
• Reason for submission and specific questions pertinent to the case
• Date and time animal was last seen alive and by whom
• Date and time animal was found to be deceased and by whom
• Location of the body at time of discovery
• Position of the body at the time of discovery
• Description of immediate environment in which the body was found including: temperature, access to food, water, and shelter, presence of bodily fluids, and other evidence associated with the remains
• Date, time, and location from which the animal was recovered
• Presence and location of livor mortis: visible, well developed, or fixed
• Temperature of the body (i.e., does it feel warm or cold)
• Presence and location of rigor mortis: beginning, complete, or passing
• Insect activity and whether there was collection of entomology samples in the field
• Medical history
• Medications present on scene
• Number of animals present on scene and overall conditions
• How the body was handled, packaged, transported, and stored prior to receipt
• Crime scene photos and video
• Crime scene reports

There are some obvious differences between a typical human crime scene and an animal crime scene investigation. On the human side, there are often specialized forensic professionals available to assist on scene, usually one or few victims involved, the cases involve a single species, and law enforcement has access to local and state labs for the analysis of collected evidence. However, on the animal side, there are often no supporting forensic specialists available to assist on scene, the crime scenes can involve few to numerous victims of varying species, and law enforcement typically has to try to enlist the assistance of veterinary schools, private labs, or other forensic professionals to assist with the analysis of evidence [3].

Regardless of the species of victim or type of crime, the objectives of any crime scene investigation are to identify, collect, preserve, interpret, and reconstruct all relevant physical evidence at a crime scene [10]. Everyone involved with the process of collecting and processing evidence needs to have a solid understanding of what exactly their role will be, the specific evidence they will be responsible for, how it should be documented, and how to best collect and preserve it. The role of the forensic veterinarian or pathologist on scene is to assist law enforcement in identifying, collecting, and preserving evidence related to the animal specific crime(s) [3].

A crime scene may unfold in a variety of ways. Although law enforcement agencies are accustomed to working a variety of crime types, animal cases are often new and unique. In addition to a possible lack of knowledge about the crime type, there may also be a lack of personnel and resources because the agency is too overtasked with the day-to-day dealings with human cases. It is also not uncommon for individuals involved with animal crime scenes to become wrapped up in the rescue of the animals and to forget that the situation is a criminal event. Crime scenes are chaotic by nature. When hundreds of animals, dozens of responders, potentially disruptive owners, and the media are added to the situation, it can quickly become disastrous if it is not approached in a calm and controlled manner [3].

2.3 Processing of a Crime Scene

Once entry has been made onto the crime scene property, law enforcement officers will be in charge of taking the necessary steps to ensure the safety and security of all people and evidence present. This typically occurs prior to the forensic veterinarian's arrival. Although there is always concern for the victims, the initial concern on a crime scene should always be the safety of the responding officers and all other subsequent responders. Once the area has been secured, documentation should begin. It is recommended that documentation of the scene occurs in phases. The initial phase should consist of documenting the condition in which the scene was found upon arrival. This will include overall photographs and possibly video of the exterior and interior portions of all areas of the property covered under the search warrant [3].

The next phase consists of documenting all animals and their environments. The first step of this phase is to identify animals in critical condition. This may be referred to as "critical triage." Triage on scene is a rapid visual sorting of animals for examination and treatment priority based on their medical condition. If an animal is found to be in critical condition, it should immediately be given a unique animal identification number, documented in situ, and removed in an expeditious manner in order to provide timely medical treatment and further documentation of the animal's condition. Documentation should include photographs as well as written notes. It is important to remember that each animal is an individual item of evidence, and if there are multiple animals present, each animal should be assigned a unique identification number. The animal identification numbers should not repeat any item numbers that are used to identify pieces of non-animal physical evidence. Clinical forensic exams typically do not occur in the field given the uncontrolled environment and lack of access to necessary equipment. However, cursory or brief examinations may be conducted in the field in order to provide necessary treatments prior to transport, as well as to document transitory evidence, such as mild dehydration [3].

It is imperative that the living conditions of each animal not be altered in any way until they have been observed by a veterinarian, documented, and photographed.- Once critical animals have been identified, documented, and are receiving medical care, non-critical animals should be more closely assessed and their environment documented. This may be referred to as "intake triage" as it corresponds with the animal intake procedure of assigning a unique animal identification number, taking identifying photographs, and preparing the animal for transport. During this assessment, the identification of animals needing a more thorough examination, application of treatments required before transport or documentation of transitory evidence, and possible collection of trace evidence may occur. The living environment will hold information that will contradict or agree with the animal's physical examination findings; therefore, it is important to be able to accurately illustrate the living conditions from which each animal came and demonstrate how the environment directly affected the animal. All animals should be photographed in situ and their locations on scene documented by sketch or other mapping technique [3].

Deceased animals are considered non-critical and are often given a unique non-animal physical evidence item number, rather than an animal identification number, when seized in conjunction with live animals. Numbering deceased animals differently from live animals helps to eliminate confusion. Deceased animals, as with live animals, should be photographed in situ prior to being altered in any way and their location documented on the scene sketch or otherwise mapped. Written notes should also be taken to document how the body was found and the immediate environment. A forensic necropsy, similar to clinical forensic exams, typically does not occur in the field due to the uncontrolled environment and lack of access to necessary equipment. However, a cursory external examination may be conducted in the field in order to document transitory evidence, such as rigor mortis, and to identify and possibly collect any obvious trace or entomological evidence. Law enforcement should question any potential owners, witnesses, or suspects concerning when the animal was last seen alive and found to be deceased and by whom. Any available medical history or records should also be obtained [3].

Following the removal of each animal, a more thorough documentation of the living space can be completed. Although some documentation will have occurred during the intake process, it is often difficult to capture all elements of the living space when the animal is still in situ. For this reason, it is recommended that midrange and close-up photos of the living space be taken following animal removal. These photographs should include but are not limited to any food or water containers, the presence or lack of food and water, shelter construction and possible hazards, feces, urine, vomitus, wire flooring, and additional photos as requested by the forensic veterinarian. It may also be necessary to record the dimensions of living enclosures. These can also be documented on the sketch showing the location of each animal [3].

In addition to recognizing and documenting animal evidence, the forensic veterinarian or pathologist can assist law enforcement with the identification of non-animal medical and non-medical evidence. This could include items such as medications, supplements, surgical supplies, and fighting paraphernalia. Some items of evidence may be overlooked by law enforcement officers who are not familiar with the particular crime type. For example, the metal tubs pictured in the photo (Fig. 2.2) were overlooked by law enforcement with respect to a dogfighting investigation. Tubs such as these are utilized to wash the dogs just prior to a fight and were consistent with the opinion that the dogs at the scene were most likely being utilized for the purposes of fighting. However, without knowledge of dogfighting paraphernalia, items such as these are easily overlooked [3].

Similarly, a forensic veterinarian or pathologist can assist law enforcement with the potential evidentiary value of an item. Although officers may

Fig. 2.2 Metal washtubs found on the scene of a dogfighting investigation [3]

recognize an item as evidence, they may not be aware of its full evidentiary value. An example could be anabolic steroids recovered from a dogfighting crime scene. If the investigator is unaware of forensic testing available to detect the presence of anabolic steroids in the body, then the fact that steroids were found on scene will likely not be passed on to the forensic veterinarian or pathologist, and possible evidence of the drug in an animal's system will be lost. However, if present on scene, the forensic veterinarian will be knowledgeable of additional diagnostics and/or forensic testing that could be performed and can therefore pass along this information about the steroids in order to ensure the appropriate testing is performed [3].

Law enforcement may also need the assistance of the forensic veterinarian or pathologist to ensure the proper packaging and preservation of deceased victims. On a human death scene, the process of collecting remains is handled by the medical examiner or coroner's office, making the handling of deceased remains relatively uncommon for law enforcement officers and crime scene analysts. Again, deceased animals should not be altered in any way until the veterinarian has had an opportunity to assess the animal and its immediate environment. Once cleared by the veterinarian, the body may be properly packaged and removed. Forensic necropsies ideally should not occur in the field, as doing so may lead to loss or contamination of evidence [4]. Therefore, it is most likely that a body will have to be packaged prior to leaving the scene. The body should be packaged in a manner that will preserve it until the examination can be conducted. The forensic veterinarian or pathologist can provide guidance in how to collect, package, and store the body prior to submitting it for necropsy. The veterinarian or pathologist can also advise of possible zoonotic hazards and of the proper personal protection equipment (PPE) that should be utilized [3].

In some cases, it may be necessary for the forensic veterinarian or pathologist to collect trace evidence that is loosely adhered to the body and will likely be lost in transit. The phrase "trace evidence" is used to describe any evidence that is very small, possibly even microscopic, and includes items such as foreign hair, fibers, and paint flecks. If trace evidence is visible on a body with just the naked eye, it is recommended that it be collected prior to the body being packaged. The visible evidence should be gently collected with tweezers or forceps and sealed in a small container such as a glassine envelope or paper bindle. Items collected from distinctly different regions of the body should be packaged separately. If trace evidence is suspected, but not clearly visible, the body should be packaged in such a way that any evidence that becomes dislodged during transit will be contained in the packaging [3]. To ensure this, the following steps should be taken:

1. Handle the body as little as possible.
2. Place paper bags on the feet or head on scene and secure them in place with rubber bands.
3. Wrap the body in a clean sheet prior to placing it in a heavy plastic bag [3, 4, 14].

2.4 Evidence Authentication

The forensic veterinarian and pathologist must be competent in not only the identification of evidence from the body but also in its collection and preservation. Evidence is commonly lost either because an individual is not aware of its presence and potential value or they fail to properly collect and preserve the item [15]. The body is an item of evidence and everything collected from the body is evidence. In order for evidence to be authenticated in court, it must be accounted for at all times. This is done to prove that the item was not tampered with and is in fact the item that was removed from the crime scene or animal. In addition to witness testimony, a chain of custody and the evidence packaging are used to prove authentication. A chain of custody is the chronological documentation of every person who has had contact with the item from the time it was seized until it is disposed of. This includes how the item was packaged, the persons involved, dates, times and purposes of all transfers. An evidence receipt (Fig. 2.3) is the common means for documenting all of this information. It is imperative that all bodies be submitted by law enforcement with the original evidence receipt. A body should not be accepted without signing and dating an evidence receipt, which serves as the chain of custody. The original evidence receipt should be maintained with the item of evidence (i.e., the body) and copies retained by anyone who has signed the receipt [3].

The second means of authentication is the proper and complete sealing of the container in which the evidence is packaged. Evidence should be sealed with friable evidence tape, which is signed with the packager's initials and the date.

Primary Agency ____________________
Agency Case # ____________________

Evidence Receipt

☐ **Osteology** ☐ **Crime Scene** ☐ **Necropsy** ☐ **Photography**
☐ **Serology/DNA** ☐ **Toxicology** ☐ **Entomology** ☐ **Other:** ____________

ITEM #	DESCRIPTION OF ITEM

I hereby acknowledge that the above list represents all property released from my possession and that I have received a copy of this receipt.

Print Name: ________________________ *Signature:* ________________________ *Date:* __________

RECEIVED BY:	REASON	DATE & TIME

Send Report To: ______________________________
____
____

Page _____ of _____

Fig. 2.3 Sample evidence receipt used to track the chain of custody

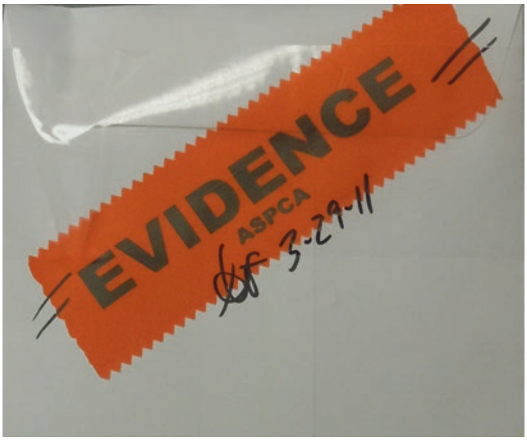


Fig. 2.4 Evidence container secured with packing tape and token evidence seal [3]

These markings should cross over the evidence tape and carry onto the package. This is done as an additional indicator of tampering because it is unlikely that the tape could be replaced in the exact same position should it be moved. If the container is bulky, like a large bag or cooler, it is acceptable to initially seal the container with clear, 2-in. packing tape for added strength and security. Pieces of marked evidence tape can then be used as token seals across the packing tape (Fig. 2.4). Additional protocols should be in place to ensure that evidence remains secure and unaltered. Items should be signed in and out of storage, and access to the storage areas should be restricted and monitored [3].

Before opening a sealed container, written and possibly photographic documentation should be made of how the body was packaged upon receipt. Photographing the outer packaging is not mandatory, but is recommended under certain circumstances, for example, if the packaging does not match what is documented on the evidence receipt, if the package has passed through undocumented hands (i.e., a courier or delivery service), and/or if there is apparent damage to the packaging. Upon opening and removing the packaging materials, all bags, wrappings, or other items accompanying the body should be maintained as they may prove to contain valuable evidence [3].

2.5 Evidence Documentation

It is important to understand that simply having an item of evidence is not enough for that item to be useful to the investigation. Having only the item of evidence with no information regarding its context and its relation to the crime scene or body and other items has been equated to having only *pieces* of a puzzle. Contextual information is necessary to put the pieces together into an overall picture [9]. That said, there is an opinion that providing the forensic veterinarian or pathologist key information concerning the deceased, including crime scene information, may bias the interpretation of postmortem finding. Known as "cognitive biases," these are considered to be unintentional errors in reasoning. There are several types of cognitive bias to consider; however, there are two which are most likely to be encountered by a forensic veterinarian or pathologist looking at forensic cases. "Contextual bias" occurs when irrelevant information, or the way in which information is presented, influences an opinion [16]. An example of this would be an investigator bringing a deceased animal for necropsy and stating that the animal must have been mutilated by a human because there were no predators seen in the area. Depending on the types of wounds, this may be an easy scenario to accept if a thorough examination is not conducted.

The second type of bias is "confirmation bias." This can occur when information is interpreted or new evidence is sought out, to confirm pre-existing assumptions because "people tend to more easily see and give more weight to information which is consistent with what they already believe…." [16] An example would be multiple bodies submitted by the same owner, of which are examined and found to have died from gunshot wounds. Due to conformational bias, additional bodies may not be fully examined because an assumption is made that they, like the others, died as a result of gunshot wounds.

There is no doubt that biases can, and do, occur. There have been several suggestions as to how to prevent this potential issue, including giving examiners no information about the case or only giving them enough of the essential facts necessary to complete the analysis. The debate on cognitive bias and its solution is far from settled. However, if analysis is based on science and fact, rather than speculation and the attempt to match findings to the provided information, the provision of such information should not be an issue as it may be necessary to correctly interpret findings at necropsy.

As previously stated, the body is evidence and needs to be documented before being altered in any way. Before any internal or external examination begins, the body should be thoroughly documented—to include photographs, written notes, diagrams, and radiographs if applicable. Notes should be all inclusive, beginning with the date and time of case notification and ending with the date and time it is closed. While "all-inclusive" may seem very broad, it is the best way to ensure that all pertinent information is documented accurately. In addition to information related to a postmortem examination, information such as the law enforcement case number, contact information for the investigators and forensic veterinarian involved, and a brief narrative of the information obtained regarding the crime scene findings should be included. Notes should be written in chronological order as events occur—not be written from memory after the fact. If a detail is left out, it can be added to the notes later on and dated as such. Corrections made to notes are also perfectly acceptable. However it is recommended that corrected information have a single line drawn through it rather than being completely scratched out. The correct information is then written adjacent and initialed. This ensures that no information is lost, should the corrected information turn out to be needed. Case documentation should never be discarded until it has been confirmed that the case has been adjudicated and all appeals processes finalized. Even then, if storage permits, it is recommended that the case file be retained. If original documentation is illegible or become contaminated, it is acceptable to recopy or type notes, but the originals should be maintained (in a sealed plastic bag in the case of contamination). It is likely that finalized notes will include more information than is needed for a final report. It is not necessary to include all of the information from the notes in the final report. However, all information that will end up in a final report should be documented in the notes [3].

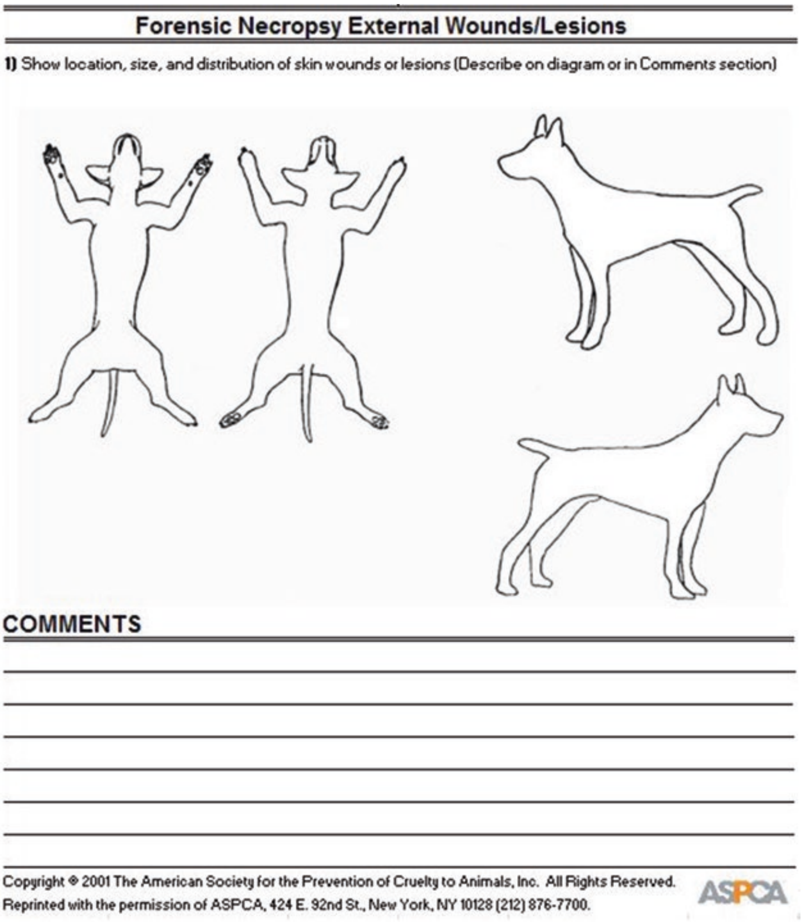
Photographs are another fundamental component of a forensic necropsy [3]. Forensic photography in this capacity is utilized for several purposes:

1. Identify the victim.
2. Demonstrate the condition of the evidence at the time of discovery.
3. Record and document evidence that cannot be preserved or left unaltered.
4. Allow for later review of the evidence.
5. Illustrate and supplement a written report.
6. Demonstrate the absence or presence of alleged findings.
7. Present in a court of law, the items of evidence as they were found, thereby validating the testimony being presented [3, 15].

Because the opinion of the forensic veterinarian and pathologist should be based in science and remain unbiased, it is important that the photographs be a clear and accurate representation of what was observed. Photographs should include both expected and unexpected conditions, as well as those which support and contradict theories. One way to verify accuracy is to utilize an L-shaped forensic scale or ruler. The forensic ruler should be placed flat and on the same plane as the item being photographed—this will prevent size distortion between the scale and the area being photographed. Whether or not the scale is uneven can be determined by looking at the circle with cross hairs. If the ruler is not level, the circle will appear elongated, rather than round. The ruler can also be used to verify proper color and exposure, as it clearly identifies black, white, and 18% gray. Finally, the ruler provides measurement as well as demonstrates that the photo is in focus, as the ruler can be clearly read [3].

All photographs from the crime scene and necropsy are also considered evidence and must be maintained in a manner that ensures their integrity. Digital media cards should be cleared and formatted prior to beginning a new case, and only one case should be captured at one time on a card. If possible, it is recommended that images be taken using a RAW file format. RAW images are uncompressed and unprocessed sensor data. However, because RAW images are data files, they must be processed by a RAW image file converter which is typically included in the camera software [17]. RAW files are also read only and incapable of being altered without first being saved as a JPG or other image format. While this may seem cumbersome, having RAW formatted images ensures that unchanged originals will be available for presentation in a courtroom. Photographs should never be deleted. If images are of poor quality or taken by mistake, they should be retained and kept in the sequence in which they were taken. Deleting images can create a gap in the metadata of the media card which could be called into questioned. Once the media card is full, or no further photographs are needed, all of the images should be downloaded to a "Master" file located on a secure digital storage device. If a RAW format was used to take the images, they must be retained in this format, as well as be converted to a readable format such as JPEG or TIFF. The converted images should be stored in a "Copy" file and will be the photographs that are not only viewable by standard image programs but will also be capable of being enhanced. Reproductions of the "Master" and "Copy" files can then be placed on a CD or other external storage device to be placed into case folders or given to law enforcement or attorneys. How the original digital media card is handled once the images are downloaded and securely saved will be at the discretion of agency or institution in charge. A review of procedures followed by law enforcement agencies shows that both the practices of retaining original digital media cards and reformatting the cards for reuse are followed. If the original card is retained, this should be documented in the case notes and the card appropriately packaged and stored as an item of evidence. Retaining the original card allows the original images to be available should the downloaded and saved images ever be destroyed. The downside, however, is the continual expense of purchasing new cards. If the original card is to be reused, it is recommended that the reformatting procedure not occur until it has been confirmed that the images have been fully downloaded and a copy of them produced. This requires visually checking each image saved to ensure that all images are accounted for [3].

Just as on a crime scene, all items of evidence located during a forensic necropsy must be documented in situ prior to collection or alteration. In addition to photographs, it is also recommended that evidence is diagrammed. Knowing the exact location evidence was recovered from is crucial information, making diagramming a central step in the recovery of evidence. The location of evidence (including collectible items and lesions) should be measured and sketched onto a body diagram (Fig. 2.5). This information can later be utilized to reconstruct the lesions on the body, just as evidence can be reconstructed at the crime scene. Additionally, diagrams will ultimately complement the report by combining key information from photographs and notes and, thereby, allowing readers to understand the interrelationship between the evidence and the body without difficulty [3, 9, 18].



Forensic Necropsy External Wounds/Lesions

1) Show location, size, and distribution of skin wounds or lesions (Describe on diagram or in Comments section)

COMMENTS

Fig. 2.5 Sample canine body diagram [3]

2.6 Evidence Present on the Body

In order to determine what may be important evidence to collect, the forensic necropsy should be approached within the context of potential criminal prosecution. Questions must be considered and answers sought within this context. These questions will likely be determined by the applicable laws. Therefore, the forensic veterinarian or pathologist should discuss the relevant questions at hand with the submitting agency.

The types of evidence most commonly associated with a body include biological, toxicological, entomological, trace, and firearm evidence.

Biological evidence, such as tissue, blood, and urine, are commonly collected during a forensic necropsy. Biological evidence can be utilized to identify the victim or place the victim in a particular location (i.e., the trunk of a suspect's vehicle). Of particular use is DNA evidence, which can be obtained from a variety of biological samples. Buccal swabs are commonly utilized and can easily be used by DNA analysts to compare the victim's DNA to unknown biological substances found at the scene, like blood on the walls of a fighting pit. Buccal swabs can be obtained by utilizing a sterile cotton or sponge swab or cytology brush. With newly gloved hands, the swab or brush is placed, one at a time, against the buccal mucosa of the cheek and swirled for 10 seconds. It is recommended to collect at least two samples [3].

Toxicological evidence, such as the stomach contents, liver, and kidney, may also be collected during a forensic necropsy. Specimens such as these can be utilized to detect the presence of drugs in the animals system. Unfortunately, there is no fully comprehensive toxicological screen available. However, in cases where there is a suspicion of the specific or type of substance utilized, these can be tested for individually. Such substances may be present on the crime scene or at the suspect's disposal. There also may be a history or characteristic lesions observed which may assist in determining the type or specific substance to test for. The forensic veterinarian or pathologist should work closely with a toxicologist in order to determine the most appropriate samples to obtain and testing to pursue [3].

Entomological evidence, such as puparia, insect larvae, and eggs, may be present on a body. Entomological evidence can provide valuable information concerning the postmortem interval and may need to be collected. Because there is a variety of collection and preservation methods used which are dependent on the type of evidence being collected, it is recommended that the forensic veterinarian or pathologist work with a forensic entomologist to determine what information the samples may provide, as well as proper collection, sample storage, and submission [3].

Trace evidence, such as foreign hair, fibers, and paint flecks, are minute and commonly overlooked or lost. Visible items should be carefully collected either tweezers and placed in an appropriately sized, clean paper or glassine envelope. If trace evidence is not readily visible, but is suspected, tape can applied to the body, allowing particles to adhere to it when it is removed. The tape should then be placed adhesive side down on a clear backing such as a plastic transparency sheet. Multiple strips of tape can be secured on the same backing as long as they are properly labeled as the areas from which they were collected. This evidence should be submitted to law enforcement or a private laboratory for further analysis if warranted. It may be necessary to request trace and biological analysis if there are hairs collected which still retain follicular tissue containing DNA [3].

Firearm evidence, such as projectiles, projectile fragments, jacket, or wadding, may be present in the body. Radiographs should be utilized to assist in locating these items which should be collected if present. Firearm evidence should only be handled with a gloved hand or plastic instruments. Metal instruments should never come into contact with these items as they may damage the microscopic evidence (rifling) which can be used to relate the projectile back to a specific firearm. Firearm evidence should be gently cleaned with water, allowed to air dry, and then placed in an appropriate sized paper box or other rigid container. This evidence should be turned back over to law enforcement for further analysis if warranted [3].

No matter how decomposed, damaged, or mutilated the body, it is always advantageous to conduct a necropsy [2]. In situations of advanced decomposition and/or in the presence of trauma, it is recommended that the body be macerated and the skeletal remains examined, following examination of the available tissues. It is possible that minor injuries, such as cut marks, may not be visualized on radiographs. It is also possible, due to extensive tissue trauma or injury, that individual defects and sequence of injuries cannot be determined with the tissue still on the body. In such cases, it is recommended that the forensic veterinarian or pathologist work with an osteologist or forensic anthropologist. Although forensic anthropologists are frequently concerned with determining the identity of a human individual, they are also extensively trained and experienced in the identification and analysis of skeletal trauma [3].

In addition to the typical items and tools associated with a necropsy, there are several others that are recommended to have on hand to assist in the collection and preservation of evidence (Table 2.2). The use of an alternative light source (ALS) may also help to identify physical evidence present on the body. An ALS consists of a light source (such as a laser or incandescent bulb) and a combination of filters that allow selected wavelengths of light to be emitted. Certain substances, such as biological fluids or fibers, will fluoresce at a given wavelength, allowing these items to be more readily located and collected [3].

In addition to in situ documentation, described above, the item of evidence itself should also be measured and described, and close-up photographs taken following removal from the body.

Table 2.2 Suggested items to have on hand for evidence collection and packaging in addition to typical necropsy supplies [3]

• Tyvek tags
• Paper evidence bags of various sizes
• Paper envelopes of various sizes
• Glassine envelopes
• Paper boxes of various sizes
• Evidence tape
• Packing tape
• Permanent marker
• Plastic jars of various sizes
• Uncoated paint cans
• Glass jars or vials
• Sterile cotton or sponge tipped applicators
• Sterile saline or sterile water
• Fecal containers
• Plastic bags of various sizes
• Camera
• Forensic rulers or scales of various sizes
• Plastic tweezers
• Identification board or card
• Evidence receipts
• Body diagrams of the species which you commonly encounter

To prevent cross contamination, each item collected from the body should be packaged separately in a new unused container. However, similar items collected from the same body region may be packaged together—for example, two buccal swabs collected from the same animal. A plastic bag should not be utilized to package evidence that is wet or of a biological nature, unless the item will be immediately refrigerated or frozen. Plastic traps moisture leading to the formation of mold, mildew, and other processes that can deteriorate the specimen, often times rendering it useless. Moist items, such as a DNA sample in the form of a buccal swab, should be collected and then allowed to air dry before being placed into a paper container, such as a swab box or envelope. Some items, such as those containing accelerants, are unique and will require atypical packaging materials. Accelerants are volatile substances and may dissipate if not properly stored. Therefore, these items need to be stored in a special container designed for arson evidence, a glass jar or uncoated paint can. It is recommended that a reference manual for evidence packaging, such as *Crime Scene Investigation: A Guide for Law Enforcement*, published by the National Forensic Science Technology Center, is consulted for general and unique packaging guidelines [3, 19].

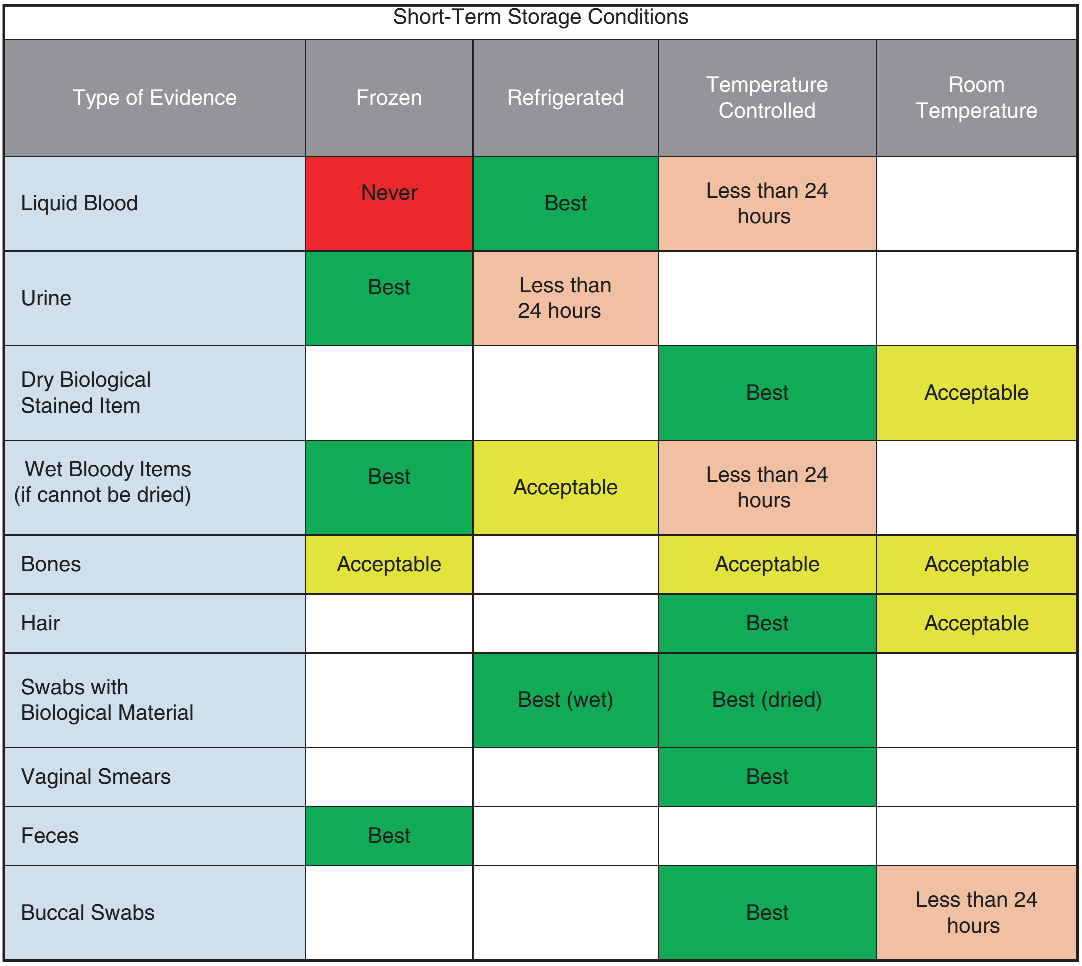
Each item of evidence collected should also be properly identified, labeled, and secured. The container label should include:

1. The case number
2. The item number
3. The investigating agency
4. Location of collection
5. Description of the item
6. Name of the individual who collected the item
7. The date and time the item was collected

To prevent loss, contamination, and access by unauthorized individuals, evidence must also be securely and properly sealed with evidence tape as described above. As previously mentioned items of evidence also need to be housed in a monitored and secure area. The storage area(s) should have limited access which is monitored by some design of documentation when the area is entered and exited [3].

Storage areas will need to consist of three different environments—dry room temperature, refrigerated, and freezer—and can consist of entire secured rooms or individual cabinets with locking capabilities. The type of storage environment utilized will be dependent on the individual types of evidence encountered, with the primary concern being to prevent, or at least delay, the degradation of the evidence. Generally speaking, items that are normally found in a dry and room temperature environment should be stored in the same. This would include items such as projectiles, most trace evidence, entomology, and personal items such as collars. Prior to storing in a room temperature environment, it may be necessary to air dry some items first. Storing wet items cannot only lead to mold growth inside plastic packages but also leakage in paper packaging. Most specimens collected for toxicological analysis are wet biologicals and should therefore be either refrigerated or frozen. Insects that are to be used for toxicological analysis should also be frozen, if this can be accomplished quickly. Otherwise, they should be preserved in 80% ethyl alcohol. The storage of biological specimens will vary, basically depending on whether the sample is wet or dry. It is commonly believed that all biologicals should all be stored in refrigerated temperatures. However, a guide titled *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers* was published in 2013 by the Technical Working Group on Biological Evidence Preservation that clarifies this misconception. The guide includes a matrix of the best and acceptable storage conditions for biological specimens based on the type of evidence and whether the storage is temporary or long term (Fig. 2.6). It should be noted that freezing of insects is specifically for DNA recovery and toxicological purposes and should not be done to insects being used for analysis of postmortem interval [3].

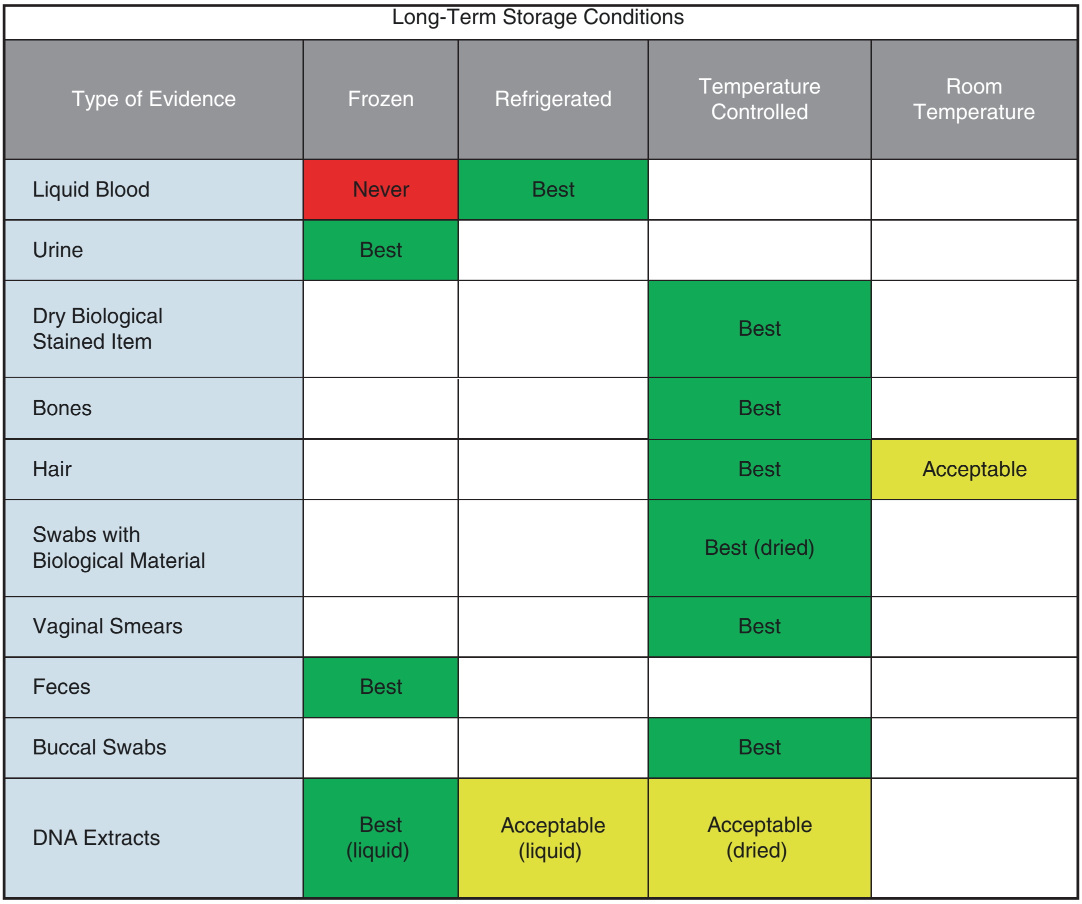
It should always be remembered that even though evidence may be in the custody of the forensic veterinarian or pathologist, it is not theirs to do with at will. The forensic veterinarian or pathologist is providing a contract service for the submitting agency, on evidence that belongs to that agency. None of the evidence, including



Short-Term Storage Conditions

Type of Evidence	Frozen	Refrigerated	Temperature Controlled	Room Temperature
Liquid Blood	Never	Best	Less than 24 hours	
Urine	Best	Less than 24 hours		
Dry Biological Stained Item			Best	Acceptable
Wet Bloody Items (if cannot be dried)	Best	Acceptable	Less than 24 hours	
Bones	Acceptable		Acceptable	Acceptable
Hair			Best	Acceptable
Swabs with Biological Material		Best (wet)	Best (dried)	
Vaginal Smears			Best	
Feces	Best			
Buccal Swabs			Best	Less than 24 hours

Fig. 2.6 Long- and short-term storage matrices (public domain images—reproduced with permissions from the National Institute of Technology) [20]



Long-Term Storage Conditions

Type of Evidence	Frozen	Refrigerated	Temperature Controlled	Room Temperature
Liquid Blood	Never	Best		
Urine	Best			
Dry Biological Stained Item			Best	
Bones			Best	
Hair			Best	Acceptable
Swabs with Biological Material			Best (dried)	
Vaginal Smears			Best	
Feces	Best			
Buccal Swabs			Best	
DNA Extracts	Best (liquid)	Acceptable (liquid)	Acceptable (dried)	

Fig. 2.6 (continued)

the body, should be disposed of until written consent has been obtained from the submitting agency. Depending on the details of the needs of the case, desires of the submitting agency, and the laws of the jurisdiction, obtaining consent for disposal could occur quickly or take an extended amount of time. Once permission is obtained, disposal of all evidence should be documented on the original evidence receipt and retained in the case file.

Conclusion

A forensic necropsy must withstand scrutiny in the courtroom and therefore should be approached within the context of potential criminal prosecution. For this purpose, it should be recognized that a forensic necropsy does not begin on the table. Rather, it commences at the crime scene [1–4]. The forensic veterinarian or pathologist must recognize the significance of the crime scene findings and how they may be used in rendering an analytical opinion [3, 5, 8]. If the forensic veterinarian or pathologist is not present on scene, they should discuss the findings with someone who was(ideally another veterinarian) and request copies of crime scene documentation and reports [3]. In doing so, this will ensure that the forensic veterinarian or pathologist receives all of the available information which can be used to interpret findings at necropsy and determine the cause of death [3, 5, 8]. In addition to the crime scene findings, the forensic veterinarian or pathologist must also understand the appropriate methods and techniques of identifying, collecting, and preserving veterinary forensic evidence [3]. Aside from the postmortem examination, proper evidence handling is paramount to a successful forensic analysis.

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Performing the Forensic Necropsy

3

Rebecca Kagan and Jason W. Brooks

3.1 The Forensic Necropsy

The forensic necropsy is a careful examination and documentation of the body, the purpose of which is to discover the cause and manner of death for the court. While this procedure is similar in some ways to the investigation of natural death as occurs daily in diagnostic or clinical cases, there are critical distinct differences in the approach, training of the examiner, techniques, and documentation that must be very strongly considered by all parties involved with any such investigation. The basis of any necropsy is a thorough examination using sound technique. While the focus and documentation practices may differ between forensic necropsies and diagnostic necropsies, the examination procedure is fundamentally the same and should follow established veterinary necropsy guidelines [1].

3.1.1 Initial Evaluation and External Examination

The goals of the external examination in a forensic case are essentially to establish or confirm identity (breed, species, and/or individual), document the nutritional condition and hygienic status of the body, document postmortem preservation, document external lesions, and collect trace evidence. Prior to starting the external exam, notation should be made regarding the manner and condition in which the carcass was received. Document the packaging, labeling, and any seals that may be present. If possible, radiographs of the carcass should be taken prior to removal from the packaging to avoid loss of radiographically visible evidence (such as metal) that might be only loosely attached to the body or contained within packaging materials (Fig. 3.1). All packaging, including any seals, should be saved and resealed with the carcass remains. At a minimum, packaging should be described in the report and photographed (Fig. 3.2).

Once removed from the packaging, all surfaces of the body should be photographed with a label and scale. Any potentially sensitive evidence (such as trace fibers or fingerprints) should be retrieved prior to further manipulation of the carcass. Following initial establishing photography, examination via alternate light source is recommended if this imaging modality is avail-

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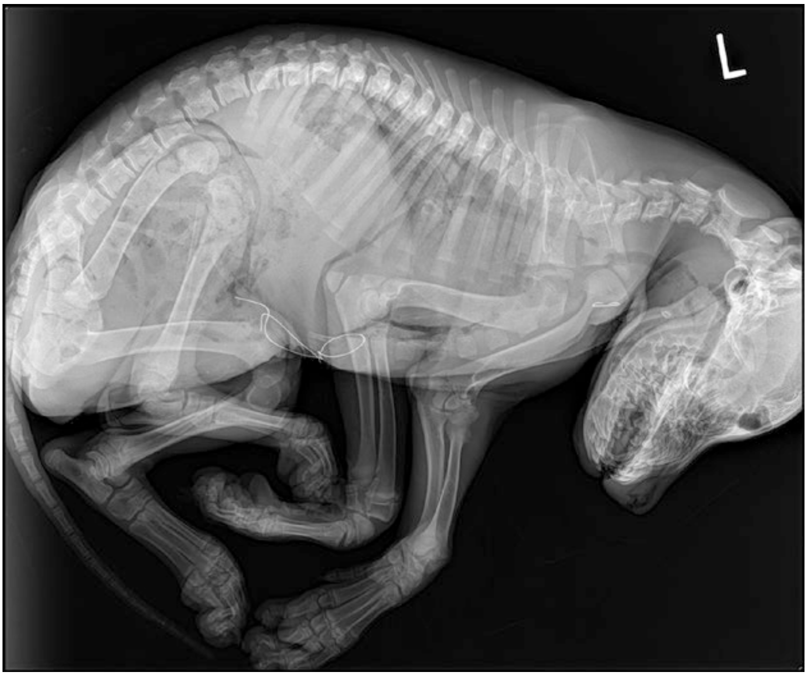

Fig. 3.1 Radiograph of a dog upon presentation. Dog was radiographed within the original bags in which it was submitted. Metallic bag closures are visible and patient positioning is not optimal within the bag, but sufficient to identify radiopaque items contained within the packaging material

Fig. 3.2 Initial photos should be taken of the carcass as received within any packaging materials, including any seals and tags. All photographs should include a label with the case number (identifying information was cropped out of this photo for publication)

able (see Sect. 3.2.4). Document species, breed, and sex, estimate age if possible, and measure body weight and body length (crown to rump and/or total length). Be aware of species-specific features that may be relevant (e.g., the presence or absence of a tail kink in a Florida panther). Search for and document identifiers such as tattoos, microchips, radio collars, or leg bands, and photograph and retain any identification devices.

Consider collecting samples for DNA analysis, including hair with intact roots and/or swabs containing mucosal epithelial cells (see Sect. 3.4.2.3). If the body was heavily soiled or requires rinsing for further evaluation, this may be done after initial examination and evidence collection as previously described. Establishing photographs of the body may be retaken following washing of the body.

Observation should be made as to the condition of the hair coat, skin, nails, teeth, and external orifices. In birds be sure to document areas of feather breakage, loss, or other abnormalities. The nutritional condition of the carcass should be described, preferably by assigning a body condition score if such a system exists for the species being examined. It is advised to specify the scoring system used, as multiple scoring systems exist for some species (Fig. 3.3). The Purina Body Condition System and the Tufts Animal Care and Condition (TACC) scale are commonly used in dogs. The body should be examined for any postmortem changes including livor mortis, rigor mortis, decomposition, or insect development. Core body temperature may be measured and recorded if the time of death is suspected to be less than 48 h or if the body temperature has not yet equilibrated to ambient temperature after death. If external wounds are present, they should be photographed both with and without a scale. Fur or feathers may be removed to allow for better visualization. If multiple external wounds are present, it may be helpful to designate a number to each for descriptive and photographic purposes. The body should be palpated for bone fractures or deformities that are not grossly evident.

Prior to proceeding with the necropsy, the carcass should be radiographed to assist with the detection and location of bone fractures, metallic projectiles, or other medical conditions. As previously described, radiographs may have already been taken with the body still contained inside of any packaging materials. At this stage, radiography outside of any packaging materials is advised to allow for optimal positioning and avoid any artefactual interference created by packaging. If radiography is delayed until after internal examination, significant artifacts from tissue manipulation may result, causing difficulty in interpretation.

Following radiography, the hair coat or feathers should be either removed to permit visualization of the skin or the skin should be reflected to permit visualization of the subcutis. Areas of bruising or small penetrating wounds obscured by fur or feathers will be more easily found (Fig. 3.4). If wound tracts are present, the placement of probes in through-and-through wound tracts as visual aids should be done with caution or not at all. Aggressive probing can result in iatrogenic damage that may be hard to differentiate from the actual wound path. It is preferable to delay probe placement until after the body has been opened and the wound path identified (Fig. 3.5). See Chap. 7 for additional details.

3.1.2 Internal Examination

A complete internal examination, including removal of and dissection of all internal organs, should be performed as for a comprehensive rou-

[redacted]

Fig. 3.3 An emaciated dog with external evidence of medical intervention. The carcass should be evaluated for body condition, hygienic status, and identifying features as well as any evidence of medical or surgical intervention

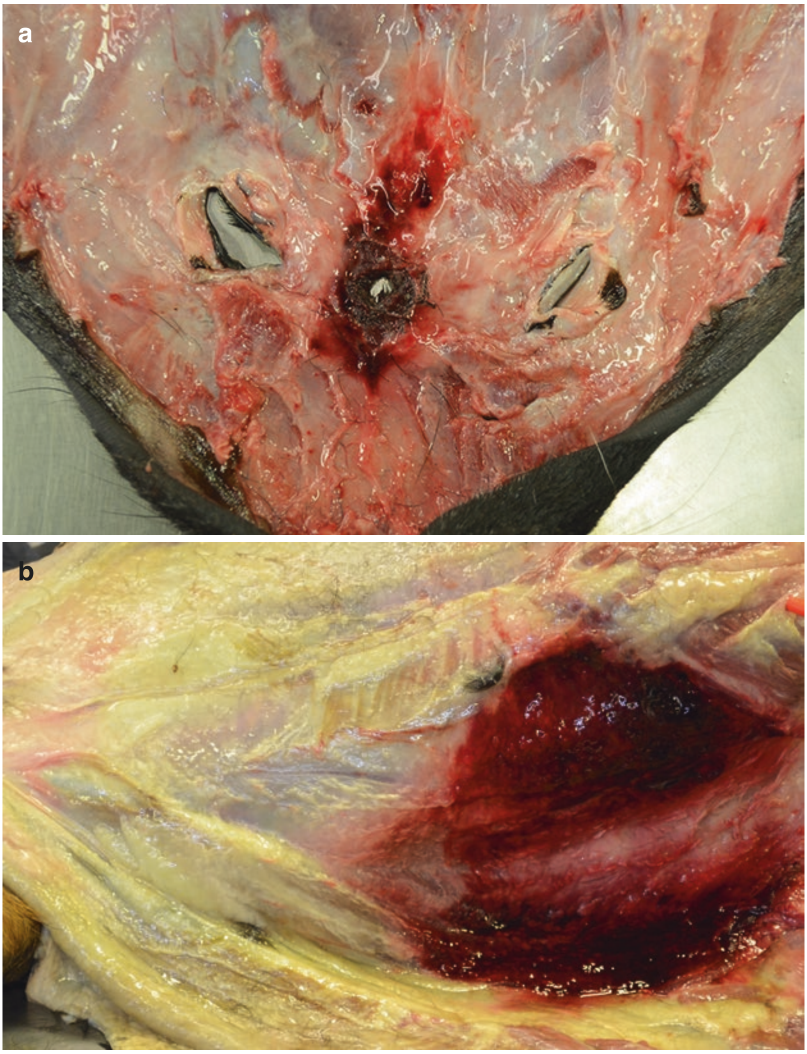

Fig. 3.4 (**a**) Reflected skin of the head showing focal subcutaneous hemorrhage surrounding a gunshot wound between the eyes of a dog. (**b**) Reflected skin of the abdomen and chest showing focally extensive subcutaneous hemorrhage surrounding a gunshot wound to the chest of a dog

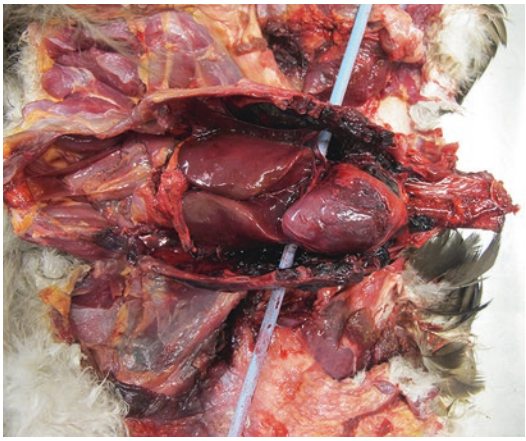

Fig. 3.5 A probe (*a blue dowel*) has been placed to demonstrate the path of a bullet wound in a golden eagle. In this case the probe was inserted after the body cavity was opened and the wound tract examined

tine diagnostic necropsy. Special attention should be given to areas in which wounds, medical or surgical intervention, or other abnormalities are located. Note what is normal as well as abnormal. The content of each organ should be examined, measured, described, collected, and preserved. The bone marrow may be removed from long bones such as the femur for bone marrow fat analysis. Tissues should be collected for ancillary testing and, when appropriate, any evidence should be collected and preserved.

Additional evidentiary items from within the body should be collected if encountered. Most commonly, projectiles or fragments may be present within body tissues or cavities. These may be readily retrieved or may require the assistance of radiographs or a handheld metal detector for location. Metallic projectiles and fragments should be handled only with blunt, nonmetallic instruments or by hand to prevent the creation of artifactual tool marks.

3.1.3 Histopathology

The National Association of Medical Examiners recommends that histologic examination be performed in any non-skeletonized case for which the cause of death is not evident on gross examination [2]. Microscopic examination of tissues can be used to establish a timeline for injury, exclude other possible causes of death, identify extenuating circumstances, and assess overall health at the time of death [3, 4]. Particularly in wild or exotic species, histopathology may also be valuable for the monitoring of population health. Whether or not to pursue histopathology in a forensic case is generally left to the discretion of the pathologist. If the pathologist decides not to do a histological examination, she or he should be prepared to explain this decision in court.

As with other diagnostic tests, the examiner should keep in mind that the opposing council may ask that their expert witness also be allowed to examine the case. Slides, formalin-fixed tissues, or blocks may need to be made available upon request. To this end, samples prepared for histopathology should be good quality, representative samples. Consider also saving additional tissues as sealed evidence, either frozen or preserved in formalin.

3.2 Special Techniques

3.2.1 Decomposed Remains

Poor postmortem preservation does not necessarily preclude a complete and successful necropsy. For example, bullet fragments or certain toxins may be detected long after death (Fig. 3.6). Trauma involving bones could still be apparent. As in any non-hospital death, the state of postmortem preservation is not always ideal in cases submitted for necropsy. In forensic cases especially, bodies may not be submitted until long after death. Attempts might have been made to conceal the death or, often in wildlife cases, the animal may have died in a remote location and/or have been exposed to harsh environmental conditions. In some cases, owners or law enforcement officers are uncertain about requesting a necropsy and may cause delay; sometimes bodies are frozen or buried before a decision is made regarding whether or not to request a necropsy. Birds and other small animals tend to decompose quickly, necessitating more rapid discovery and recovery than may be possible. The examiner will be expected to interpret lesions and postmortem changes in bodies that are desiccated, have been frozen and thawed, macerated, or scavenged.

Fig. 3.6 Example of a markedly decomposed, largely skeletonized, Mexican gray wolf (*Canis lupus baileyi*). Despite the poor carcass preservation, metal fragments were identified in association with acute fractures allowing for a diagnosis of gunshot injury

3.2.2 Desiccated Carcasses

In human remains, the tissue desiccation may begin as early as 3 days after exposure [5]. Exposure to a hot, dry environment can quickly leave the examiner with a stiff mummified animal to necropsy. Radiographic imaging, including computed tomography (CT), is an extremely useful tool for examining and documenting trauma in these types of carcasses. For a more thorough examination of the skeleton and remaining internal structures, soaking the body in water overnight or longer can rehydrate soft tissue enough to allow for manipulation and internal examination. Regardless of the condition of the body, the pathologist should strive to perform as complete an exam as possible and collect any trace evidence prior to immersion.

3.2.3 Skeletal Preparation

Examination of cleaned bones can allow for a better characterization and documentation of skeletal trauma, particularly in areas such as the spine and skull where intimate attachments to muscle and other soft tissues obscure the bone. When cleaned and reconstructed, bullet holes, blunt force trauma impact sites, and tool marks are among the features that may be revealed (Fig. 3.7).

Methods of skeletonization vary. Ideally, cleaning will be rapid and minimally destructive to bone and use readily available materials. If skeletal preparation is a frequent necessity, a dermestid beetle colony may be a valuable addition to the forensic laboratory (Fig. 3.8). Dermestid beetles (Coleoptera, Dermestidae) are often used by museums and taxidermists to prepare skeletons. Both the larvae and adults eat soft tissue. A healthy colony can skeletonize a small bird in as little as 24 hours, while large specimens may take several days to weeks. The beetles do not cause any damage to the bones, though they may start to eat hair and other keratinized structures if the carcass is not removed in time. Beetle-assisted preparation has the added advantage of preserving DNA [6]. Dermestid beetles can be purchased from scientific supply companies. Colonies require appropriate humidity, light and dark exposure, and substrate, and they may require additional food if casework is not sufficient. Ample online resources exist to instruct in the establishment of a colony.

Other skeletonization techniques involve variations of maceration to remove soft tissues. Immersion in warm or cool water requires little specialized equipment or materials but is time

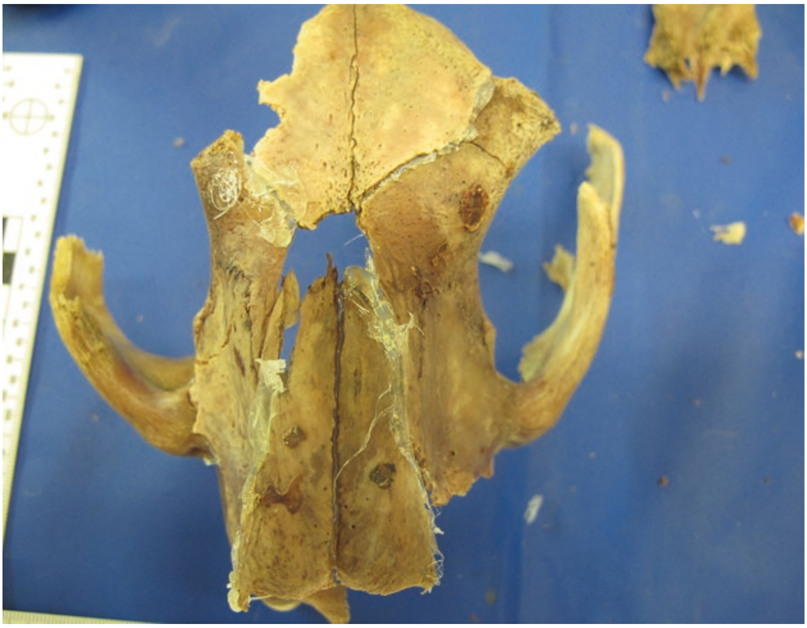

Fig. 3.7 Reconstruction of a Florida panther (*Puma concolor coryi*) skull. A non-exiting (penetrating) bullet wound from a high velocity rifle shattered the skull. Tissues were skeletonized using a dermestid beetle colony and the bullet entry hole was reconstructed using hot glue

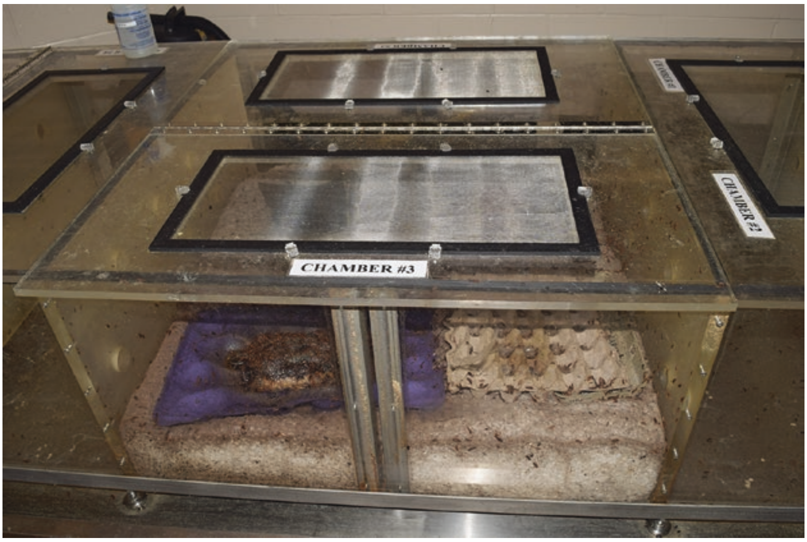

Fig. 3.8 A dermestid beetle colony in action. The beetles live on a bedding of loose batting. The colony is housed in a locked room with limited access

consuming and often malodorous. Simmering is more rapid but must be monitored to avoid overcooking, which can soften and warp the bone. The addition of detergent and baking soda (20 mL of each per 2 L of water) reduces odor and speeds the process. A second round of simmering in diluted liquid ammonia (150 mL per 2 L of water) acts as a degreaser [7]. In the author's experience, using this method on a skinned and partially defleshed small mammal or bird skeleton can result in a cleaned skeleton in 6–8 hours. Other maceration techniques use protease and lipase enzymes or household bleach [8]. Since maceration techniques involving heat treatment may destroy DNA, samples should be retrieved ahead of time for storage in the event that genetic analysis is requested at a future date [9].

If bleach is to be used as a finishing technique, it should be done with caution. Bleach readily degrades calcium, resulting in chalky surface residue. A gentler method of whitening can be achieved by soaking the bones in over-the-counter hydrogen peroxide solution (Fig. 3.9). Following this with exposure to direct sunlight will whiten bones even further. Keep in mind when using any of these techniques that evidence security needs to remain a priority. Samples should not be accessible to outside parties, should ideally kept in a locked cabinet or secure room, and should only be handled by persons within the chain of custody.

3.2.4 Alternate Light Source Exam

Alternate light sources are used in crime scene investigation and fingerprint examination but can also be useful during the necropsy (Fig. 3.10). Though high-end alternate light source equipment may be too costly for many labs, cheaper portable models are available. For the pathologist routinely involved in forensic necropsies, this equipment can be a worthwhile investment.

Examination of the body with light of different wavelengths utilizes both the principles of photoluminescence (objects absorbing light of one color and emitting light of a different color) and absorption. Blood, for example, appears black when viewed under light at approximately 415 nm [10]. Conversely, the author (RAK) has observed that areas in the subcutis and muscle that appear blanched in visible light suggestive of constriction become highlighted under 415 nm light conditions due to lack of blood. Saliva, semen, and urine will photoluminesce in the 400–500 nm range depending on the filter used and the substrate (Fig. 3.11) [11, 12]. Other applications include detection of trace fiber on feathers and hair coats and detection of burned keratin in electrocution and flame burn cases [13]. Ultraviolet light detects markers in antifreeze and M-44 cyanide bait [14].

Photographing items being examined in this way requires that the filter be placed between the

Fig. 3.9 Black bear (*Ursus americanus*) bones before and after (*inset*) treatment with hydrogen peroxide. Though whitening is not necessary, if desired, hydrogen peroxide is a good alternative to harsher bleach treatments

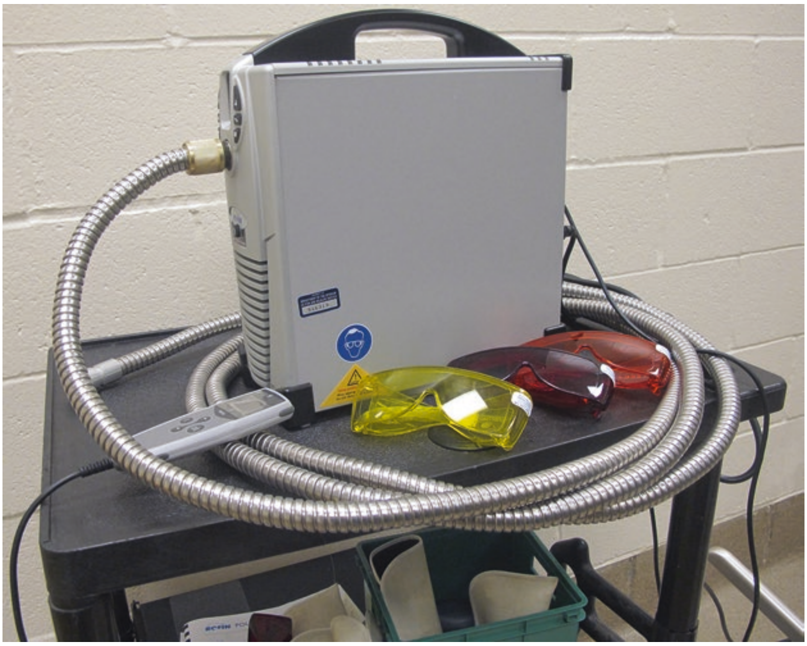

Fig. 3.10 A portable alternate light source and accompanying *red*, *yellow* and *orange* filters

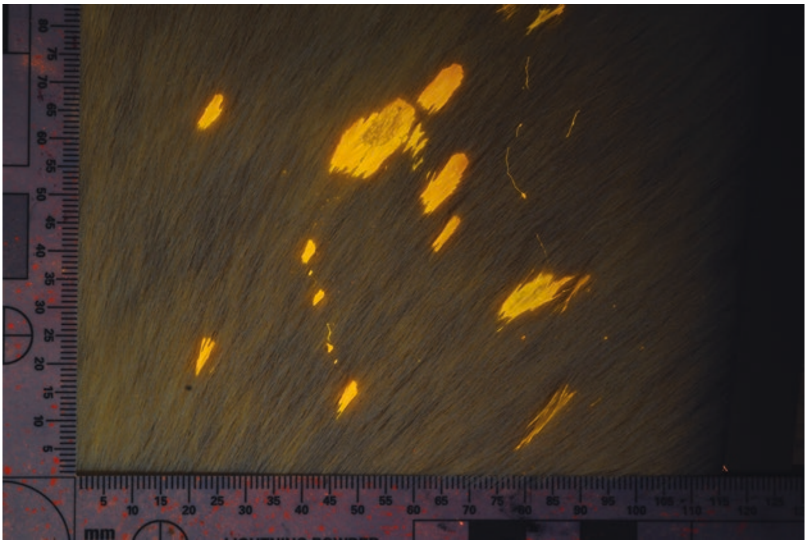

Fig. 3.11 Human semen on cow hide demonstrating luminescence under *blue* light with and *orange* bypass filter

lens and the light source. Colored filters that attach directly to the lens can be purchased for different camera types. A tripod is also useful given the low light conditions requiring slower shutter speeds.

3.3 Imaging

3.3.1 Photography

As a necropsy necessarily results in disturbance and deconstruction of the evidence, photographic and radiographic images are what remain as visual documentation of the body as received, the necropsy process, and in situ findings. As opposed to the traditional necropsy, where photos may only be taken to document particular findings of interest, photography for a forensic necropsy should be as comprehensive as possible.

A basic approach to forensic necropsy photography involves first taking images of the body as received (ideally in the sealed packaging), followed by whole-body images. All images should be labeled with the case identification and date. When there is a specific injury or other area of interest, the larger area should be photographed followed by close-up shots. Distracting unnecessary elements such as gloves or instruments should be kept out of the frame. Scales should be used when appropriate. The proper method for scale bar use involves keeping the scale in the same plane as the area of interest and not covering any part of the subject. If a marker or scale is used and overlaps any portion of the body, a photo should be taken with and without the marker [15]. Tabletop markers to identify right, left, dorsal, ventral, etc. may also be helpful. A copy stand with dedicated lighting is recommended for photography of individual samples, or a tripod may be used for items unable to be accommodated by the copy stand (Fig. 3.12).

If using digital photography, unintentional, out of focus, or otherwise flawed images should be kept and not deleted so there are no gaps in the numbering to give cause for questions. Instead, errors should be noted in writing by the examiner or technician. Images should be archived in a database or on CD/DVD, but should not be kept stored on the camera. A written photo log should be kept to record case number, date, time, number of photos taken, and storage method.

3.3.2 Radiography

Radiography has a long history of use in forensic cases, dating back almost to the year that Röntgen developed the technique [16]. The pathologist who does not already take radiographs as a routine part of the exam should seriously consider

Fig. 3.12 Copy stand with dedicated lighting and digital camera mounted to allow for photography of individual items

doing so. The pathologist is in the rather unique position of being able to open the body to identify or confirm findings rather than having to rely heavily on radiographs as in a living victim. Despite this, radiographs can be of use. Radiographs not only aid in diagnosis and trace metal retrieval but also result in an image that can be used in court to explain the findings. Radiographs of gunshot wounds in particular can make a powerful impact on a jury, tend to be easier for the layperson to understand, and are generally less disturbing than necropsy photos [17]. The pathologist should take care to speak only within her or his scope of training. For cases in which the conclusions rest solely or heavily on the radiographic findings, consultation with a radiology specialist should be considered.

The use of radiography as a means to identify the body is less often necessary in nonhuman casework, but can be helpful, particularly if the presence or absence of microchips, old fractures, or metal implants can be ascertained. Growth plate closure and other age-related changes to bone may also help in determining signalment and identity. In cases of abuse, radiographs are used to determine the scope of the injuries and help in aging fractures [18].

In cases of gunshot injury, radiography is used to determine the presence and location of the bullet or bullet fragments (Fig. 3.13). Gunshot wounds are not always straightforward. Grossly, they may resemble blunt force trauma or sharp force injury. Bullets can end up lodging in sites distant to the entry wound depending on what internal structures they have encountered. When bullets shatter, the resulting “lead snowstorm” can help to trace the trajectory. In cases of shotgun injury, where pellets are too numerous to retrieve, numbers can be more easily assessed with radiographs. Drawing conclusions regarding bullet caliber, shotgun range of fire, and shot pellet size based on radiographic images is not recommended [16].

As previously stated, radiographs should be taken of the body in the packaging and then again of the body after having been removed from packaging and properly positioned. Having an image of the body as received allows the pathologist to find any trace metal that may have fallen off into the packaging and account for any iatrogenic fractures that may occur during manipulation. Once the body is removed and photographed, full body radiographs with standard orthogonal positioning will follow. All images should be labeled with the case identification and date and tabletop markers used. It is advisable to make notes as to machine settings, the number of images taken, and individuals in attendance in case this information is requested in court.

Fig. 3.13 Radiograph of a dog in left lateral recumbency with two metallic projectiles (.25 caliber bullets) located within the soft tissues of the ventral neck

Regarding metadata and long-term storage, recommendations for radiographic images follow those of photographs.

3.3.3 Computed Tomography and Magnetic Resonance Imaging

It is the fortunate pathologist who also has access to CT, MRI, and other advanced imaging modalities. Though still not well explored in the field of veterinary forensic pathology, there is much possibility. Multi-slice computed tomography examination can help to reconstruct projectile injuries and shows promise as aids in the diagnosis of fluid inhalation and gas emboli [19–21]. A project known as the "Virtopsy® Project" has studied the combined use of CT and MRI as a supplement to the traditional autopsy [17]. One of the main advantages of cross-sectional imaging is the ability to visualize things like complex fractures or fluid distribution in situ. This is a decided advantage as once a body is opened during an autopsy, bone fragments will be disturbed and gases and fluids will shift [20]. CT allows for more accurate measurement of projectile fragments and can even be used to distinguish between types of materials [17].

3.4 Evidence Handling

3.4.1 The Chain of Custody

The chain of custody is a written or electronic record that documents the movement of a piece of evidence. At its simplest, the chain of custody is a written document with point-to-point signatures, dates, and times. High-volume forensic labs may use a bar code or radio-frequency identification system [22]. Ideally, a chain of custody will be unbroken from the time the evidence is collected to the time that it is archived or disposed of. If a body or other piece of evidence enters a facility without a chain of custody, one should be started upon arrival (Appendix A). The chain of custody answers the question of "who" and "where" at any given time, documenting how long and when the evidence was in any one person's charge. If additional evidence is generated (such as stomach contents taken from a dog), a separate chain of custody should be established for that separated item.

3.4.2 Trace Evidence Collection

Ideally, you will already be in contact with a lab to analyze trace evidence. In that case, collection and submission should meet their specifications. In the event that evidence is being collected for potential future use or an unknown third party, below are some general suggestions for best results. Please also refer to the respective chapters in this book.

Trace evidence in this context refers to anything separated from the body for additional analysis. That includes tissue samples, swabs, ballistic evidence, etc. The pathologist should photograph the trace evidence and note the details of collection, including location of the body. All trace evidence should be then be appropriately labeled and sealed and a chain of custody initiated for each piece. Labeling, at a minimum, needs to include the case and item number. If the item is bagged in multiple layers, identifying information should be included on the innermost bag. To appropriately seal evidence, the opening of the container should be closed with tape and the collector's initials and date written over the tape (Fig. 3.14). If the package has multiple openings, all will need to be sealed.

3.4.2.1 Ballistic Evidence

Bullets, other projectiles, and projectile fragments should be collected gently to avoid marring rifling and other identifying markings. Do not use metal forceps; plastic forceps can be purchased for this purpose but gloved fingers also suffice (Fig. 3.15). Cleaning of projectile evidence, if deemed necessary, should be done gently and the evidence air-dried before packaging. Package in breathable material. Gunshot residue can be collected on a tape lift, by shaving the area in question or submitting the entire section of the skin. Depending on the crime laboratory, gunshot residue collection kits may be available. Additional methods for gunshot residue collection and analysis are currently being developed for use on haired skin (see Chap. 7) [23].

3.4.2.2 Fingerprints

If evidence is to be collected for fingerprint analysis, that should be done early on in the necropsy, preferably right after external photo documentation. The item in question should be handled as little as possible and only with gloved hands. It

Fig. 3.14 Properly sealed, internally generated trace evidence has the opening taped, initialed and dated (sealed) as well as item identification on the innermost packaging and an attached evidence tag with the internal chain of custody

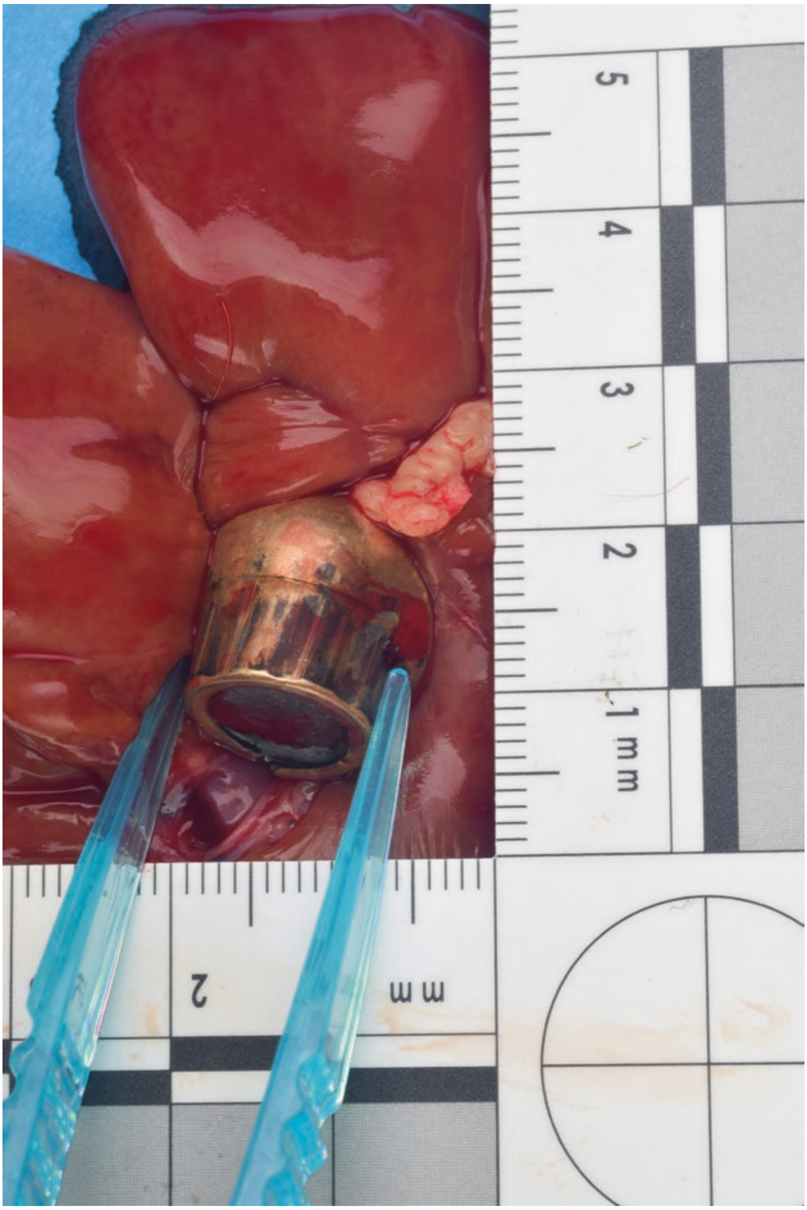

Fig. 3.15 A .45 caliber bullet being removed from the liver by the use of plastic forceps. Use only manual recovery or plastic or rubber tipped instruments to recover projectiles to avoid creating artifacts that may interfere with ballistic analyses

should be packaged in such a way that the item has minimal contact with the packaging material (Fig. 3.16). Packaging should be breathable.

3.4.2.3 DNA Analysis

Sampling of tissues for DNA analysis may be done for individual matching, species determination, or identification of fluid or other trace evidence. The pathologist should be aware of potential sources of contamination (self, concomitant necropsies, dirty instruments, etc.) and strive to eliminate or mitigate them. Tissue samples taken during the necropsy should be kept frozen. Soft tissues are excellent sources of DNA; however, bone, antler, horn, and hair (if the follicle is attached) also work well. Teeth, hair shafts, and mature feather shafts have only small concentrations of DNA. Dry tissues should be kept dry. Wet tissues should be frozen. Swabs can be dried and stored in envelopes or bags or they can

Fig. 3.16 Fingerprint evidence should be collected and packaged to minimize surface contact. In these sample cases, the soda can was picked up by placing a pencil in the opening and securing it to a cardboard sleeve. The tape does not touch the can and the cardboard only contacts the top and bottom rims. The *arrow* is secured to cardboard with zip ties. Cardboard collars around the ends of the *arrow* and cardboard "cushions" between the zip ties and the packaging reduce contact. Further packaging would be in a sealed, breathable container (such as a cardboard box)

be placed in empty vials and frozen, but do consult with the receiving lab for preferred medium and any other submission requirements.

3.4.2.4 Toxicology Samples

In general, samples for toxicology analysis should be packaged in glass, hard plastic vials, or aluminum foil and kept chilled or frozen. At least half of the sample should be saved back, particularly if the test is consumptive. Consultation with the toxicology lab ahead of time will ensure best results.

3.4.2.5 Forensic Entomology

Collection for forensic entomology is best done in the field, but that does not preclude sampling during the necropsy. Take a representative sampling of maggots. Typically 50–100 are collected. Be sure to include the largest and/or eldest identifiable individuals and remember that the oldest developing insects may be pupae. If the body has been submitted frozen, the maggots may be refrozen. If the body has been submitted fresh, maggots can be killed by immersing them in hot (not boiling) water and then placed in ethanol for submission. Collection and submission of an additional sample of live maggots may be helpful as species identification is made easier when adults are available. At a minimum, containers and a food source can be kept at the ready. Chat with the forensic entomologist ahead of time to confirm whether or not any of the maggots should be submitted alive.

Information the forensic entomologist will need to know to make the time of colonization determination minimally includes location where the body was found (preferably GPS coordinates), the date the body was found, the date the insects were collected, and the conditions the body was kept in between collection and necropsy. Storage temperatures will affect maggot growth, so refrigeration conditions should be noted.

The results you will get from the entomologist will reflect time of colonization but not necessarily the time of death. This is an important distinction to keep in mind. If insects did not immediately have access to the body, the time of colonization and the time of death may not be the same. For a more in-depth discussion of the role of forensic entomology in the veterinary necropsy and techniques for the pathologist, refer to Chap. 4 or other reference books on the subject [24–26].

3.4.2.6 Miscellaneous Trace Evidence

As a general rule, if something is dry, save it dry. Samples degrade more rapidly when they are kept in a damp environment. Fabric with trace evidence on it can be submitted whole. Trace fibers can be folded into a paper envelope or picked up on a tape lift that is then secured to a piece of clear plastic. If evidence such as bone or rope is cut by the pathologist, the cut end should be made apparent by marking it with tape. These are guidelines for some of the more commonly encountered pieces of trace evidence. As stated earlier, establishing a relationship with a crime lab ahead of time is the best way to ensure good collection technique.

3.4.3 Disposition of the Carcass, Samples, and Evidence

The carcass is evidence and needs to be treated as such through all stages of the investigation. When the necropsy is complete, all tissues should be repackaged, sealed, and appropriately stored. Ideally, storage both before and after the necropsy takes place in a locked refrigerator or freezer with minimal allowed access. It is best not to destroy any evidence until the case is closed and permission to dispose is given by the submitter. Premature disposal could be viewed with suspicion by the opposing council. It may also be considered obstructive; experts from the opposing council should also have the opportunity to analyze the evidence. Document the disposal on the chain of custody record and keep all of the paperwork indefinitely.

Legal Note The findings and conclusions in this chapter are those of the author and do not necessarily represent the views of the US Fish and Wildlife Service.

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4

Postmortem Changes and Estimating the Postmortem Interval

Jason W. Brooks and Lerah Sutton

4.1 Introduction

Death is defined as the cessation of the physiological processes that maintain cellular integrity and function. Almost immediately following death, an unavoidable, irreversible, and progressive sequence of physical and chemical changes begins in the body. While these changes occur in a predictable order of progression, the rate at which they develop is subject to great variability due to many circumstantial and environmental factors. A knowledge of the expected progression of postmortem changes is essential for the proper interpretation of gross and microscopic pathology at necropsy. A thorough understanding of such processes by a well-trained pathologist will minimize the risk of misinterpreting postmortem artifacts as lesions and will reduce the misidentification of actual lesions that may be obscured or distorted by postmortem changes. In addition, the estimation of the postmortem interval (PMI), also known as time since death, is highly dependent upon knowledge of these postmortem processes.

Estimating the postmortem interval is important in many investigations of human deaths, and it is similarly important in some animal forensic investigations. An estimation of the PMI may permit the investigator to include or exclude individuals from a pool of suspects and may corroborate witness testimony or other evidence while providing a more complete timeframe for the events that occurred during an alleged crime [1]. While much research on PMI in human and animal subjects has been conducted using controlled studies, limited data on PMI in animals are available from case-based studies. Furthermore, the interpretation of the data generated by many animal studies is difficult due to the great variation in methodology and species studied, inhibiting the formation of general conclusions about the relatedness of the findings of multiple studies. As a result, veterinary pathologists may attempt to extrapolate from the human data when attempting to estimate time since death in animal cases. It is essential to consider that many of these methods have not been validated in most animal species, and this lack of validation presents a significant barrier to the use of these techniques in court for cases involving animal crimes. Conversely, some of the methods used and conclusions drawn from human studies may, in fact, be applicable to animal subjects, although some form of verification may be warranted.

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The accuracy of PMI estimations is notoriously poor, and it has not significantly improved over many decades of research. Perhaps most importantly, no single method can be reliably used alone to accurately estimate the time since death [1–3]. Any such estimate is highly dependent on multiple factors, and is subject to increasing error with increasing time since death, necessitating great caution when formulating any estimate of PMI [4].

It is essential for the pathologist to understand the expected progression of postmortem changes in order to properly interpret the presence or absence of lesions at necropsy and to estimate the time since death. The pathologist should be aware that the changes described in this chapter, although addressed individually in the text, in nature will occur simultaneously and at different rates. It is of critical importance to note that these processes are subject to the effects of environmental conditions such as temperature, oxygen tension, insect and scavenger activity, and other factors [1].

4.2 Postmortem Changes

4.2.1 Algor Mortis

The cooling of the body after death is known as algor mortis. According to the principles of physics, the body may be approximated as a cylindrical mass of water subject to thermodynamic laws that influence its rate of cooling [5]. There is disagreement, however, regarding the applicability of certain thermodynamic principles to biological organisms, resulting in the proposal of alternative models of postmortem cooling of the body [6, 7]. Further complicating the identification of an ideal cooling model, it has been observed that many intrinsic and extrinsic variables heavily influence the rate of body cooling. Despite decades of research on the topic, an ideal temperature decay model has not been identified for any species and, if such a model existed, would be expected to vary across species. Some general conclusions have been drawn, however, and algor mortis may be used to aid in the estimation of the postmortem interval, although ideally it should not be used alone to estimate the time of death. As a generality, the postmortem period can be subdivided into the early and late postmortem periods, defined as the periods prior to which and after which the body has nearly equilibrated to ambient temperature, respectively [5, 8, 9].

Using algor mortis to estimate PMI is based on the concept that the body begins to cool after death due to the cessation of homeostatic cellular activities that generate heat and maintain body temperature. Following death, because no additional heat is created by physiologic processes, the body begins to lose heat to the environment, and the core temperature begins to decrease. In some cases, however, there is a delay in the internal cooling of the body. Such a delay is not entirely understood and has been observed both in human bodies and inanimate objects. The delay has been variably attributed to the establishment of a temperature gradient to permit heat transfer or to postmortem aerobic or anaerobic metabolism or intestinal bacterial metabolic processes [10]. When observed, the resultant cooling delay is known as a lag phase or temperature plateau effect (TPE) and has caused much difficulty in the modeling of postmortem temperature decay [11]. The TPE varies widely between studies and appears to be dependent upon such factors as animal species, cause of death, body region, body size, surface insulation, and environmental conditions [11, 12]. A number of mathematical models have been developed to predict body cooling, many of which have incorporated the TPE. Early simplistic models used very basic formulae for linear decay known as "rules of thumb." One such simple model states that the body cools at a rate of 1.5 °F per hour. This was later revised to account for more sigmoidal decay by estimating body cooling at 1.5–2.0 °F (0.83–1.11 °C) per hours for the first 12 h and 1 °F (0.55 °C) per hour thereafter [2, 13]. Another simple "rule of thumb" model states that the body cools at a rate of 1 °C (1.8 °F) per hour after death with an additional factor of 3 h to account for the TPE [14]. A two-exponential model eventually defined the sigmoid cooling curve mathematically and became the basis upon which the commonly used nomogram was developed [7, 15]. While numerous other formulae were developed, a comparison of the error associated with eight different methods showed the most consistent estimates with the smallest error in human bodies were produced by the use of the two previously stated "rule of thumb" models and the original two-exponential model [16].

The applicability of these cooling models for use in veterinary cases remains uncertain, although some studies suggest that cooling differs across species and that the application of such models may be inappropriate. In various animal studies, the TPE was not consistently observed in animal carcasses, resulting in a rate and pattern of cooling that differs from that observed in studies of human bodies. Studies of dogs and deer failed to produce any TPE, and studies of pigs produced only minimal TPE in few animals [6, 17–21]. Furthermore, one study in dogs demonstrated an average rate of cooling of 0.5 °C (0.9 °F) per hour; this differs significantly from the 0.83 to 1.11 °C (1.5–2.0 °F) hour rate of cooling predicted for human bodies [17]. Overall, animal studies suggest that the cooling of animal carcasses differs from that of human bodies, and methods for estimating PMI based on temperature may not be applicable across species.

4.2.2 Livor Mortis

The purple-red discoloration of the soft tissues due to postmortem gravity-dependent pooling of blood is livor mortis. Livor mortis may be observed on the external body surfaces such as the skin and mucous membranes or on the internal body surfaces such as the abdominal or thoracic viscera. Although readily visible on human skin, lividity is often not prominent in the skin of many animals even after shaving the hair, though it may be visible on the skin of pigs, the pinnae of dogs, and in the lungs of most animal species (Fig. 4.1) [18]. Livor mortis commonly develops within 30 min to 2 h after death in humans, although its onset in animals is not well established [13]. In early stage livor mortis, the discoloration of the tissue is the result of simple pooling of blood within the vessels at gravity-dependent locations. At this stage of development, digital pressure or repositioning of the body will force

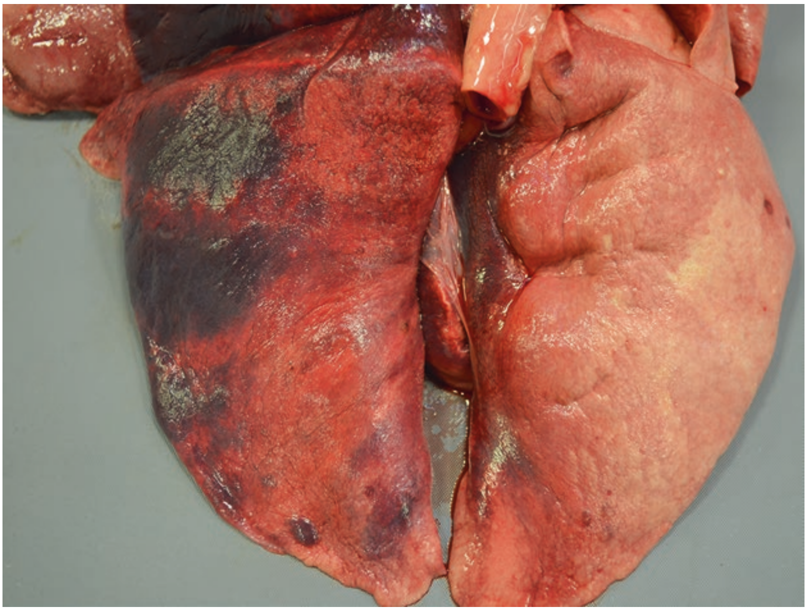


Fig. 4.1 Lividity in the left lung of a dog

blood away from the compressed area, resulting in focal blanching until the displaced blood is again permitted to flow back into the gravity-dependent areas. This early stage is referred to as non-fixed livor mortis. In addition to digital pressure, blood will also be forced away from areas in firm contact with the ground or other objects, and such materials may create patterns of blanching in the skin that match the contour of the affecting object [22]. Later in its development, at approximately 8–12 h in humans, hemolysis and decomposition of vessel walls result in the leaking of blood into surrounding tissues. Thus, at this later stage, lividity will no longer blanch in response to digital pressure [13]. At this stage, it is referred to as fixed livor mortis. This may be an important consideration for cases in which the position of the body at the crime scene may be relevant to the outcome of the case [1]. If the pattern of lividity observed at the time of discovery is not consistent with the position of the body with respect to gravity, then the pathologist should consider whether the body may have been moved after lividity became fixed [22].

4.2.3 Rigor Mortis

The state of postmortem muscle rigidity is known as rigor mortis (Fig. 4.2). Following death, a series of biochemical reactions begins to occur in the skeletal and cardiac muscle fibers. The molecular source of energy for muscular contraction, adenosine triphosphate (ATP), continues to be consumed by the muscle cells after death, resulting in cross-bridge formation between myosin and actin fibers. When the limited supply of ATP is exhausted, however, the regeneration of new ATP is no longer possible after death. Because ATP is required for the decoupling of actin and myosin fibers and the resultant relaxation of the muscle, relaxation can no longer occur after ATP is depleted. This results in the muscle fibers remaining in a state of permanent contraction, unable to relax due to the lack of additional ATP [23]. As this process develops following death, the muscles of the body will become increasingly rigid until all ATP is depleted, and they will remain in this state until the rigidity is either disrupted by force or early decomposition, thereby disrupting the integrity of the myosin and actin filaments [13].

The progression of rigor mortis is variable; however, there are well-established intervals for the expected onset and resolution of postmortem rigidity in humans. Rigor mortis typically begins at approximately 2–6 h after death and persists for roughly 36 h until it begins to slowly resolve. This progression, however, is dependent upon ambient temperature and patient factors such as antemortem activity and cause of death which may affect muscle metabolism and core body

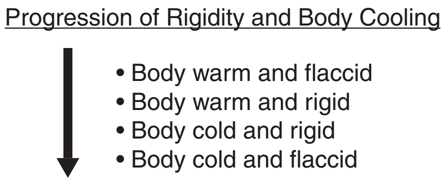
Fig. 4.2 Rigor mortis in a cat. The limbs are rigid and resist flexion

temperature at the time of death [13, 23]. In humans, rigor mortis affects all muscles in the body uniformly and simultaneously, although the contraction is often most easily visible in the small muscle groups of the body. The jaw often appears to be affected first, later progressing to the larger muscles of the upper extremities and then to the lower extremities, with resolution following in a similar pattern [13]. Bodies with greater muscle mass tend to display more prominent rigor mortis. Therefore, healthy adult males may be expected to display more pronounced rigor mortis while little or no rigor may be observed in juvenile or aged patients with little muscle mass [24]. The time course and pattern of development of rigor mortis in animals is not well established. One study in dogs reported the diffuse onset of rigor mortis at less than 1 day after death, with persistence in the hind limbs and jaw until 7 days after death [18].

While the rate of onset and resolution of rigor mortis is variable and dependent on multiple factors, the following rule of thumb has been observed for human bodies based on the association between rigor mortis and algor mortis (Fig. 4.3) [23]. This rule of thumb was not based on animal studies; however, the general progression of body cooling during the onset and resolution of rigor mortis is well documented and practical for field interpretation (Fig. 4.4). The interval assigned to each of these four stages is, however, expected to vary across species.

4.2.4 Desiccation

The postmortem drying of mucous membranes and delicate skin surfaces may result in changes of color or texture. Desiccation begins upon death and may progress rapidly in mucous membranes which, in life, are moist and sensitive to drying. This drying effect is often most prominent in the eye in humans, resulting in a horizontal band of red to brown-black discoloration of the sclera, known as tache noir, where the eyelids fail to close (Fig. 4.5) [22]. This manifestation is often not prominent in animals due to a relatively larger cornea and smaller exposed sclera. The development of desiccation in skin is typically slower, as skin surfaces are not normally moist and are more resilient to drying. The skin surfaces most often affected are thin, delicate areas such as the lips and genitalia. The gross appearance of desiccated surfaces is dark red to black with an irregular surface. Desiccation of skin and mucous membranes appears to be a less prominent feature in most animal bodies as compared to human bodies.



- Body warm and flaccid
- Body warm and rigid
- Body cold and rigid
- Body cold and flaccid

Fig. 4.3 Association between rigor mortis and algor mortis

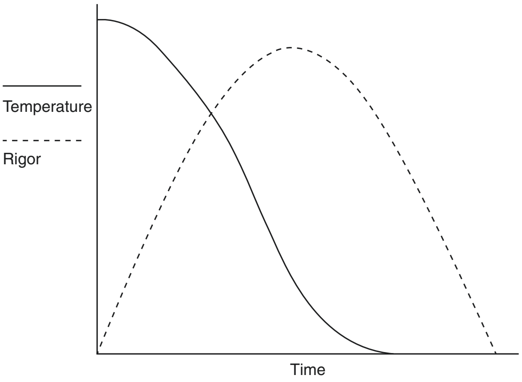


Fig. 4.4 Graphic depiction of the progression of rigor mortis and body temperature change

4.2.5 Decomposition

The decomposition of the soft tissues of the body is the most distinctive postmortem change. Decomposition is the result of two parallel and often simultaneous processes, autolysis and putrefaction. The process of decomposition begins on a molecular level almost immediately after death due to the failure to maintain the integrity of cellular membranes. As cells degrade and eventually

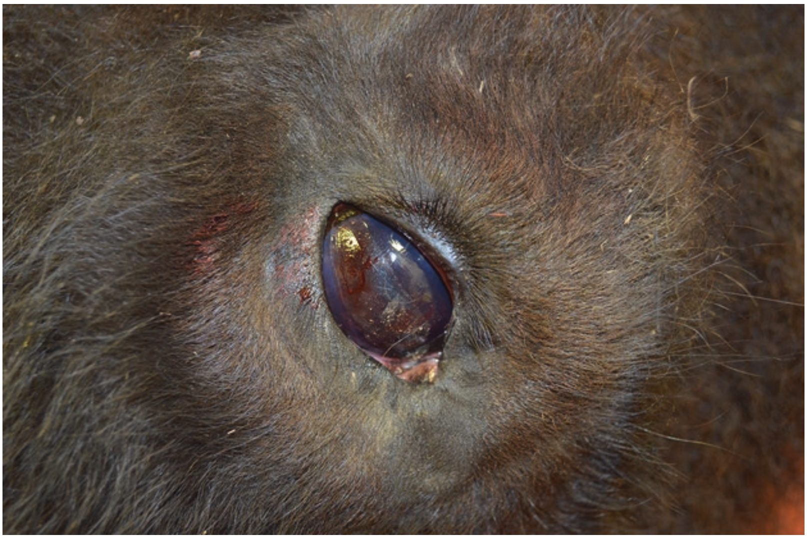


Fig. 4.5 Desiccation of the cornea of a steer resembling tache noir

rupture, they spill proteolytic enzymes that further degrade exposed cell surfaces and connective tissue fibers. The resultant chain reaction of degradative effects due to the digestion of tissues by intrinsic enzymes is autolysis. At approximately the same time, a second pathway of decomposition may begin due to bacterial proliferation and consumption referred to as putrefaction. Autolysis often precedes bacterial putrefaction, with the intrinsic degradation of cellular and interstitial components creating ideal conditions for bacterial proliferation. While decomposition has been extensively studied in animals and humans, its progression is known to be affected by many variables.

Despite the notable variations in rate of decomposition, there is a somewhat predictable sequence of stages through which most bodies progress after death if unimpeded by artificial means of preservation. It is of critical importance to note that many of these changes and processes are occurring simultaneously, and the development of one change may affect the progression or the appearance of other changes.

Classically, the phases of decomposition have been categorized as fresh, bloat, decay, and dry [25]. These stages have been defined as follows [25, 26]:

- Fresh stage—death until bloating begins (4–36 days)
- Bloated stage—onset of bloating until resolution of bloating (3–19 additional days)
- Decay stage—resolution of bloating until drying of carcass (6–183 additional days)
- Dry stage—drying of carcass until no evidence of carrion insects (13–27 additional days)

These stages, however, have been problematic for investigators due to the lack of clearly defined starting and ending points; therefore, Vass summarized the data in the literature and presented a table based on these four stages using degree of decomposition [27]. Prior to the summary by Vass, other investigators developed modifications of Reed’s four-stage scale, initially using a five-stage scale and later a six-stage scale [28–30]. The six-stage scale of Wilson considers the following stages: fresh, primary bloat, secondary bloat, active decay, advanced decay, and skeletonization. Both the five- and six-stage scales contain numerous grossly descriptive starting and ending points for each stage. The six-stage scale may be more appropriate for buried carcasses or those with a shorter PMI, while the five-stage scale may be better suited to exposed carcasses or those with a longer

PMI. The five-stage scale is summarized as follows (Fig. 4.6) [28]:

- Fresh—no discoloration or insect activity (0–5 days postmortem)
- Early decomposition—gray to green discoloration, bloating, post-bloating rupture, skin slippage, hair loss (1–21 days postmortem)
- Advanced decomposition—moist decomposition of tissues, sagging of flesh, caving in of abdomen, extensive insect activity, bone exposure of less than half of the skeleton, mummification (3 days–18 months)
- Skeletonization—bones with some body fluids present or tissue covering less than half of the skeleton, dry bones (13 days–3 years)
- Extreme decomposition—skeletonization with bleaching or exfoliation or metaphyseal loss or cancellous exposure (2 months–3 years)

In an attempt to explain the variation inherent in the rate of decomposition, Megyesi developed a

Fig. 4.6 Pig (**a**) Fresh—no discoloration or insect activity; (**b**) Early decomposition—gray to green discoloration, bloating, post-bloating rupture; (**c**) Advanced decomposition—moist decomposition of tissues, sagging of flesh, caving in of abdomen, extensive insect activity, bone exposure of less than half of the skeleton; (**d**) Skeletonization—bones with some body fluids present or tissue covering less than half of the skeleton; (**e**) Extreme decomposition—skeletonization with bleaching

method based on accumulated degree days (ADD) by scoring decomposition and accounting for time and temperatures to which human remains were exposed [29]. This study showed that ADD was responsible for 80% of the variation with which the bodies decomposed, supporting the conclusion that accumulated temperature over time was more critical than time alone. Vass also supported the ADD theory and proposed a universal formula for the estimation of PMI that was not dependent on geographic or climate-related factors [27]. However, Cockle demonstrated that the two universal formulae presented by Vass were not reliable and suggested that no universal formula could be expected to reliably estimate PMI [31]. One study emphasized the importance of climate in the accuracy of the ADD method, citing the low correlation between ADD and observed decomposition score in a study using ten human bodies in a semiarid subtropical climate [32]. Moffatt has recently suggested adjustments to the model used by Megyesi for improving the calculation of ADD from total body score (TBS) for an improved estimation of PMI, and Nawrocka recommended refinements to the TBS scoring system [33, 34].

The utility of TBS provides a unique perspective in which a four-stage model—fresh, early decomposition, advanced decomposition, and skeletonization—is broken down into more specific and descriptive units of measure. Rather than measuring the entire body as a whole, new methods for postmortem interval estimation, such as those developed by Megyesi, utilize TBS and ADD in conjunction to better associate temperature units with degree of decomposition [29]. Field research using this method indicates that PMI is more accurately estimated when decomposition is associated with environmental conditions. Traditionally, estimation and evaluation of degree of decomposition has been a qualitative process; that is, a visual evaluation of the body is conducted and the stage of decomposition is therein assessed. However, this lends itself to numerous problems as decomposition is grossly affected by external variables, particularly temperature and environmental changes. The body size also plays a role in the rate of decomposition. A study conducted by Sutherland using the TBS/ADD method shows that smaller pigs decompose faster than larger pigs [35]. The TBS method is beneficial because it allows for a quantitative approach to a traditionally qualitative method of PMI estimation; however, the method is not yet refined enough for field application. It assessed three anatomical regions of the body independently: the head and neck, trunk/torso, and limbs/extremities. In many cases, due largely to the influence of insects colonizing the remains, one area of the body will exhibit a more advanced stage of decomposition than others (i.e., the head may be skeletonized, while the torso may still exhibit bloat). In such cases, using only an overall four-, five-, or six-stage model assessment of degree of decomposition would certainly be conflicting and inaccurate. The independent scoring of each major area of the body allows for a more accurate and precise measurement of degree of decomposition [35]. New and ongoing research by this author at the University of Florida shows that even when specimens are of similar size and are placed in the same location to decompose, seasonal environmental changes and amount of precipitation lead to dramatic differences in the time it takes for remains to fully skeletonize. In the summer with consistent afternoon precipitation, remains can be fully skeletonized within 2 weeks. In colder winter and early spring months in the absence of precipitation, it can take up to 4 months for the remains to skeletonize. Likewise, in the same temperature conditions, precipitation alone can alter the rate of decomposition due to the onset of mummification of the remains in hot and dry conditions (Sutton, 2016).

The rate at which decomposition occurs appears to be affected by multiple variables, of which, temperature and moisture are the most completely understood. Additional variables that may affect decomposition rate include cause of death, disposition of the body (e.g., buried, submerged in water, enclosed in bag, encased in concrete), external covering and insulation by clothing or hair coat, insect activity, scavenger activity, trauma, and other factors [31, 36]. These variables are too numerous to be fully discussed here, but a few key findings are summarized. Although it has been well documented that locations of bodily trauma provide alternate sites for insect colonization and may therefore affect the pattern of decomposition, it has been determined that the presence of trauma from gunshot wounds in pigs does not significantly affect the overall rate of decomposition [37, 38]. Additionally, the presence of clothing on pig carcasses slightly but significantly slowed the rate of decomposition, although by providing more oviposition sites, the clothing may have changed the pattern of decomposition [39]. Exposure to insects and the heat generated by insect masses has been demonstrated to be a key factor in decomposition rate. A study using rabbits showed that rabbits continuously exposed to insects decomposed more rapidly than those exposed to insects and later buried [40]. Both of these groups decomposed more rapidly than rabbits that were never exposed to insects, both above ground and buried. Exposed surface remains have generally been estimated to decompose two times faster than submerged remains and eight times faster than buried remains [4]. A TBS system has recently been developed for use in submerged human bodies although none has been specifically developed for use in buried remains [41]. Scavenger damage to carcasses has also been showed to significantly affect decomposition and the estimation of PMI. One study showed that wolf damage to deer carcasses resulted in an increased rate of decomposition with consumption and movement of the carcass, reduction in carcass size, and an altered succession of insects [42]. Accelerated decomposition of human bodies was reported under a variety of conditions including high ambient temperatures (as a result of natural weather conditions, fire, indoor heating, bath/sauna), high body temperature (as a result of fever or drug use), sepsis, diabetes mellitus, and obesity [43].

4.2.6 Mummification

Under dry ambient conditions, regardless of temperature, in which there is low humidity and sufficient ventilation with limited insect activity, the body may become generally desiccated rather than progressing through the typical stages of decomposition [22]. In this case, the skin often becomes tight, yellow-brown to black, and may have a leathery or parchment paper consistency [2]. As a result of exposure to such dry conditions, the processes of autolysis and putrefaction are inhibited, and the tissues become dehydrated. This drying results in visible changes to the body including contraction or wrinkling of skin, retraction of the nailbeds and finger tips, and contraction of the erector pili muscles (Fig. 4.7) [22]. The time required for mummification to occur is variable, although it may generally be considered to require at least several weeks [23].

Fig. 4.7 Mummification of a dog resulting in black leathery skin. This dog was recently removed from the environment in which it mummified and was thrown into a dumpster which allowed for colonization by blow flies

4.3 Estimating the Postmortem Interval

A small number of techniques are likely to be of practical use for estimating the postmortem interval in veterinary cases. Methods used by the pathologist to estimate PMI will vary according to the actual postmortem interval (Fig. 4.8).

4.3.1 Gross Changes

The body begins to decompose almost immediately after death, first through autolysis and later through bacterial putrefaction. Although decomposition requires at least several hours before becoming grossly evident, other more visible changes may occur earlier such as rigor mortis and livor mortis. While these processes are greatly affected by numerous variables as previously discussed, a typical progression of gross changes over time can be anticipated (Fig. 4.9).

During the course of course of decomposition, the body undergoes a somewhat predictable sequence of alterations including changes in color, temporary muscle rigidity, distention with free gas, production of purge fluid, slippage of epidermis, destruction of soft tissues, and eventual destruction of bone. The purple-red gravity-dependent livor mortis that develops in the first few hours is due to postmortem settling of blood within the vasculature. Liver mortis may be visible within 30 min postmortem and typically becomes fully developed at approximately 10–12 h after death in humans; however, lividity is not a prominent feature in most animal carcasses [1, 22]. Following its full development, livor mortis becomes fixed and can no longer be blanched with pressure. The time required for fixation to occur is variable and may range from 12 h to 3 days in humans [23]. Rigor mortis typically begins at roughly 2–6 h postmortem, becomes fully developed by 6–12 h, and then begins to resolve at approximately 36 h in humans [23]. Rigor mortis may be interpreted in conjunction with body temperature to yield a postmortem window, although the timing of such windows may be species specific.

With increasing time postmortem, the effects of autolysis and putrefaction become increasingly evident. Color changes to the skin and soft tissues often become visible at approximately 24–30 h, often first observed as green discoloration of the abdominal skin as a result of the denaturation of hemoglobin to biliverdin and its reaction with hydrogen cyanide, a prominent putrefactive gas [2]. It has been reported that this green abdominal

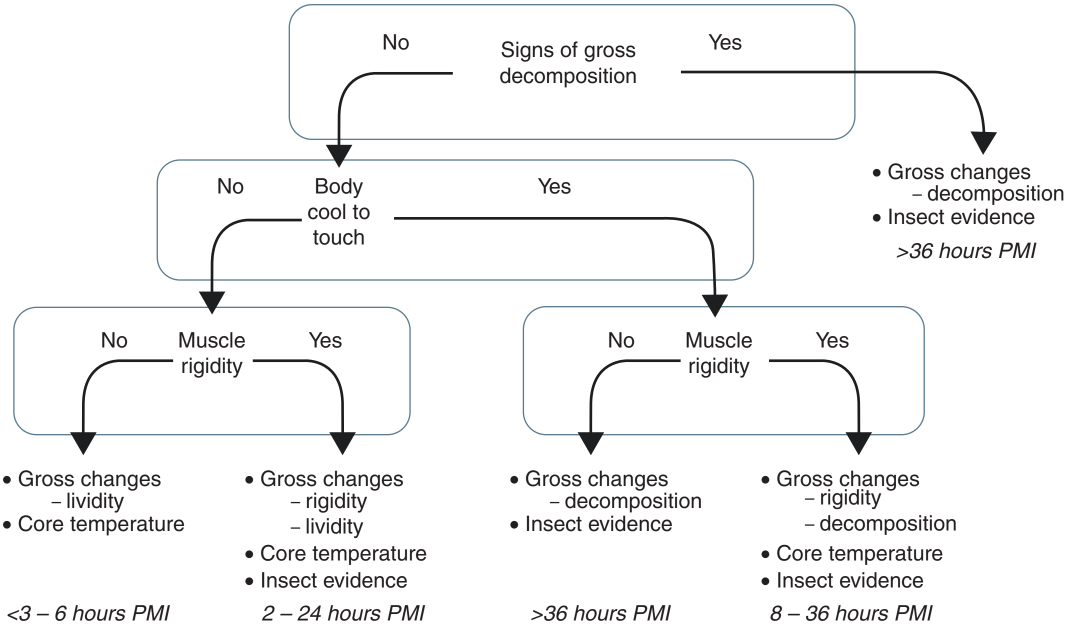


Fig. 4.8 Methods used to estimate postmortem interval according to actual time since death

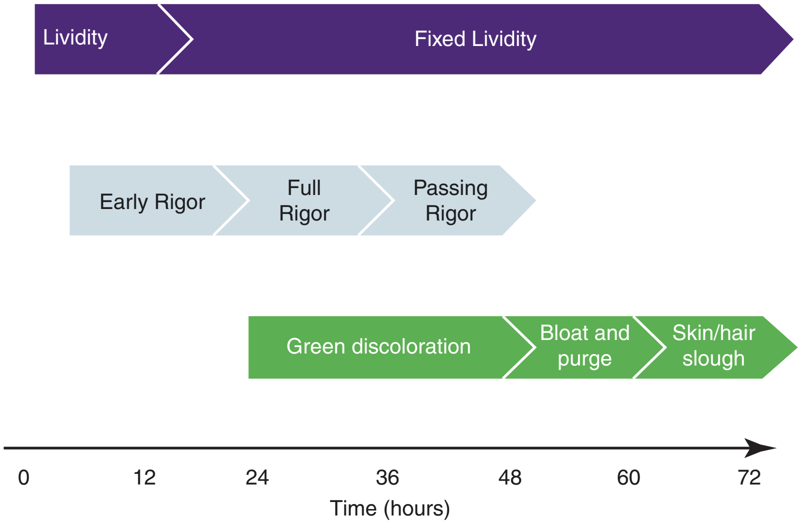


Fig. 4.9 Gross postmortem changes expected over time

discoloration may fail to develop in malnourished dogs and cats [44]. In addition to color changes, body cavities and internal organs often become distended by gases as a result of bacterial putrefaction. These gases may include methane, carbon dioxide, hydrogen, ammonia, hydrogen sulfide, and mercaptans and are largely responsible for the odor produced by the decomposing body [2]. Gaseous distention in most animal carcasses is most notable in the abdomen and may result in bulging or prolapse of the rectum. A variety of soft tissues may be affected by gaseous distention which often develops at roughly 60–72 h postmortem in humans; however, this may occur significantly faster or slower depending on animal species and environmental conditions. In ruminants, for example, abdominal distention may occur remarkably early, especially during high ambient temperatures [44]. Alternatively, small monogastric animals maintained at low ambient temperatures may develop no appreciable abdominal distention.

As decomposition progresses, the integrity of the skin becomes compromised and may result in slippage of the epidermis or sloughing of body hair. In humans, cutaneous vesicles filled with fluid or gas may form, although this feature is not commonly observed in animal carcasses. Red-brown fluid produced by autolysis and decomposition, known as purge fluid, often exudes from the mouth and nose and later may exude from the anus or other orifices [22]. In dogs, pink discoloration of the teeth may be observed several days to weeks postmortem [18]. Over the successive weeks, the continued degradation of the soft tissues by bacterial putrefaction, insect activity, and scavenger damage is almost certain but highly variable. These processes will lead to exposure of bone by several days to months after death, resulting in skeletonized remains.

Temperature is known to have a significant effect on autolysis and putrefaction, and either high or low ambient temperatures may affect the rate or pattern of decomposition. In general, increasing environmental temperatures result in an increase in the overall rate of decomposition. Freezing of the carcass, however, may both decrease the rate of decomposition as well as alter the pattern in which decomposition occurs [45]. Frozen carcasses have demonstrated a slower overall rate of decomposition, less internal putrefaction, and more external desiccation and oviposition by blowflies when compared to fresh carcasses [45].

More recently developed strategies have attempted to quantify the process of decomposition and standardize the time it takes to reach different stages of decomposition using thermal units rather than calendar days. These studies use total body score (TBS), which quantifies stages of decomposition independently in three-body regions (head, torso, limbs/extremities). Accumulated degree days (ADD)—a method most commonly used by entomologists to predict and determine age and development of arthropods—is used in this study to associate TBS with the time it takes to reach each stage of decomposition in thermal units within a 24-h period of time. In order to calculate ADD, one simply adds the sum of the average daily temperature of all days during the period of interest, including only days on which the average temperature was above 0 °C, the threshold at which decomposition can occur. In this way, a sum of thermal units accumulated over time is calculated. According to this theory, a body exposed for 2 days at 15 °C (ADD = 30) receives exposure to the same number of thermal units as a body exposed for a single day at 30 °C (ADD = 30). It is expected that ongoing research will further refine the application of TBS/ADD methods, but they are currently of little practical use in the field. These methods do, however, show great potential to become the standard of practice in the near future.

Although temperature and other environmental conditions are important factors in estimating degree of decomposition, new research suggests that the species itself may play a larger role in rate of decomposition which could alter the way future decomposition research is conducted. It has generally been accepted that pigs represent a suitable substitute for humans in decomposition research due in part to their size, distribution of hair, and insect preference toward them as compared to human carcasses. However, this type of research does not typically take into account the preference of animal scavengers and the ways in which scavenging affects rate of decomposition. A study conducted at the University of Tennessee compared the rates of decomposition of rabbits, pigs, and humans and, uniquely, included animal scavenging as a factor. It suggested that, as consistently documented in literature, blowfly colonization was more prevalent in warmer months and that animal scavenging was a more significant factor in the winter when insect activity was slower. It also suggested that the pattern of animal scavenging differed between species. Pigs tended to experience animal scavenging in the torso/trunk region, whereas humans were often scavenged at the limbs (AAFS proceedings, 2016). In a forensic investigation, taphonomic changes including the evidence of animal scavenging of the remains will certainly be addressed. As a result, more research is needed to fully understand the implications of species differences as they relate to animal scavenger preference.

4.3.2 Temperature Changes

The use of a postmortem temperature decay model is a common method for estimating PMI in human bodies; however, any such estimation should be considered in the context of the species in question rather than through simple extrapolation from data derived from human studies [46]. The ideal approach for the veterinary investigator is to obtain data from controlled studies and case-based data collected from the species in question. Unfortunately, only a small number of such studies currently exist, and the data generated are of limited utility for practical application. Therefore, the veterinary investigator may consider the following practical methods for interpreting core temperatures in the field for the estimation of PMI. The first three methods were developed based on studies of human bodies, while the fourth method was developed based on data collected from dogs. Prudence should be used in the application of these methods. It is generally advised that the investigator considers the range of estimates produced by several of these methods:

1. The "rule of thumb" which states that the body cools at a rate of 1 °C per hour after death, plus a factor of 3 h to account for the TPE [14]. This can be expressed as:

$$PMI\left(\text{in hours}\right) = 37^{\circ}\text{C} - rectal\ temperature^{\circ}\text{C} + 3 \quad (4.1)$$

2. The reported average rate of cooling of 1.5–2.0 °F (0.83–1.11 °C) for the first 12 h after death, followed by 1 °F (0.55 °C) per hour thereafter [2, 13]. This can be expressed as:

[Approximated as *PMI* (in hours) = (37 °C–*rectal temperature* °C)/0.83] (4.2)

$$PMI\left(\text{in hours}\right) = \left(98.6^{\circ}\text{F} - rectal\ temperature^{\circ}\text{F}\right)/1.5$$

3. A two-exponential model refined by Henssge and presented in the form of an easily used nomogram instead of a calculation [5]. The nomogram is available for use on many websites and is widely published in many textbooks. Its use requires only a single rectal temperature, ambient temperature, and body weight and allows for correction due to clothing and environmental conditions.
4. Rectal temperature decreased 0.5 °C per hour in dogs. Body weight and body volume were inversely proportional to the rate of cooling, although sex, body mass density, and hair coat density were found to have no effect [17].

For application in animals whose core body temperature differs from that of humans, these formulas should be adjusted by replacing the 37 °C (98.6 °F) with the expected core temperature for the species in question.

The veterinary investigator must consider that there is a degree of error inherent to each of the methods even when applied to the species for which they were developed. In general, there is at least a 2-h margin of error in the first 6 h postmortem, at least a 3-h margin of error in the subsequent 14 h, and at least a 4.5-h margin of error in the following 10 h [47]. The nomogram method specifically results in an error of 2.8–7 h in human bodies, and no studies have evaluated its validity in animal carcasses [15, 19, 23]. After approximately 24 h, when the body temperature equilibrates to the environmental temperature, these methods are no longer useful. Thus, the investigator should consider the limitations and inherent error of such methods and ideally consider other methods concurrently, particularly when used in court as evidence for estimating time of death [11, 48].

Models continue to be improved and new temperature-based methods have been more recently developed; however, none is yet practical for field application. While many methods have been investigated, perhaps the most promising are methods based upon recording of multiple rectal temperatures or temperature measurements from the eye or ear. One recently developed method is based on recording at least three rectal temperatures and using a nonlinear least squares method to calculate the estimated PMI with a decreased margin of error as compared to the nomogram method [49]. Ear temperature measurements in human bodies by a probe inserted into the ear canal have been well correlated to PMI when environmental temperature was above 15 °C, showing no plateau effect, and are superior to the nomogram method or other traditional "rule of thumb" formulae for estimating PMI [50, 51]. Eye and orbit soft tissue temperatures in humans measured by a pin probe inserted into the globe through the sclera show a faster postmortem temperature decrease compared to rectal temperatures, no plateau effect, and no effect due to body mass and reduced error compared to rectal temperature [52]. Similarly, ocular temperature was most accurate in estimating PMI in pigs during the first 13 h after death [6]. Thus, research continues, and models for PMI estimation based on ear or ocular temperatures or multiple rectal temperatures are likely to be refined in the near future.

4.3.3 Entomology

The careful evaluation of a body and its immediate environment and proper sampling of appropriate insect life stages may provide valuable information for the estimation of PMI. Insect evidence may, in some cases, provide the best estimate of the time of death or time of tissue colonization such as may occur antemortem with an untreated wound; however, one must carefully consider that the life processes of the relevant insects are highly susceptible to variations in environmental conditions and temperature [38]. The identification and further evaluation of insects falls beyond the scope of practice of most veterinary pathologists and is covered elsewhere and therefore is not discussed here [53]. Proper collection and preservation of insects from the body and/or the scene, however, is a skill that should be mastered by the pathologist for use in cases in which entomological evidence is expected to be of critical importance. While there are many guidelines regarding proper collection and preservation of entomological samples in the current literature, a basic overview is detailed here for use by a veterinary pathologist.

Adult insects should be collected on the scene if possible as they will quickly dissipate when human interaction with the remains begins. Adults can be collected using an insect net with a "figure 8" sweeping motion. Once collected, they should be preserved through placement into a screw top vial filled with either KAA (a solution of kerosene, acetic acid, and alcohol) or EtOH. A live collection of adults is not necessary. Larval specimens (i.e., maggots) should be collected both alive and deceased and can often be collected from the laboratory if an on-scene collection was not conducted. Ensure that collection of soft bodied insects such as maggots is done with featherweight forceps to prevent damage to the larvae. Blanching the maggots before preservation is recommended to prevent the maggots themselves from decomposing which may make a species identification difficult. Place the maggots into very hot water for 15 s, and then remove and place into KAA or EtOH. If possible, 50–60 individuals should be collected and preserved for the deceased larvae collection. If a sufficient number of larvae are present, a live collection should be made as well. Collect the maggots with featherweight forceps and place them into a plastic container. Within the plastic container, a soil substitute should be placed on the bottom; soil itself can be used or a soil substitute such as vermiculite is also acceptable. A food source such as beef liver or even cat food should be placed in a small amount of tinfoil and placed on top of the soil substitute. Place the live maggots directly onto the food source inside the tinfoil, and gently close up the foil, making a pouch to shelter the maggots for transport. Do not close up the foil so tightly that the maggots will not receive airflow. Place a lid on the plastic container that is holding the soil, tinfoil, food, and maggots, and punch small holes to allow airflow but not so large that the maggots will be able to escape. Label all containers with case number, collector's name, time, date, and location of collection. An entomologist will need all this information to properly write their report. If a collection can be made on scene, pupae should be collected as well. They are small and dark in color with a rigid outer casing. These should be collected the same way as the larval specimens with the exception of blanching in water, which is not necessary for pupae. Eggs may be present on the body but may be difficult to collect. They are typically off-white in color and are present in clumps in the hair on the head of the body, often near the eyes, ears, nose, or mouth. These should be documented photographically if collection is not possible. As with pupae, they should be collected the same as larval specimens.

From a practical perspective, basic forensic entomological findings may be interpreted by the pathologist according to the following guidelines. Typically, the first insects to arrive upon the dead carcass in a terrestrial environment are the blowflies [54]. The delay between death and the initial arrival of blowflies is highly variable and dependent upon many factors but may occur within seconds to minutes [2]. This is a critical feature in the interpretation of entomological evidence and its utility in estimating the minimum time since death [23, 38]. Adult female blowflies will deposit eggs on the body in predictable locations, including the orifices of the head (eyes, nose, mouth, ears) and the anogenital region; any deviation in this pattern is suggestive of trauma, as insects will also colonize other body areas containing injured skin or exposed body fluids [38, 55]. The rate of development of the subsequent life stages is highly dependent upon environmental conditions and is most precisely interpreted in terms of accumulated degree hours or days [2, 38]. As a very general model, Saukko's estimates of life-stage times are presented, although these must be used with great caution [23]. Eggs hatch into the first-stage maggots, or first instar, typically within approximately 8–14 h of oviposition. First instar then molt to second instar after an additional 8–14 h. Second instar feed for 2–3 days before molting to third instar, after which time they feed for approximately 6 days before leaving the host to pupate. Pupae emerge as adult flies after roughly 12 days. Thus, the entire life cycle of the blowfly from egg to adult is approximately 18–24 days, depending on conditions. Following the colonization of the body by blowflies, additional insect species typically arrive, including other species of flies and beetles. As previously stated, proper collection and preservation is critical for the analysis of entomological evidence, and proper insect identification and appropriate environmental data are essential for the estimation of minimum time since death.

4.4 Ancillary Methods

4.4.1 Botany

The ubiquitous nature of plants in the environment makes botanical evidence potentially useful for establishing a geographic association between a victim and a suspect or the scene of a crime and can even be useful in locating burial sites and assisting in the estimation of the PMI [56, 57]. Plant evidence may be found on or inside of the deceased body, in the area surrounding the deceased body, or on the suspect's body and may range from entire plants or leaves, to small plant fragments or microscopic pollen or algae [58]. A competent forensic botanist, if provided with appropriate samples, may be able to identify plant species based on taxonomy or DNA sequencing which may indicate the area from which the plant fragment originated based on known geographic distributions of plant species. The life stage of the plant specimen may also assist in determining the time of year in which the plant structure was likely to have existed in nature. This may assist in the estimation of PMI or in the validation of witness testimony. Additionally, plant evidence may contribute in rough approximation of the PMI by estimating the minimum amount of time required for a plant to grow to the observed stage within the body or on the grave of a buried body. In order to properly interpret botanical evidence, it is essential that appropriate collection techniques are used and that samples are evaluated by a forensic botanist. Proper collection of plant evidence are reviewed elsewhere [58, 59]. In general, appropriate samples include such materials as reference plant samples from the crime scene; trace evidence samples including whole plants or plant portions such as seeds, flowers, leaves, or plant fragments; and pollen, spores, or algae from soil or water.

4.4.2 Postmortem Chemistry

The chemical analysis of body fluids for the estimation of PMI has been attempted for many decades; however, an ideal method has yet to be identified. Decades of research on various bodily fluids have generated data are imprecise, unreliable, and impractical for use in the field [60]. These methods, therefore, are not commonly used in modern investigations. While early studies focused on analysis of serum, more recent studies have observed that vitreous humor is less subject to autolysis [60]. Decreases in vitreous humor glucose and increases in potassium and hypoxanthine have been well documented [61–63]. However, a more recent resurgence of interest in vitreous chemistry using newer analytical techniques has narrowed the focus to vitreous potassium, sodium, and glucose as potential indicators of PMI [64–67]. One investigator has recently developed a web-based application for performing the calculations to interpret vitreous potassium for the estimation of the PMI [64]. It is expected that these methods will become more refined and their precision improved, thus rendering them a useful adjunct to PMI estimation.

4.4.3 Ocular Tonometry

An array of ocular changes have been correlated with PMI, including changes in corneal opacity, pupillary diameter, retinal vessel striation, retinal color, and intraocular pressure [68, 69]. Of these, corneal opacity and intraocular pressure are the most strongly associated with PMI. Corneal opacity was found to increase significantly at greater than 8 h after death; however, the opacity of the cornea is difficult to objectively assess and quantitate [69]. Intraocular pressure, however, is easily quantitated by a handheld tonometer and has been shown to decrease significantly over the first 12 h after death. Additional research is needed before this technique can be applied in the field.

4.4.4 Molecular Methods

Techniques to detect changes in nucleic acids and proteins have been investigated for their utility in estimating time since death. The degradation of either DNA or RNA through quantitative amplification of target genes or by assessment of nucleic acid integrity has been evaluated. Both DNA and RNA have demonstrated time-dependent decreases in integrity; however, these decreases differ between tissues and environmental conditions such as temperature [70, 71]. The rate of DNA or RNA degradation is generally lower at lower temperatures and occurs at different rates in different tissues [71–74]. Overall, methods of PMI estimation based on the assessment of DNA or RNA integrity show great promise, but such methods are not currently practical for field application.

Beyond nucleic acids, changes in protein profiles or levels of other organic compounds in tissues during the postmortem period may aid in estimation of the PMI. Such compounds may be measured by western blotting, matrix-assisted laser desorption ionization mass spectroscopy (MALDI-MS), ^{1}H magnetic resonance spectroscopy, and other methods. A number of specific proteins have been observed to decrease in relative amounts with increasing time postmortem, including various intermediate filaments such as desmin and troponin, calpain, calcineurin A, protein phosphatase 2A, and N-acetylaspartate, while some compounds were found to increase as decomposition progressed, such as butyrate and acetate [60, 75–80]. Although these methods may contribute to the understanding of decomposition and postmortem chemistry, they do not currently provide any practical value to the estimation of PMI.

4.4.5 Microbial Assay

Current DNA sequencing technologies permit wide-scale analysis of entire communities of bacteria and their genes within ecosystems. Such a collection of bacterial genes from organisms residing in a specific ecological niche is referred to as the microbiome. Analyses of organismal microbiomes, particularly the gastrointestinal tract microbiome, have shown dramatic changes in bacterial populations at various postmortem intervals and at different locations within the body [81–83]. Furthermore, the composition of bacterial communities within the soil underneath decomposing human remains has been found to change at various stages of decomposition [84]. These findings may lead to the development of methods for the estimation of PMI based on the composition of bacterial populations either within or on the body or the surrounding environment; however, they are currently not of practical use.

4.4.6 Microscopic Changes

Although not often attempted for the estimation of the PMI, predictable changes in microanatomy or ultrastructural architecture at various stages postmortem have been reported. The pathologist must be aware of the critical importance of effective fixation and variation between different fixatives to allow for proper interpretation of histopathologic findings [85]. Within 1–3 days after death, gas bubbles, saprophytic bacteria, loss of cellular stain uptake, and loss of tissue architecture are observed [86]. Because of the great variability in the onset of autolysis and decomposition, no timeframe has been produced for such changes in most tissues. Therefore, histology currently has limited utility in the estimation of PMI. One study in dogs, however, presented histologic changes observed at various intervals postmortem, while another study in rats correlated microscopic changes in the testes with postmortem interval [18, 85]. Immunohistochemical staining patterns were used in canine B and T lymphocytes and in human pancreatic and thyroid cells for insulin, glucagon, thyroglobulin, and calcitonin; however, these methods lacked precision [18, 87–90]. Ultrastructural changes in dog myocardial cells examined by transmission electron microscopy during the first 4 h after death were correlated with PMI due to changes in mitochondrial structure that occurred within 15–45 min postmortem [91]. Although these microscopic tissue changes alone are not likely to yield a reasonable estimation of PMI, these methods may be used to provide supporting evidence in some cases.

4.4.7 Radionuclide-Based Methods

For cases involving skeletonized remains, special techniques for aging bone have been attempted. Such methods have included analysis of radionuclides such as ^{210}Pb and ^{210}Po, radiocarbon ^{14}C dating, citrate content, nitrogen content, and several other methods [92–94]. Application of these techniques is typically beyond the scope of the pathologist.

4.5 Summary

Time of death estimation remains a central topic in forensic medical research, and it is evident that an ongoing study is needed. Techniques capable of improving accuracy in the estimation of the postmortem interval will undoubtedly assist in veterinary forensic investigations and would likely be investigated for their applicability to human forensic investigations. Investigators are advised to consider that the methods discussed in this chapter remain imprecise and that no single method, or even a combination of methods, can be used to reliably estimate PMI with great accuracy. Caution must be exercised when attempting to extrapolate the data and formulas based on human studies for use in animal cases.

In practice, the pathologist will likely find only a small set of these findings to be of use for application in veterinary cases. Although nearly all animal cases submitted to the pathologist are likely to arrive during the late postmortem period, the pathologist should be familiar with techniques applicable to both the early and late postmortem period. In most cases, it is likely that PMI estimation in the early postmortem period will be based upon gross changes including quality of rigor mortis and early decomposition, rectal temperature decrease, and perhaps early insect evidence. It is likely that PMI estimation in the late post mortem period will be based upon entomology and gross changes including the quality of rigor mortis and the stage of decomposition (Table 4.1).

Table 4.1 Summary of commonly observed changes in the early and late postmortem periods for use in estimating the postmortem interval

Early postmortem period (Approximately <24–36 h PM)
Core temperature decrease
A decrease of approximately 0.5–1.5 °C per hour may be expected
Gross changes
Livor mortis
• Onset (30 min–2 h)
• Fixed and persistent (beginning at 8–72 h)
Rigor mortis
• Onset (2–6 h PM)
• Fully developed (6–36 h PM)
• Resolving (>36 h PM)
Decomposition
• No discoloration or insect activity (0–5 days PM)
• Bloating (as soon as 1 day)
• Discoloration: grey–green (as soon as 1 day)
• Skin/hair sloughing (as soon as 1 day)
Entomological evidence
Blowfly Life Stages (reported as time since oviposition)
• Eggs (highly variable—may be deposited at any time after death)
• 1st instar (8–14 h)
• 2nd instar (16–28 h)
Late postmortem period (Approximately >24–36 h PM)
Core temperature decrease
Equilibrates to environment in approximately 24–48 h PM
Gross changes
Livor mortis
• Fixed and persistent (beginning at 8–72 h)
Rigor mortis
• Fully developed (6–36 h PM)
• Resolving (>36 h PM)
Decomposition
• Bloating (onset at 48–72 h, resolution by 7–13 days)
• Discoloration: grey–green (onset at 24–30 h)
• Skin/hair sloughing (onset 1–5 days)
• Purge fluid (onset at 48–72 h)

(continued)

Table 4.1 (continued)

• Soft tissue destruction (3 days–3 years)
• Skeletonization (13 weeks–3 years)
Entomological evidence
Blowfly Life Stages (reported as time since oviposition)
• Eggs (highly variable—may be within minutes of death)
• 1st instar (8–14 h)
• 2nd instar (16–28 h)
• 3rd instar (3–4 days)
• Pupa (9–10 days)
• Adult (18–24 days)
Decomposition is generally slowed by 50% or more during submersion or burial

These observations are highly variable and dependent on multiple factors and should be considered only as a guideline only for very general estimation. *PM* postmortem

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Blunt Force Trauma

5

Robert Reisman

5.1 Introduction

Blunt force trauma is a common cause of accidental injury in animals and is the most common cause of non-accidental injury in animals. Common causes of accidental blunt force trauma injuries are moving vehicle accidents, falls including high-rise falls in urban environments, and athletic activity. One of the goals of a forensic necropsy of a blunt force trauma case would be to draw a conclusion as to whether the reported explanation of accidental injury makes sense or, if the pattern and type of injuries are more consistent with a non-accidental cause.

In all cases of non-accidental injury, a violent person is the perpetrator. Violence is a major public health problem. It is a problem that can be addressed by veterinarians. In 2006, the One Health Initiative presented a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals, and the environment [1]. "Together, the three make up the One Health triad, and the health of each is inextricably connected to the others in the triad" [2]. Generally, infectious disease is considered to be the central focus of this type of interdisciplinary effort; however, violence is also a "leading worldwide public health problem" [3]. In 1996 at the 49th World Health Assembly in Geneva, Switzerland, member states were urged to "assess the problem of violence in their own territory and to communicate to WHO their information about this problem and their approach to it" [3].

Interpersonal violence (violence between two humans) is defined as "the intentional use of physical force or power, threatened or actual, against another person or against a group or community that results in, or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment, or deprivation" [4]. In the last 25 years, the "Link" [5] between interpersonal violence and animal abuse has been well documented by social science research [6]. The "Link" is a perfect example of the One Health Initiative.

Family violence refers to the situation in which there is interpersonal violence in a family. There can be one or multiple perpetrators and one or more victims. Victims can be animals, children, intimate partners (domestic violence), and the elderly and/or disabled individuals. Violent perpetrators can be adult men and women, adolescents, and children. There is a societal need for forensic veterinarians and veterinary pathologists to investigate cases of non-accidental injury of an animal because the animal cruelty prosecution enhances the prosecution of violent offenders. In January of 2014, NYPD became the primary responder for complaints of animal cruelty in New York City. Since then, there have been more than 80 cases in which an animal has been injured,

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and there has been concurrent human violence. Several of these were domestic violence cases, and in some of these cases, there was a successful animal cruelty prosecution but no concurrent assault prosecution for interpersonal violence (the abused woman refused to testify against her abuser—not an uncommon situation).

In a study of 53 New Jersey pet-owning households in which there was child abuse and neglect, there was animal abuse in 60% of the households. Children, who are abused, may abuse their pets. This was true in 26% of the households. Animal abuse occurred in 88% of the households where there was physical child abuse (21 families) [7]. The inclusion of an animal cruelty prosecution, separate from the need to protect animals, frequently benefits human victims as well.

Violence toward animals is common. The most common type of violence toward animals is blunt force trauma. The forensic veterinary evaluation of an animal with blunt force trauma injuries should identify the type, severity, and pattern of injuries. In the case of a deceased animal, the forensic necropsy should determine cause of death, and finally, the injuries should be considered in the context of cause; is the type, severity, and pattern of injuries consistent with an accident or a non-accidental event?

5.2 Forensic Evaluation of an Animal: Goals

A forensic evaluation is performed when there is the suspicion that a crime has been committed. As animal cruelty is a legal determination, the purpose of the veterinary forensic evaluation is to collect information about the animal's health that will assist in making the legal determination. The forensic veterinary medical investigation is a parallel and independent investigation from the law enforcement investigation. The veterinary forensic investigation is an impartial search for the truth. The veterinarian's responsibility is to make an unbiased assessment of the animal's health.

Like any veterinary examination, the first step in a forensic evaluation is the medical history. A unique aspect of the medical history in a forensic evaluation (as compared to a non-forensic evaluation) is crime scene investigation. The veterinarian should take advantage of opportunities to participate in crime scene investigation. If the animal(s) is brought to the veterinarian by law enforcement, there should be a discussion of the crime scene. Photographs at the crime scene are important because they document and give context to the traumatic event (if indeed, the location of where the animal was found is the location where the crime took place).

Key points in the medical history may help the veterinarian understand the circumstances that resulted in the animal's injuries. This history includes reviewing any previous medical records for the injured animals. Veterinarians should make concerted and determined efforts to review an animal's previous medical records and the medical records for other animals from the same household. A pattern of repeated traumatic events may come to light. This would be powerful evidence that an animal is the victim of non-accidental injury (NAI).

Litigation of criminal cases can last for years, the endpoint being a trial, and evidence may be discovered at any time during this period. Law enforcement and the district attorney should inform the veterinarian of the discovery of any evidence that is informative about the actual traumatic event.

It is not often that a veterinarian gets to witness the traumatic event that caused an animal's injuries. Video surveillance recordings and the ubiquitous smartphone have changed that, and the veterinarian now has the occasional opportunity to witness the traumatic event that caused the animal's injuries. This is educational. If a veterinarian sees a video recording of an animal being thrown to the ground, the veterinarian will have the opportunity to witness the type of impact that can cause, for example, a fractured femoral diaphysis (true case).

5.3 Non-accidental Injury (NAI) and Accidental Injury (AI)

Traumatic injuries can be classified as accidental injuries (AI) or non-accidental injuries (NAI). In some instances, the traumatic event is witnessed; a dog is hit by a car, a kitten is thrown against the wall. The cause of the animal's injuries is known. An explanation by an individual of the cause of a traumatic event(s) that resulted in an animal's injuries may be true or false. The explanation may be given to law enforcement (when they respond to an allegation of animal cruelty) or to the staff at an animal facility (animal hospital or animal shelter) when an animal is brought for care. When the explanation is given to law enforcement, the veterinarian's role is to identify the type and severity of the animal's injuries. If possible the veterinarian should draw a conclusion as to whether the pattern, type, and severity of the injuries is explained by the reported event (i.e., accidental or non-accidental). In cases where the event is non-accidental, the veterinarian is conducting a forensic veterinary examination and functioning as a forensic veterinarian or veterinary pathologist. They are collecting information that may one day be presented in court. Veterinary evaluations may or may not support a finding of animal abuse. Law enforcement and the prosecutor's office rely on the veterinarian to make an accurate determination.

For cases in which the explanation of an animal's injuries is an accidental traumatic event and the type and severity of the injuries are not consistent with the reported event, there may be a suspicion of abuse. A legal case may be initiated by the veterinarian or other staff as complainant. A veterinarian who reports suspected animal abuse will be making a "good faith" report of suspected animal cruelty or "recognizing and reporting" suspected animal cruelty.

5.4 Reasons to Suspect Non-accidental Injury When an Animal Is Injured [8]

Why would there be a suspicion of abuse if the events described assert that the injuries were caused accidentally?

5.4.1 Person(S) Behavior and/or Statements Raise Suspicions

- The person presenting the animal confesses or implicates another person.
- The history is discrepant—more than one explanation for the injury/injuries is given by one individual, or multiple people give different explanations as to cause of the animal's injury/injuries.
- The behavior of the person with the animal arouses suspicion.
- The person is reluctant to explain the animal's injuries.
- The person becomes defensive or angry when questioned.
- The person has a lack of concern for the animal.
- The animal is caught in the larger problem of family violence.

5.4.2 Clinical Picture Is Inconsistent with the Explanation Given

- The animal does not go outdoors and is not exposed to unknown causes of trauma.
- This is no history of accidental trauma (e.g., moving vehicle accident).
- Review of medical records (standard medical practice) may uncover a history of trauma (one animal, multiple animals).
  - One animal presents multiple times for traumatic injuries. This is likely a "battered animal" who has experienced repetitive injuries. Repetitive injuries can be determined two ways:

    Medical records (from one or more animal hospitals) show that an animal has presented repeatedly for traumatic injuries.

    A veterinary evaluation determines that an animal has traumatic injuries of different ages.
  - Multiple animals in the same household have experienced traumatic injuries. What is the nature of the injuries? What is the age of the animals that have been injured? If all the animals are young, it should be considered that in addition to any live injured animals, other animals may have been killed.
- The account of the traumatic event does not explain the observed injury/injuries:
  - The severity of the injury/injuries is greater than would occur by the force of the impact of the described traumatic event.

  - The number of injuries could not occur because of the described event. Accidents tend to have one major impact and possibly a minor secondary impact.
  - The pattern or distribution of injuries in NAI differs from pattern of injuries typically seen in cases of accidental trauma. Blunt force trauma due to falls to the ground or similar “benign” circumstances should be distributed over bony prominences of the body and have a pattern of injuries along one plane of impact of the body as the result of a single major impact. Injuries to recessed or protected parts of the body are suspicious for assault [9].
  - There are injuries of different ages (repetitive injuries).
- The injury type may vary with each episode, but fractures feature prominently; head trauma (+/− skull fractures), rib fractures, and/or femur fractures are present [10, 11].

5.5 The Case of the Midwestern Twenty Something Who Didn’t Seem Interested in the Circumstances That Resulted in Her Cat’s Broken Leg

It was a bitter cold, wintry evening in New York City when a young woman with an injured cat showed up at the animal hospital. The examining veterinarian had a sense that the woman’s lack of interest in how her cat broke its leg was important. After all, the cat lived exclusively in a NYC studio apartment and never went outdoors. The veterinarian did not suspect that the young woman had hurt her cat; however, she felt like there was more to the story and that this may not have been a benign event. How does an indoor cat in a small Manhattan apartment break its leg? The veterinarian contacted a mental health professional who was available to interview the woman. The veterinarian could also have contacted the police. Although questions asked during medical history taking may provide useful information about the event that caused the animal’s injuries, it is not the responsibility of the veterinarian to determine the human circumstances of the event. That responsibility belongs to the police.

The psychologist discovered that the woman, who had moved to New York from a Midwestern state a few years earlier, had fallen on tough times and unfortunately turned to prostitution. A sad tale of young life in the big city. It was one of the young woman’s “clients” who had kicked the cat and fractured its leg. Because of this intervention, the woman got back in touch with her family, from whom she had become estranged. Her family, happy to be reconnected, paid for the cat’s care, and the woman moved back home. Two lives saved, because a veterinarian recognized a problem with the woman’s lack of interest in the cause of her cat’s injuries.

5.6 The Case of the Boyfriend Who Was a Serial Animal Killer

A man calls his girlfriend at 7 pm on Sunday night to let her know that the Dachshund puppy she recently gave him for his birthday (think of the significance of bringing a live being into their new “family”) was not acting normally. The woman asked him to take the puppy to the veterinary hospital. Hours later, she called to find out the results of the doctor visit. To her dismay, she found out that he had not taken the puppy in for medical care. Voices were raised and he agreed to take the puppy to the hospital.

When he arrived at the veterinary hospital, he explained that the puppy had fallen while being bathed. The puppy had six rib fractures (Fig. 5.1), a hemothorax and a hemoabdomen, injuries that were inconsistent with the reported event. A fall from a few feet could not have resulted in an impact with adequate force to cause the observed injuries. The puppy died 20 h later, despite intensive medical care.

During the investigation, it was determined that the woman had given her boyfriend another Dachshund puppy months earlier that had also died. Exhumation of the puppy’s body by law enforcement allowed an exam that identified a skull fracture (Fig. 5.2), with

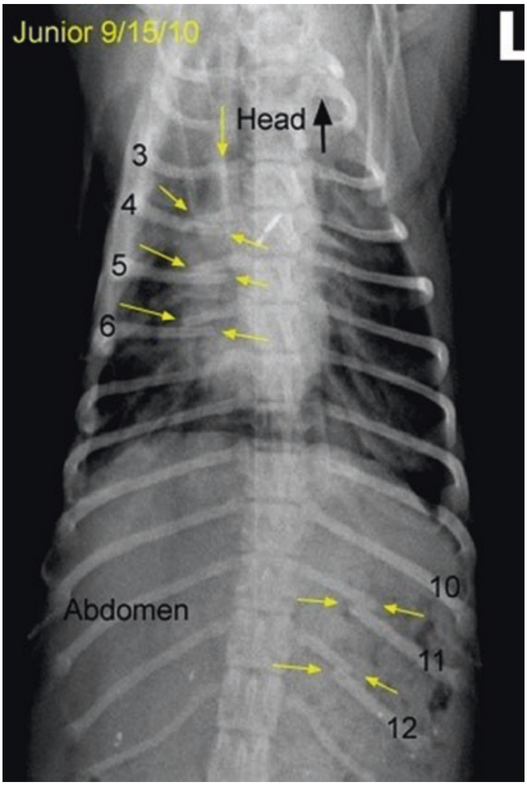

Fig. 5.1 Two groups of rib fractures representing at least two impacts in the puppy that was reported to have fallen while being given a bath

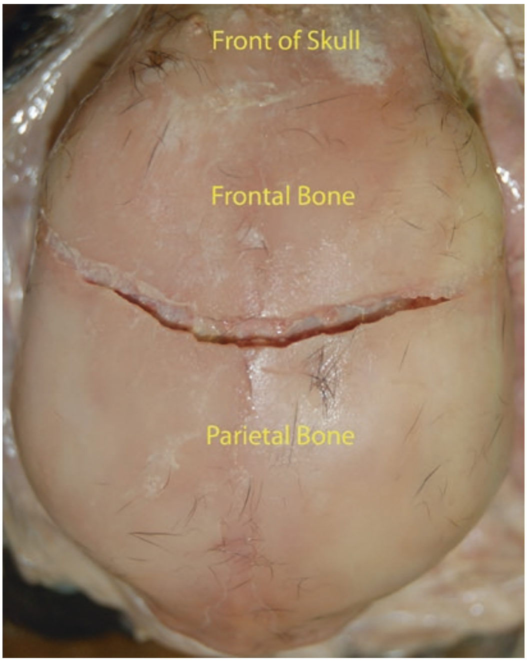

Fig. 5.2 The skull fracture of the exhumed puppy. Adobe Photoshop Elements is a good software program for labeling photographs and doing basic exposure enhancement. For the benefit of non-medically trained people working on the case, medical terms should be defined and images labeled

an assumed associated traumatic brain injury. The individual was found guilty of felony (puppy that was said to have fallen during the bath) and misdemeanor (exhumed puppy with fractured skull) animal cruelty.

5.7 Differentiating Accidental from Non-accidental Injury

There are different ways a veterinarian can differentiate AI from NAI. Biomechanics, the physics of forces and their effect on living tissues, can answer questions about the type of force that would be necessary to cause observed injuries. It is a biomechanical assessment on the part of the veterinarian that is the basis for concluding that a 6-month-old puppy's six rib fractures, hemothorax, and hemoabdomen were not caused by a fall in the bathtub (Sect. 5.6). The force of an impact from falling 3 ft. could not have caused the identified injuries.

The types of questions that should be attempted to be answered by physical examination (live animal examination and/or necropsy) to differentiate accidental injury from non-accidental injury are:

1. What injuries are present?
2. What is the nature of the injuries? Blunt force trauma, sharp force trauma, or animal attack?
3. What is the distribution of the injuries on the body (specific areas or anatomic parts)?
4. Are the identified injuries the same age, or is there evidence that they occurred at different times?
5. What is the blunt force mechanism of injury? Energy transfer, acceleration/deceleration, or physical disruption of tissue (crushing injury)? See discussion of Mechanism of Injury, Sect. 5.17.

5.8 Tissue Injury

Tissue injury can be defined as damage to the body tissue that results in tissue dysfunction. Injury causes pain, distraction, and incapacity [12]. What are the consequences of injury? Does pain and distraction due to injury cause reduced appetite and resulting weight loss? The "severity of an injury" is a characterization that may assist in creating a treatment plan or understanding cause of death. No classification scheme is perfect; however, a systematic approach to assessing injured tissues is superior to a haphazard assessment. Classification schemes may vary based on the tissue involved and whether the animal is alive or deceased. The basic elements of a classification scheme (see Table 5.1) for injury severity should include.

(1) structural involvement/disruption, (2) physical signs (including pain) in live animals, and (3) level of dysfunction [13]. This type of classification can guide description of injured tissue in live and deceased animals. For example, when there is musculoskeletal injury, if significant swelling and inflammation of associated soft tissue is not present, then a low-energy impact is likely.

Table 5.1 Classification of human ligament injury severity [13] as seen in Whiting 2008 [12]

Grade	Severity	Structural involvement	Exam	Performance deficit tissue dysfunction
1	Mild	Negligible	No visible injury, mild local inflammation and pain[a], joint stable	Minimal to a few days
2	Moderate	Partial	Visible swelling, marked pain, +/− stability	Up to 6 weeks (may be modified protective support)
3	Severe	Complete	Gross swelling, marked pain, antalgic posture[b], unstable	Indefinite. Minimum of 6–8 weeks

[a] Pain has been substituted for the terms tender or tenderness used in Whiting 2008 [14]

[b] Antalgic posture—a posture or gait assumed to lessen pain

5.9 Blunt Force Trauma

Blunt force trauma injuries (soft tissue and bone) are produced by the impact of an animal's body against a blunt surface or by the impact of an object with a blunt surface against an animal's body. Blunt force trauma injuries occur during accidental and non-accidental events. Common causes of accidental blunt force trauma injuries are moving vehicle accidents, falls including high-rise falls in urban environments, and athletic activity. Specific types and patterns of injuries characterize each type of event. Pelvic fractures, for instance, are the most common bone fractures in motor vehicle accidents, occurring about 20% of the time [11, 15]. Friction abrasions of the skin and focal full-thickness skin defects are also commonly observed motor vehicle accident injuries (Fig. 5.16–5.20). Blunt force impacts are the most common cause of non-accidental injury of animals. An area of recent and current research is the type and nature of injuries associated with various accidental and non-accidental events [10, 11, 16].

In physical abuse cases, the animal's injuries may result from a single event in time or numerous separate events over time. "Battered pets" [10, 17] experience repeated traumatic events (repetitive injuries) [8]. The term battered pets was taken from the term "battered-child syndrome" introduced in the historic 1962 paper on physicians' responses to non-accidental injuries in children [18].

5.10 Understanding Blunt Force Tissue Injury

Veterinarians are trained to diagnose injury and disease. They are the most qualified individuals to understand the correlation between activity, age, and injury in animals. Biomechanical engineers are trained to perform calculations that correlate tissue injury to the forces that produce injury [19]. This requires knowledge of the mechanical laws of physics (Newton's laws) and material science (body tissues have specific material properties), dimensions of the affected tissues, and physical properties of tissues in terms of stresses, strains, and strength.

Biomechanics is used to understand the effect of forces on living tissue. Veterinarians are not physicists with expertise in biomechanics; however, a basic understanding of the biomechanics of injury will help them draw conclusions about the type of forces that were applied to soft tissue and bone consistent with observed injuries. Ideally, a discipline focused on the biomechanics of animal injury will develop.

5.11 Biomechanics of Blunt Force Trauma

Classical mechanics [12] is the branch of physical science that focuses on forces and energy and their effect on inorganic and organic objects. Some basic terms to define are motion, speed, velocity, and acceleration. Motion occurs when an object changes its position relative to a reference point during a certain time interval. Speed is the term used to describe change in position (units of distance) during a specified time interval (units of time). For example, a car is traveling at 50 miles/h. Velocity adds the additional factor of direction to speed. The car is traveling west at 50 miles/h. (velocity). Acceleration is the rate of change of velocity during a specific time interval, observed as speeding up or slowing down.

In classical mechanics, for a body with constant mass, the acceleration of the body is directly proportional to the force acting on the body (Newton's second law of motion). Force is any influence causing a free body to accelerate. Change in acceleration is one of the mechanisms of injury caused by the application of a blunt force to living tissue.

$F = m*a$
F = force (units = Newtons)
m = mass (units = kg)
a = acceleration (units = change in velocity/unit time)

All objects (including biological tissues) have material and structural properties that influence the effect of forces applied to these objects. Mechanical engineering principles describe the effect of dynamic forces acting on an object (with specific material and structural properties) and resultant changes in the structure's form and direction of motion.

Biomechanics [12] is the application of classical mechanics to biologic systems. Biomechanics is used to describe the effects of intrinsic and extrinsic physiological and nonphysiologic forces on living tissue (with specific material and structural properties).

The force applied to tissue is also called the load. Body tissues continuously experience loads during normal activity with no obvious injury. Internal and external forces are applied to tissue during normal movement. These loads are within a physiologic range. The probability of injury increases when loads exceed the physiological range (overload). A single overload can exceed a tissue's maximum tolerance to manage the overload. Internal forces are the effect of muscles, tendons, and ligaments on bone. External forces include gravity and the impact of an animal's paw as it strikes the ground. Kinematics is the terms used for the description of movement without regard to forces involved, and kinetics is the description of movement in the context of forces involved.

5.12 Biomechanics of Animal Tissue Injury

Testing of the biomechanical properties of all human tissue is incomplete. This has been supplemented by testing of biological materials of other animal species, and the result of these tests has then been correlated to human tissue properties. A statistical analysis of human vs. animal tissue reveals the similarity in the strength of the materials [19]. Consequently, there is already scientific information to support a discipline focused on the biomechanics of animal tissue injury (see Sect. 5.20). Biomechanical properties of tissue (e.g., bones, tendons, ligaments, cartilages, and organs) are determined by testing tissues under tension, compression, bending, impact bending, impact snapping, torsion, tearing, cleavage, shearing, crushing, as well as expansion, bursting, and extraction. Certain limits must be exceeded to create injury. To injure a ligament, its elastic limit must be exceeded. To break a bone, the applied force must exceed the yield point to cause a fracture to begin to propagate.

5.13 Injury and Force

Forces applied to a body or tissue are the most significant element to be considered when there is physical injury due to a blunt force impact. As previously stated, forces that must be considered when there is injury are (1) the forces muscle, tendon, and ligament exert on attached bones, (2) gravity, (3) normal compressive forces when an extremity strikes the ground (walking, running), and (4) the force(s) applied to tissue at the blunt force impact site. It is rare that a single force acts on an animal's body. Most injury cases involved multiple forces.

When a force is applied to an animal's body, the animal's body moves. There are two basic forms of movement when the force is applied: (1) translational or linear motion, a body moves along a straight line (rectilinear motion) or a curved line (curvilinear motion), and (2) angular or rotational motion, the body rotates about an axis of rotation. Consider, for example, a blunt force impact near a joint. Rotational movement will ensue. Movement of living organisms can be a combination of these two basic forms of movement. If a force great enough to cause injury is applied to an animal's body, the type of injury sustained is in part due to the movement generated in the animal's body [20].

Specifically, for a blunt force impact, the injuries that result are determined by:

1. The force of the impact (see different qualitative aspects of force below)
2. The characteristics of the body area that sustains the impact
3. The characteristics of the object contacting the body

5.14 Qualitative Aspects of Forces

When an injury occurs, there are specific aspects of the force that affect the type of injury, the tissues injured, and the severity of the injury [12]:

1. Magnitude of the force applied. A blunt force trauma impact of greater force causes more severe tissue disruption/injury than a blunt force impact with a smaller force.
2. The location on the body where the force is applied will affect the injuries sustained. If, for example, there is an object-tissue impact near a joint, there will be angular or rotational motion of the impacted tissues. It is important to keep in mind that there can be injuries remote from the impact site because of the different mechanisms of injury (see Sect. 5.17).
3. Direction of the force or the angle of impact. Is the impact perpendicular to the impacted tissue (creating a compressive force) or tangential (creating a torsional compressive force)?
4. How often is the force applied? Repeated force application with insufficient time for recovery (cumulative trauma injuries) may result in chronic injuries. Repeated force applications can weaken tissue and may result in what appears as an acute injury.
5. Variability of the magnitude of the force (constant or variable during application).
6. The length of time of the impact or the rate of the application of the force. If a force is applied slowly, the tissue can displace at a rate low enough to absorb and dissipate the force over a large area minimizing tissue disruption. Conversely, if a force is applied rapidly, the tissue cannot displace at a low rate and over a large area. The result is a shock or compression wave that forms and passes through the tissue resulting in tissue injury.

5.15 Types of Forces That Can Impact Tissue [20, 21]

Tensile: A force that pulls on tissue, for example, the force generated when there is motion or an impact and tendons and/or ligaments pull on a bone.

Compressive: A force that pushes on tissue, for example, when a blunt object impacts tissue or tissue impacts the ground because of a fall or being thrown to the ground. The angle of impact influences the effect of the compressive force.

Bending force: Applied to a specific focal point on the bone perpendicular to the long axis of the bone and result in transverse or short oblique fractures of the diaphysis.

Torsion: When the force applied causes tissue rotation.

Shearing: The applied force causes tissue planes to slide past one another.

Additional types of forces: tearing, cleavage, crushing, expansion, bursting, and extraction.

5.16 What Happens to a Three-Dimensional Object When a Force Is Applied? [20]

5.16.1 Strain, Shear, Stress, and Deformation

When a force (F) is applied to a three-dimensional object, the object's shape changes or is deformed in two possible ways:

1. The dimensions of the object (length, width, depth) change along the x, y, or z axes that define the shape of the object.
2. The shape of the object may be distorted, meaning there are changes in the angles between the lines that define the shape.

5.16.2 Terms Used to Describe the Effect of Forces on Objects

5.16.2.1 Strain

Strain occurs in an object (normal) when the dimensions of the object change because of the application of force. Tensile or compressive forces that stretch or compress an object cause strain. The object responds to the applied force by becoming longer and thinner (tension pulling the object apart) or shorter and wider (compression pushing on the object).

5.16.2.2 Shear

Shear refers to the effect of a force applied to an object that results in a change in angles between lines that define the object's shape. This results in distortion of the shape of an object.

5.16.2.3 Stress

Stress is equivalent to the force generated internally in bone as a load is applied (force/unit of cross-sectional area of material; Newton/m^2).

5.16.2.4 Deformation

This is a change in dimensions or shape of an object (change in angles between sides) due to the application of force. The types of forces that cause object deformation are tension (pulling on the object) which creates normal strain causing the object to become longer and thinner, compression (pushing on an object) which creates normal strain causing the object to become shorter and wider, torsion which causes shear strain changing the shape of the object by changing the angles between the sides, and bending which causes deformation that is a mix of the strains caused by the other three forces.

With the application of sufficient force, bones undergo elastic and plastic deformation. **A load-deformation curve** maps bone deformation (change in shape) as the bone is loaded (application of force). During the initial application of force, elastic deformation (reversible) occurs. With the continued application of force, a yield point is reached and **plastic deformation** (irreversible deformation) occurs. In the plastic region, the degree of bone deformation increases with continued application of force until catastrophic deformation (bone fracture) occurs when the ultimate strength of the bone is exceeded.

Forces, accelerations, stresses, and strains all behave according to the laws of physics regarding bones, muscles, tendons, ligaments, and organs. The potential for injury increases with increasing age. Increasing age of an individual results in decreased strength of joints, muscles, bones, tendons, and ligaments. When these structures are subject to excessive loading, they are subject to failure. This failure may take the form of stress, strain, rupture, or catastrophic failure as occurs with a complete fracture [19].

5.17 Mechanisms of Injury from a Blunt Force Impact

For a blunt force impact to occur, there must be motion of the blunt object or the animal or both. Kinetic energy is the energy of motion.

There are three mechanisms by which tissue (soft tissue and bone) injury occurs when there is a blunt force impact. Blunt force trauma injuries result from the effects on tissue of:

1. Energy transfer, absorption, and dissipation
2. Rapid transition from positive acceleration to negative acceleration (also called deceleration)
3. Direct physical disruption of tissue (also called "crushing" injuries)

Kinetic energy is the energy of motion. At the time of blunt force impact, there is a transfer of energy from the blunt object to tissue. Energy cannot be created or destroyed; it can only change form (conservation of energy—first law of thermodynamics). All the kinetic energy delivered to tissue is absorbed and changed. The amount of energy transferred and absorbed affects the degree of injury. The delivery of energy more than what is tolerable by tissues results in inadequate dissipation of transferred energy and tissue injury. Organ disruption such as liver lacerations and lung contusions are the types of organ injury that result from overwhelming energy absorption and inadequate energy dissipation.

In all instances when a blunt force impacts an animal's body, the body (all organs and tissues) accelerates in space. Deceleration (a change in acceleration) occurs when the animal's body and internal organs come to rest. Deceleration frequently happens suddenly, for example, when a dog's body strikes the ground after being hit by a moving vehicle. A certain velocity is reached by the animal's body when it is struck by the car. The animal's body then experiences a rapid deceleration to a velocity of zero when its body hits the ground. Internal organs are different sizes and weights and accelerate at different rates after the blunt force impact. The relative acceleration and deceleration rates of the different internal organs create a condition that results in injury. With rapid change from acceleration to deceleration, organs can be avulsed from fixed attachment points, and blood vessels can be torn. Organ disruption (liver lacerations and lung contusions) also occurs. High-rise falls have the additional force of gravity affecting the impact.

At the time of blunt force impact, there may be a direct physical disruption of tissue. This is a crushing or crushing deformation injury. This is the mechanism of injury that many people assume is the primary mechanism of injury, because it is the injury that occurs at the site of impact.

Injuries from excessive energy transfer and sudden acceleration followed by rapid deceleration may result in the most serious blunt force injuries (internal organ damage), and these injuries may be at some distance from the site of impact.

5.18 Case of a Left-Sided Impact and a Possible Additional Right-Sided Impact

During a verbal argument between a man and woman, the man took the woman's dog from the woman's hands and threw the dog to the ground. The dog was a 1.3 kg, 3-year-old, female Chihuahua. There was a single impact to the left side of the body (Fig. 5.3 and 5.4). The dog died 15 min later from a traumatic brain injury. There was no other known physical event. If there was an impact to the right side of the head (causing the observed right ocular trauma and right canine gingival laceration), it could have occurred when the woman left the room to keep her daughter from coming in the room. A right-sided impact cannot be confirmed; however, it is the best explanation for the right-sided injuries (i.e., right eye traumatic injuries and gingival laceration). In this case, as in many, the crime scene photo provided significant information about the traumatic event. It answered the question as to whether the single left-sided impact that occurred when the dog was thrown to the ground was adequate to cause the fatal injury. The answer is yes. The perpetrator was found guilty of felony animal cruelty.

5.18.1 Injuries Identified at Forensic Necropsy (Figs. 5.3 and 5.4, 5.5 and 5.6, and 5.7–5.9)

Gingival laceration (Fig. 5.6) associated with the upper right canine. Laceration is a disruption of tissue caused by a blunt force impact (see Section.

Right eye (Fig. 5.5)—Hemorrhage present in anterior chamber. Ciliary body torn. The posterior aspect of the orbit is not intact, and within the vitreous body, there is abundant hemorrhage admixed with fragments of the retina, sclera, and lens material (fragmentation of sclera, lens, and retina).

Skull (Fig. 5.9)—There are three free pieces of bone from the right and left caudal skull. The right lateral parietal area is fractured into seven smaller pieces. These injuries are due to rapid excessive energy transfer.

Brain (Fig. 5.9)—Hemorrhage is present in the meninges and the brain parenchyma on both sides of the brain. There are no macrophages present indicating the injuries are acute.

Lungs—Moderate to severe pulmonary contusions. More severe on left than right. This is an acceleration/deceleration injury.

Head, chest, and hind end injuries are common findings when small animals (i.e., dogs and cats) are thrown to the ground.

Fig. 5.3 and 5.4 Photo taken by owner soon after the dog was thrown to the ground. The pool of blood indicates that this single left-sided impact caused the fatal injuries to the soft tissues of the head, skull, and brain. There was no other witnessed traumatic impact

Fig. 5.5 and 5.6 If there was a second impact to the right side of the head (causing right ocular trauma and right canine gingival laceration), it could have occurred when the owner left the room to keep her daughter from coming in the room

Fig. 5.7–5.9 The left-sided impact that caused the fatal traumatic brain injury occurred when the dog's body struck the ground. The occipital bone and parietal bone were fractured into three large pieces. The section of the caudal right parietal bone broke into seven pieces

5.19 Characteristics of the Blunt Object

The characteristics of the blunt object that impact tissue influence the resulting injury. With the force of impact being equal, the smaller the surface area of the blunt object contacting the body, the smaller the area of the body to which the force is transferred. A force delivered to a smaller tissue area results in the energy of impact being less efficiently absorbed and dissipated by tissue than is the case with the impact of a blunt object with a larger surface area. The result is more severe tissue disruption and injury.

The impact from a blunt object that has a wider surface area, results in a force being distributed over a larger area of the body. The energy of the impact is more efficiently absorbed and dissipated by tissue, and the resulting injury is less severe.

Rib fractures are more common in physical abuse cases than motor vehicle accidents and high-rise falls. This is explained by a focused (e.g., blunt object, foot, thrown against blunt object) rather than a broad application of force during impact.

5.20 Tissue Characteristics That Influence Type and Severity of Injury [12]

The characteristics of the body area that receives a blunt force impact influence the resulting tissue injury. Material science can used to understand the effect forces have on tissues [19, 22]. Yamada (1970) provides extensive information on the effect of forces on tissues. Human and animal results come from postmortem tissues. The tissues can be prepared in different ways. "Wet compact bone" is the test material that most closely represents the effect that occurs with bone in a living person). Below is an example of the type of information that is used in biomechanical calculations for dogs and cats (see Tables 5.2 and 5.3) [22]. Table 5.4 shows human relative bone strengths:

Table 5.2 Elastic modulus of wet long bones in anteroposterior bending [22]

Species	Femur	Tibia	Humerus	Radius	Ulna
Dog	1170	1250	860	1010	1820
Cat	1300	1490	1220	1510	1320

Table 5.3 Ultimate bending strength (kg/mm^2) of wet long bones in the craniocaudal direction (CrCd) and the lateromedial (LM) directions [22]

Species	Direction	Femur	Tibia	Humerus	Radius	Ulna
Dog	CrCd	16.8 ± 0.6	17 ± 0.7	16.9 ± 0.8	17.8 ± 0.8	17.8 ±0.7
	LM	16.6 ± 0.9	17.4 ± 0.9	16.3 ± 0.7	17.7 ± 0.7	17.3 ± 0.8
Cat	CrCd	14.5 ± 0.7	17.0 ± 0.6	15.9 ± 0.9	16.9 ± 0.7	17.3 ± 0.8
	LM	14.6 ± 0.7	16.6 ± 0.6	15.2 ± 0.7	17.3 ± 0.6	17.1 ± 0.7

Table 5.4 Force required to fracture bones of the skull (human) [19, 22] derived from testing

	Fracture force in Pounds	
Bone	Range	Mean
Maxilla	140–445	258
Frontal	470–2650	1000–1710
Temporoparietal	140–3360	702–1910
Zygoma	138–780	283–516
Occipital	1150–2150	1440
Mandible	184–925	431–697

1. *Animal age* affects the strength of tissues. For example, the ultimate tensile strength of wet femoral compact bone is greatest in bone from people 20–29 years of age and least in people 60–79 years of age (70% of the strength of the younger group).
2. *The ultimate tensile strength* of wet compact bone from the middle of the femoral shaft is a little greater than that of bone from the upper or lower part of the shaft. The properties of a specific bone are not uniform over the entire bone.
3. *Ultimate percentage elongation* when a tensile force is applied to wet femoral compact bone is greatest in persons 10–19 years and least in persons 60–79 years old (85%). Specimens from small bones generally had a greater ultimate percentage elongation (i.e., they stretch more when a tensile force is applied) than specimens from large ones.
4. *Elastic modulus* represents the stiffness or elasticity of a tissue/substance which is a different property than strength. A higher elastic modulus indicates that tissue/substance is more stiff and less flexible with less deformation during the elastic portion (part of the curve where deformation is reversible) of the stress/strain (deformation/force) curve when strain (deformation) is graphed against stress (force). When a substance reaches its elastic limit, further application of force results in permanent deformation (plastic part of the stress strain curve).

   In decreasing order of stiffness in the dog: ulna, tibia, femur, radius, and humerus.
5. *Compressive strength* (humans) in the order of greatest to least: femur, tibia, humerus and fibula, ulna, and radius. Large bones generally have a greater ultimate compressive strength than the small ones.
6. The *bending strength* is greatest in the radius, ulna, and fibula and next greatest in the tibia and humerus and least in the femur. Generally, the ultimate bending strength of compact bone is greater in small bones than larger bones. Significantly, a bone will bend differently in the craniocaudal direction than the lateromedial direction.

The strength and the physical characteristics of the tissues involved influence the kind and the degree of injury produced. The volume of the tissue impacted influences the degree of tissue injury. The smaller the area of tissue impacted, the less easily the energy of the force applied is dissipated and the greater the chance of tissue injury.

Tissue strength is related to collagen, elastin, and water content. The combination of water (viscous) and fibers (elasticity) imparts what are called viscoelastic properties to tissues. The viscoelastic nature of tissue influences the relationship between the velocity with which a force is applied, the dissipation of transferred energy, the degree of compression or displacement imparted to tissue, and the degree of tissue injury. Tissues with little water and large amounts of collagen (tendon) are strong compared with a large amount of water and little collagen (liver). The capsule of an organ, which usually has a significant fibrous component, is important in protecting the organ from disruption.

The bone is a stiff material made principally of fibrous protein collagen and impregnated with a mineral closely resembling calcium phosphate. The bone also contains water, which is important mechanically. Most bone is covered by cells and has living cells and blood vessels within it. The bone being hard cannot sell or shrink; all changes in shape must take place at surfaces [23].

5.21 Tissue Response to Blunt Force Impact

Tissues may absorb energy and deform rather than be disrupted. Deformation results in areas of stress within the tissue. Whether a tissue is strong enough to absorb and dissipate the applied energy depends not only on how the force is applied but also on the stress condition of the tissue at the time the force is applied. Stress within the tissue is concentrated and intensified at sites where the characteristics of the tissue change, for example, at points where parenchymatous organs are attached by suspensory ligaments. The resistance of tissue to changing shape is a function of its internal friction, its viscosity, its elasticity, and the tendency for the tissue to resume its prestress configuration.

Tissue planes near the front of the transmitted energy wave accelerate relative to adjacent planes and may be torn apart, resulting in disruption of the tissue. Injuries occurring at low velocities are caused primarily by the extent of displacement between tissue planes. If displacement is great enough, shearing between planes results in disruption. The bones although stiffer than other tissues are also viscoelastic and follow the same rules under slow or rapid loading. During slow loading to failure, fractures propagate as a single crack and generally have only two major fragments. Fractures from rapid loading have comminutions that are caused by wave fronts propagating across numerous planes in a small area.

5.22 Types of Soft Tissue Blunt Force Trauma Injuries

More than one type of tissue injury is caused by blunt force impacts. Soft tissue injuries are contusions, abrasions, and lacerations. **Contusions** (Figs. 5.10 and 5.11, and 5.12) of the skin occur when blunt force ruptures small blood vessels in the dermis and underlying soft tissue, resulting in red blood cells moving from the injured blood vessel into the surrounding tissue (extravasation). Pulmonary contusions occur when there is disruption of the vasculature of the lung parenchyma.

The dog skin is like the human skin and that of other mammals in its composition, but not in its structure [24]. The average thickness of the skin (epidermis plus dermis) of dogs is 0.5–5 mm [24]; the human skin epidermis is on average 0.05–0.1 mm and the dermis

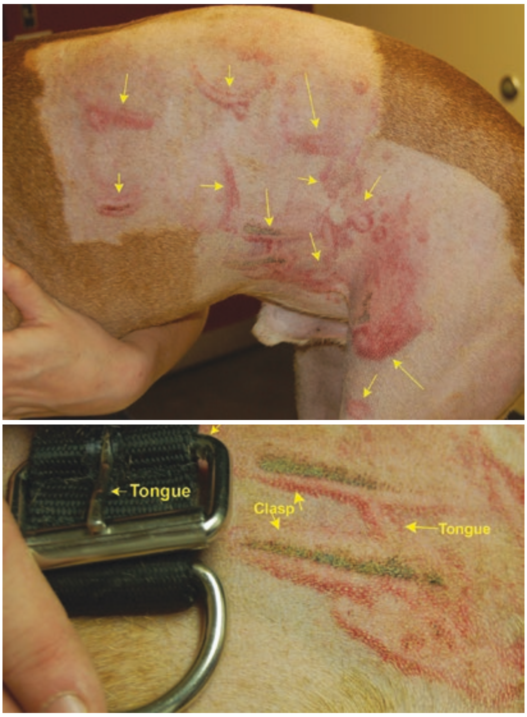

Fig. 5.10 and 5.11 This is a patterned contusion of the skin caused by the owner beating the dog with his collar buckle. Shaving the hair coat is necessary to identify and document the lesions

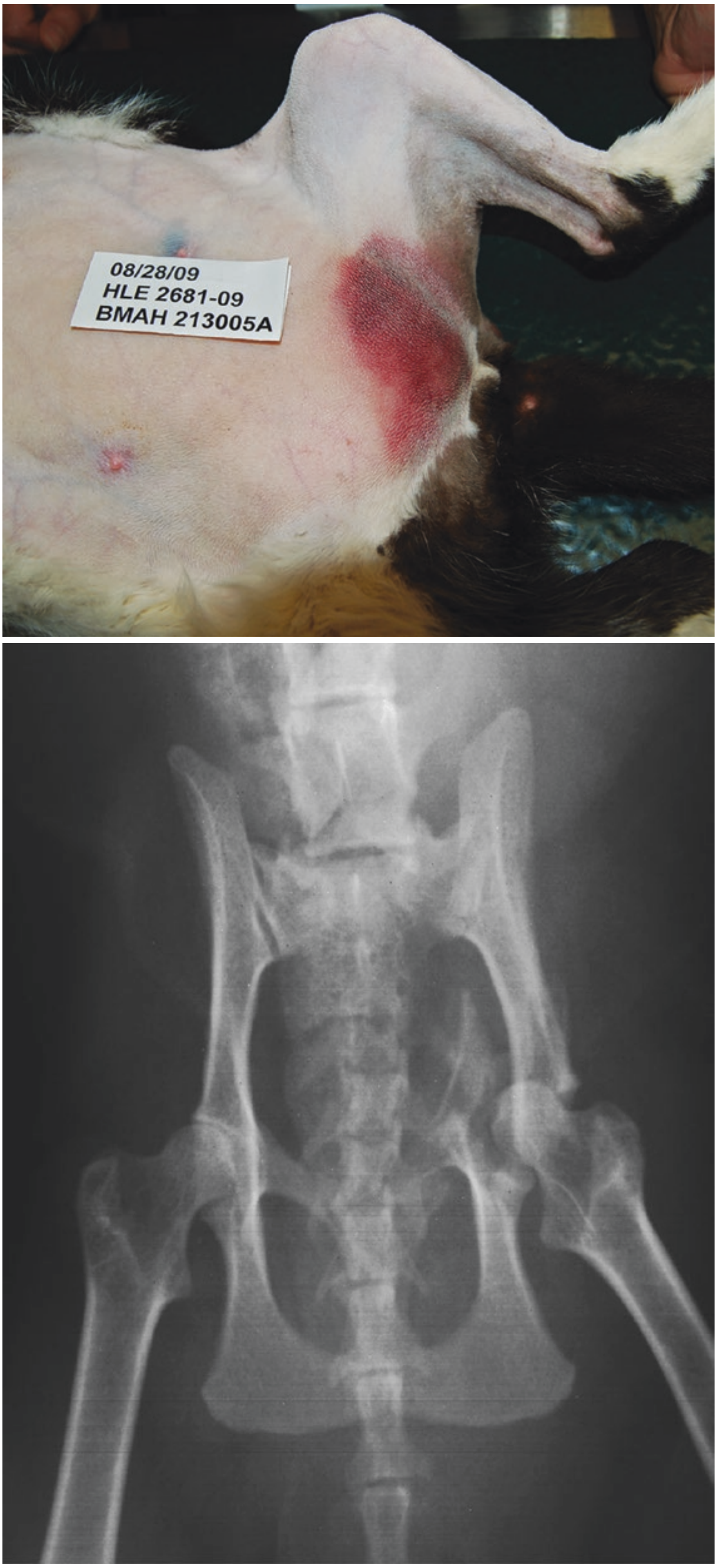

Fig. 5.12 and 5.13 An adult, female, domestic short hair cat with a single skin contusion of the caudal ventral abdomen. The blunt force impact was forceful enough to fracture the left acetabulum

0.5–5 mm [25]. Hair follicles in the dog skin are more numerous and different in composition, and the subcutaneous layer has a different structure and vascularization than the human skin [25]. The dog skin is generally more "movable" than the human skin, and its biomechanical properties should, in consequence, differ from those of other mammals [26]. For example, dog's skin bruises less easily than the human skin, and skin lacerations are less common in dogs and cats than people.

Abrasions (Figs. 5.14 and 5.15, and 5.16–5.20) occur when blunt force causes the superficial (epithelial) layer of skin to be scraped away, typically in an irregular fashion. Sometimes the pattern of the abrasion can indicate what kind of surface impacted the skin. Sometimes foreign material is embedded into the abraded surface, such as gravel from a road surface.

Lacerations represent a catastrophic disruption of tissue integrity. This tissue disruption results in an irregular splitting of tissue, frequently surrounded by a margin of contused tissue and possibly some tissue bridging across the split tissue.

Lacerations can occur to the skin, subcutaneous tissue, gingival (Fig. 5.6) and internal organs, and most notably the liver. Lacerations must be differentiated from incised tissue caused by sharp force trauma. An incised wound compared to a laceration is generally more regular in appearance, bleeds more, does not have tissue bridging across the wound, and does not have a contused margin. Lacerations, other than gingival lacerations, in the author's experience, are uncommon in blunt force trauma of dogs and cats.

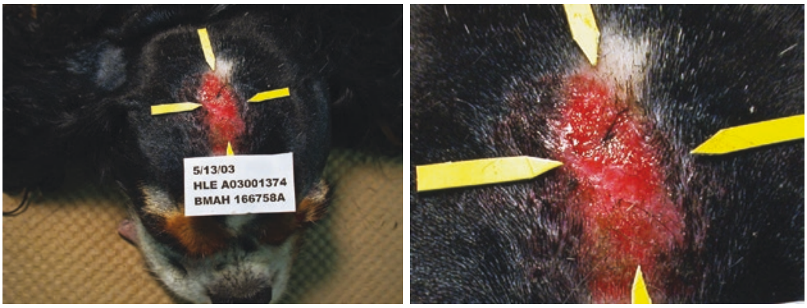

Fig. 5.14 and 5.15 This is an abrasion in a young adult, King Charles Spaniel, who was beaten to death

Fig. 5.16–5.20 Series of photos showing motor vehicle accidents skin lesions. Friction abrasion—*top right* photograph. Focal abrasions/lacerations; partial or full-thickness skin defects are common in motor vehicle accidents. Location of focal skin defects—*yellow arrows*; Close-ups of the three focal skin defects—*bottom row*

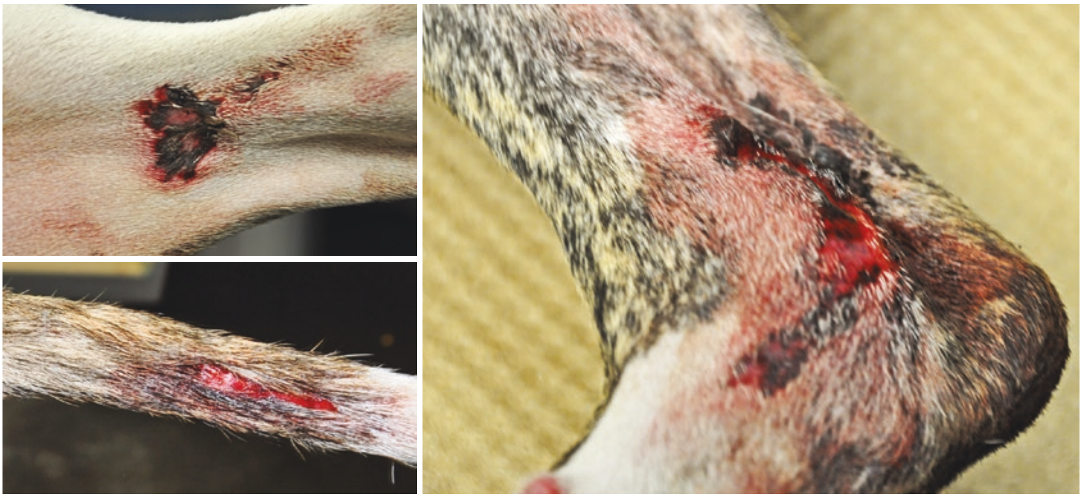

Fig. 5.16–5.20 (continued)

5.23 Case Study of the Dog with a Single Abdominal Contusion (Fig. 5.21–5.23)

A deceased, young adult, female pit bull dog was brought to the animal hospital for a necropsy. There is a single skin contusion consistent with the reported kick to this dog's abdomen. Necropsy revealed a bladder rupture and uroperitoneum as the cause of death.

5.24 Fractures [20, 21]

Bones and teeth can experience catastrophic disruption of tissue integrity (fracture) from a blunt force impact. There are two basic types of bone in an adult, the compact or cortical bone and cancellous (trabecular of spongy) bone. At the macroscopic level, the distinction between the two types is structural. The skeleton is generally made up of compact bone (80% of human skeleton). The compact bone (all long bones) provides strength and support. It has a higher elastic modulus (stiff, less deformable) than cancellous. The cancellous or spongy bone (e.g., vertebrae) is between 30 and 90% porous, is more elastic, and is generally diffused with bone marrow. It has a much lower elastic modulus (less stiff, more flexible). Larger bones, such as the femur, have both types, where the outer layers are cortical/compact and the inner portions cancellous. The vertebra is mostly composed of cancellous bone due to its function. The spinal column not only acts as a support mechanism for the upper structure of the human body but also performs a function as much as a shock absorber does in an automobile. It is movable in all three axes and capable of supporting great weight by distributing the stresses over the entire column.

What can a veterinarian say about the force that caused a bone fracture? Some language used is a powerful force that was applied to the bone to cause the fracture, or a significant force was applied to the bone to cause the fracture. Non-accidental bone fractures can be compared to accidental traumatic events such as motor vehicle accidents and high-rise falls to communicate that magnitude of the force necessary to break a bone.

Fractures fall into one of three categories based on the amount of energy that causes the structural failure: low-energy, high-energy, and very high-energy fractures. When evaluating a fracture, it is important to look at the degree of associated soft tissue injury (i.e., swelling and contusions). The greater the energy of blunt force

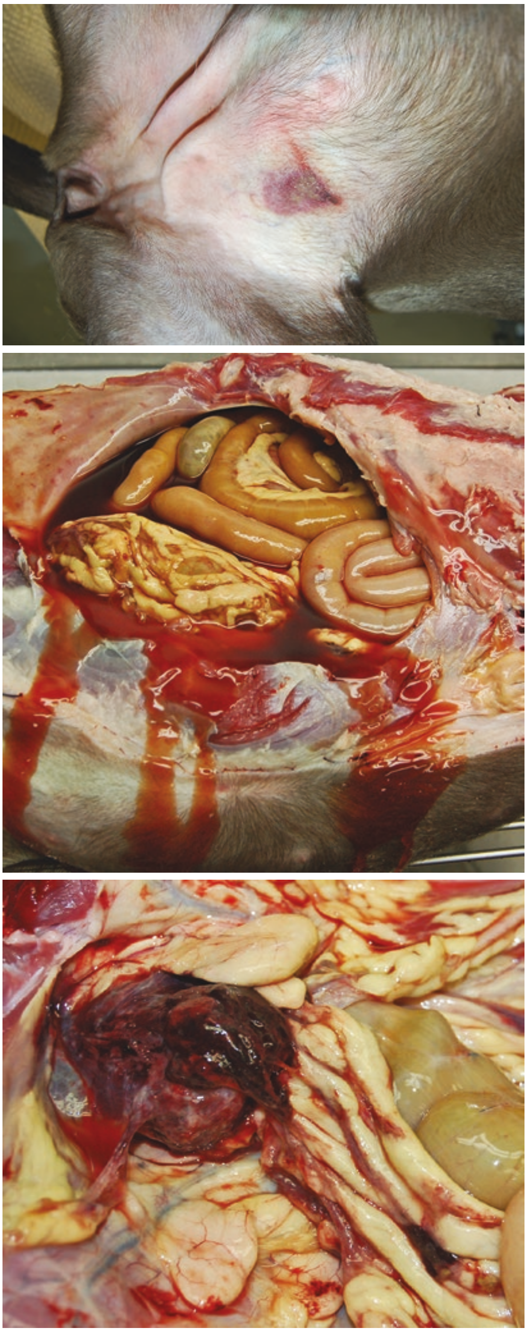

Fig. 5.21–5.23 Single blunt force impact to the caudal ventral abdomen caused the contusion, bladder rupture, and fatal uroperitoneum

impact, the greater the degree of soft tissue injury that results. A dog jumping from a low height may sustain a low-energy fracture such as a distal diaphyseal transverse radial fracture with minimal soft tissue trauma. A high-energy or very high-energy force will result in bone comminution and soft tissue trauma.

Different traumatic events result in different head/skull injuries. The dog (Sect. 5.18) thrown to the ground had an occipital bone injury. The occipital bone is a strong bone, and a powerful force is required to cause an occipital bone fracture. Occipital bone injuries are common in NAI/blunt force trauma cases as is the circumstance of a small animal being thrown to the ground or against a hard object. At the ASPCA in New York City between 2014 and 2016, there were 10 deceased dogs and cat less than 15 lb. with skull fractures. Five of these animals were witnessed being thrown to the ground or against a wall. One of the 10 animals fell from a height, and the traumatic event was not witnessed in four of the animals. High-rise falls commonly cause head trauma but typically no skull fractures other than fractures of the mandible and hard palate [27, 28]. The zygomatic bone is a weaker skull bone and should be radiographed and evaluated at necropsy in all blunt force trauma cases. The maxilla is not as strong as many other skull bones but is also not frequently fractured which is probably a result of the nature of the traumatic event.

5.25 Fracture Healing [29]

The rate of fracture healing depends on the relative position of the fracture fragments, blood supply, the presence of infection, and fracture fragment motion. A bone fracture that does not heal is called a nonunion.

There are two classes of fracture healing: primary (direct) and secondary (indirect). Primary fracture healing requires medical intervention to reduce the gap between fracture fragments to less than 0.5 mm and to immobilize the fracture site. If the fracture fragment gap is less than 0.01 mm, there is contact healing, and a new lamellar bone is formed across the fracture line parallel to the long axis of the bone. If the fracture fragment gap is greater than 0.01 mm but less than 0.5 mm than gap, healing occurs where the lamellar bone is deposited perpendicular to the long axis of the bone.

Secondary fracture healing occurs when there is inadequate fracture fragment reduction and fracture fragments are not immobilized, and/or the fractures are comminuted and the bony column cannot be reconstructed. Secondary fracture healing can occur with medical intervention that does not resolve the fracture fragment gap or adequately immobilize the fracture fragments. It also happens when there is no medical intervention. The hallmark of secondary fracture healing is callous formation. The more motion, the larger the callous.

5.26 Blood Loss (Hemorrhage)

Large volume blood loss should be quantitated if possible (see Table 5.5). The total blood volume of the dog is 8–9% of body weight (kg). The total blood volume of the cat is 5–6% of body weight (kg).

Table 5.5 Loss of blood in human beings: Physiologic effects of blood loss as a percentage of circulating blood volume

Class 1	Loss of up to 15% (approximately 10–12 mL/kg)[a] of the circulating blood volume. Clinical symptom: mild tachycardia. Blood pressure, pulse pressure, and respiratory rate are normal
Class 2	Loss of 15–30% (approximately 12–25 mL/kg)[a] of the circulating blood volume. Clinical signs: Tachycardia, tachypnea, and a decrease in pulse pressure
Class 3	Loss of 30–40% (approximately 25–32 mL/kg)[a] of the circulating blood volume. Clinical signs: Pale mucous membranes, prolonged capillary refill time, tachycardia, tachypnea, depressed attitude, and decrease in arterial blood pressure
Class 4	Loss of greater than 40% of the circulating blood volume. Life-threatening event. Clinical signs: Very pale or white mucous membranes, prolonged capillary refill time, cold extremities, tachycardia, tachypnea, rapid thready pulse, marked decrease in arterial blood pressure, delirium, and depressed attitude

From *Advanced Trauma Life* Support Student Manual, Chicago, American College of Surgeons, 1995 [30]

[a] Assumes a total blood volume of 80 mL/kg (i.e., 8% of BW)

5.27 Differentiating Accidental from Non-accidental Injury: Patterns and Types of Injuries (See Table 5.6)

Distinct patterns of injuries are known for accidental trauma that can break bones: motor vehicle accidents (MVA) and high-rise falls. Retrospective studies of motor vehicle accident cases at the University of Pennsylvania Veterinary School and Tufts Veterinary School looked in detail at types of injuries sustained [15]. Other studies have detailed high-rise fall injuries. Two recent studies have looked at the pattern of injury in NAI [10, 11].

In a study of traumatic skeletal injuries of chimpanzees, gorillas, and orangutans [33], the pattern of injuries was analyzed in the context of the lifestyle of these different primate species. Orangutans are arboreal with a solitary lifestyle and have a statistically significant low frequency of head wounds compared to chimpanzees. Chimpanzees and gorillas who have social lifestyles sustain more head wounds because of inter- and intragroup conflicts. Falls from heights explain the predominance of extremity injuries in orangutans. The head is a target in chimpanzee and gorilla intraspecies aggression as it is in human aggression, human on animal aggression, and dog on dog aggression (dog fighting).

5.28 Case Study: A 10-Year-Old Pomeranian: Cause of Death Unknown (Figs. 5.24 and 5.25, 5.26 and 5.27, and 5.28 and 5.29)

It was reported that a 10-year-old Pomeranian was found dead in the home. No traumatic event was witnessed. The external signs of trauma, as is frequently the case, do not indicate the severity of internal injury. What appear to be contusions caused by the dog being grasped around the neck are visible subcutaneous.

Table 5.6 Accidental injury and non-accidental injury

MVA								
Anatomic area	Kolata [15] MVA	Boysen [31] MVA	Streeter [32] MVA	Whitney [27] high-rise cat	Aiken [28] high-rise cat	Munro [10] NAI	Intarapanich NAI cats/dogs	Intarapanich AI cats/dogs
Skull	5%	10%[a]	5%[a]			16.4%	32%	3.1%
Scleral hemorrhage							28%	8%
Maxillofacial trauma[b]				56%	56%			
Vertebrae	6.5%		5%				10%	3.5%
Pelvis	20%	20%	20%	8%			8%	22.5%
Ribs	3%	0%			4%	15.6%	28%	8.9%
Femur	10%			28%[c]		20%		
Extremity fractures				39%				
Pulmonary injury			30%	90%	90%			43.7
Pulmonary contusions							24%	43.7%
Pneumothorax							6%	28.6%
Skin abrasions							10%	72.8%
Older fractures							22%	0.2%

[a] Head trauma
[b] No skull fractures identified
[c] 14 of 15 cats were <1 year, 9 of the 14 fractures were distal Salter II fractures, and 2 were capital physeal fractures

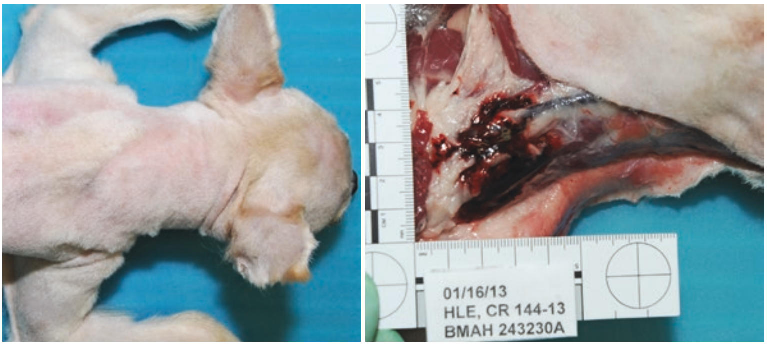

Fig. 5.24 and 5.25 Externally there is no obvious trauma to the skin. There was extensive hemorrhage of the cervical muscles as you might find if the dog was picked up by his neck

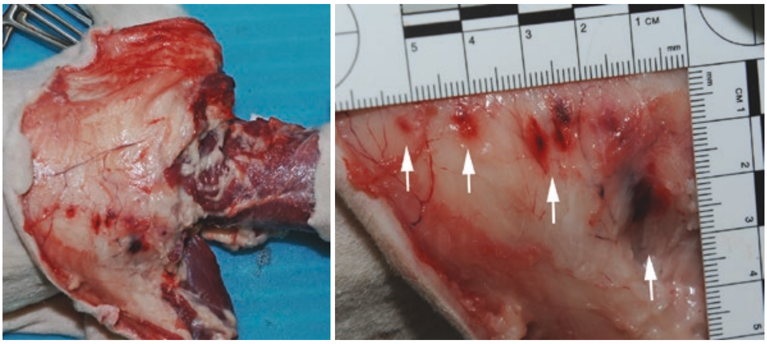

Fig. 5.26 and 5.27 The skin of the neck is reflected, and there are four discrete subcutaneous hemorrhages (the third from the left appears as two because it was incised with a scalpel) consistent with contusions from fingers grabbing the dog by its neck

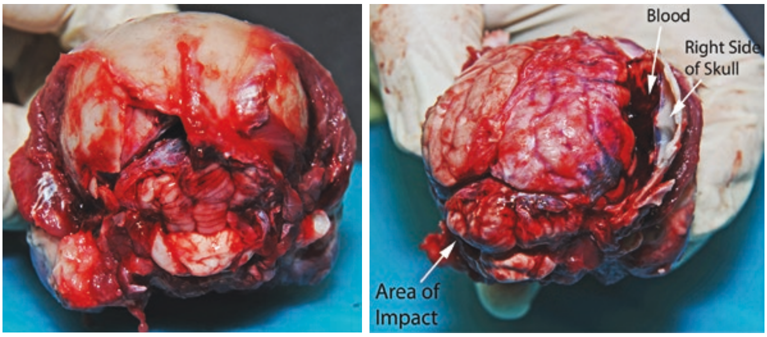

Fig. 5.28 and 5.29 The occipital bone is shattered with fracture lines extending into the parietal bone. There is a countercoup lesion on the right rostral aspect of the brain. The impact at the left caudolateral part of the skull causes a contrecoup impact of the brain rostrally

5.29 Rib Fractures

Rib fractures are a common injury in NAI/blunt force trauma cases (Table 5.3). The explanation for the disparity in the occurrence of rib fractures in NAI vs. AI (MVA and high-rise falls) is that in NAI, the blunt force trauma impact is more focused (animal is hit with an object, kicked, or thrown against an object) than the broad impact that occurs with an MVA and high-rise falls [11].

The more information that is known about a specific injury for a specific event, the more defined the difference between NAI and AI becomes. In one study [11] comparing NAI to AI, of the 38 MVA cases (8.9% of total 426 MVA cases) with rib fractures, only three dogs had rib fractures on both sides of the body (3/426; less than 1% of total MVA cases). In contrast, of the 14 NAI cases with rib fractures (14/50 = 28%), 5 (5/14 = 36%) of the cases had rib fractures bilaterally (statistically significant).

5.29.1 The Case of the "Battered" 16-Year-Old Chihuahua (Figs. 5.30, 5.31 and 5.32)

A deceased 16-year-old Chihuahua was reported to have been beaten to death by a woman's spouse. At necropsy, 18 rib fractures were identified. Because of the location and appearance of the different rib fractures, it was concluded that the fractures were caused by multiple impacts at different traumatic events in time**.** The right rib callouses are larger than most of the rib callouses on the left side of the dog's chest indicating that they occurred at an earlier traumatic event than the event that caused the left-side fractures.

5.30 Repetitive Injuries, "Battered Animals": Injuries at Different States of Healing

An animal that has been physically injured at different times is considered to have "repetitive injuries" [8, 17]. There are three reasons for a veterinarian to conclude that an animal has been injured at different times:

1. The person bringing the animal for veterinary evaluation may say that the animal has sustained physical injuries at different times.
2. The animal presents at an animal health facility or multiple animal facilities more than once with physical injuries. This requires a review of the animal's complete medical record.
3. Injuries of different ages are identified during the forensic examination.

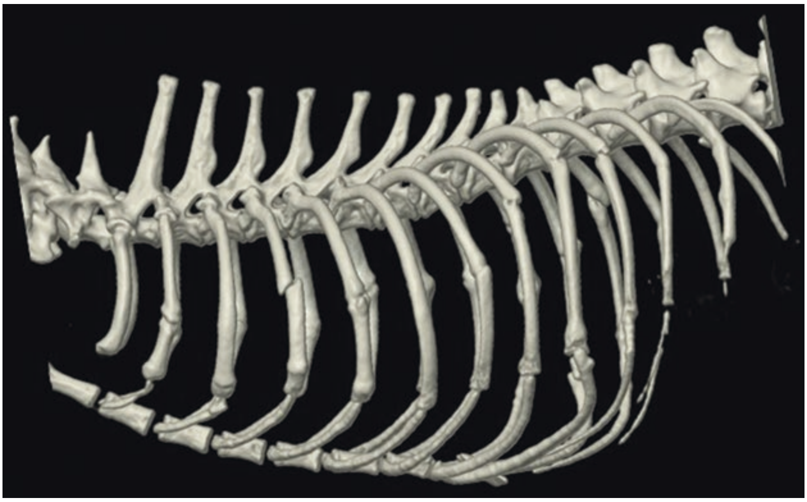

Fig. 5.30 Computed tomography scan left ribs: **A** 16-year-old Chihuahua with multiple rib fractures indicating multiple impacts at different traumatic events in time**.** Fractures are present in left ribs: 2, 4, 5, 7, 8, 9, 10, 11, and 12

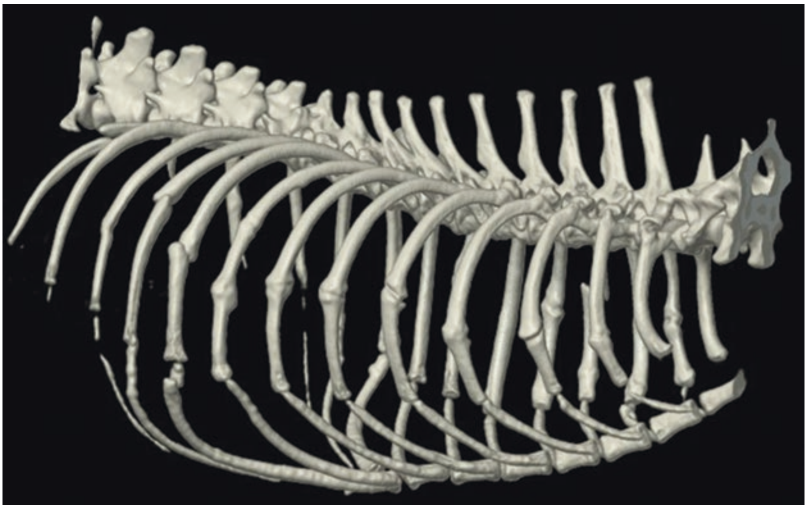

Fig. 5.31 Computed tomography scan right ribs: Fractures are present in right ribs: 2–10 and possibly 11 (right rib 11 has an abnormal contour that is consistent with an injury, but possibly not a complete fracture)

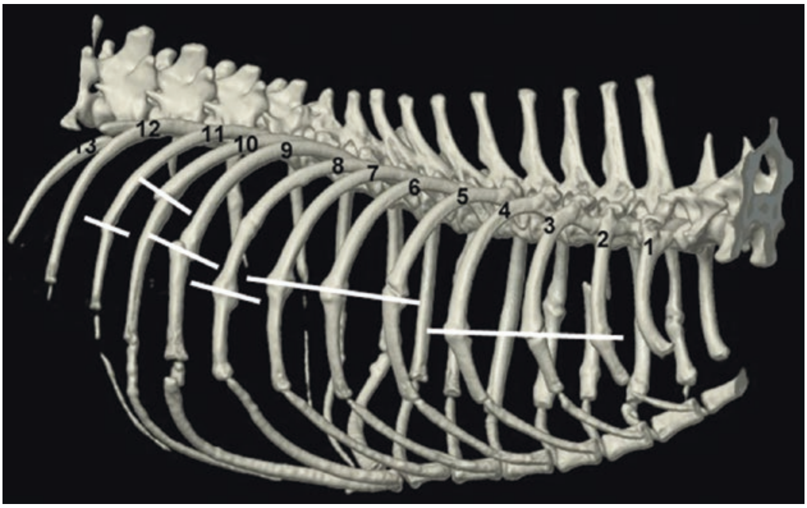

Fig. 5.32 Lines have drawn through adjacent ribs where the fracture position lines up giving a sense of the minimum number of impacts necessary to cause the injuries

5.31 Number of Impacts

In blunt force trauma, the pattern of injuries should be assessed. Depending on the location of the individual blunt force impact injuries, the number of impacts that could reasonably cause those injuries should be estimated. This should be communicated as the minimum number of blunt force impacts that occurred (as not every blunt force impact will cause an identifiable injury). Generally, accidental traumatic events cause injuries distributed in one plane of the body, as there typically is only one impact; consider, for example, a fall. The exception is motor vehicle accidents where there is a primary impact when the car hits the animal and a secondary impact when the animal's body hits the ground (when the deceleration injuries occur). The pattern of injuries should be consistent with what would be expected from the reported event, or a different explanation is necessary.

5.32 Age of Injuries

In a forensic case, questions about time are of importance, time of occurrence of an injury or injuries, and duration of a medical problem. As an example, when an animal presents with a fractured femoral neck, the age of the fracture is important. The older the wound, the more significant the neglect and the stronger the legal case. There may be injuries of different ages in a forensic case providing evidence that the animal has been hurt during more than one traumatic event (repetitive injuries) [8]. A timeline should be created showing time of occurrence of the injuries. This timeline can be consulted by law enforcement to compare to the results of their investigation determining the person or persons who had access to the animal at different times. Gross exam in combination with histopathology and radiography is routinely used to age injured or diseased tissue. Review of previous medical records will provide information about previously evaluated injuries and the approximate dates when they occurred.

5.33 The Case of the Battered 5-Month-Old Puppy

On October 25, 2010, a puppy showed up at a city shelter, this time with a distal right metaphyseal bone fracture (Fig. 5.33). No explanation was given. The puppy was taken for care to a local animal hospital that works with the city shelter. In preparation for surgery, radiographs of the injured leg and chest were taken. What was seen was unexpected. The radiographs showed bilateral femoral head osteotomies and two healed rib fractures (Figs. 5.34 and 5.35). A law enforcement was contacted because of the extensive trauma this puppy had experienced. Investigation uncovered that before the October 25, 2010 visit to the city shelter, the puppy had made two visits to a local animal hospital for traumatic injuries.

On **September 18, 2010,** a 5-month-old terrier-type puppy was brought to a NYC animal hospital with a left hind lameness. The owner reported that pots and pans fell on the dog the previous evening. Radiographs were declined and the puppy went home with an NSAID prescription. On **October 8, 2010,** the puppy presented at the same animal hospital. This time the puppy was unable to use either hind leg. Radiographs (Fig. 5.35) showed bilateral femoral neck fractures of two different ages. The left femoral neck fracture showed evidence of healing. The bone fracture ends were indistinct and there was bone sclerosis. The right femoral neck fracture had bone ends that were well defined with no bony sclerosis. Additionally, the left thigh muscles as compared to the right thigh muscles were atrophied. The left hind femoral neck fracture occurred on September 18, 2010,

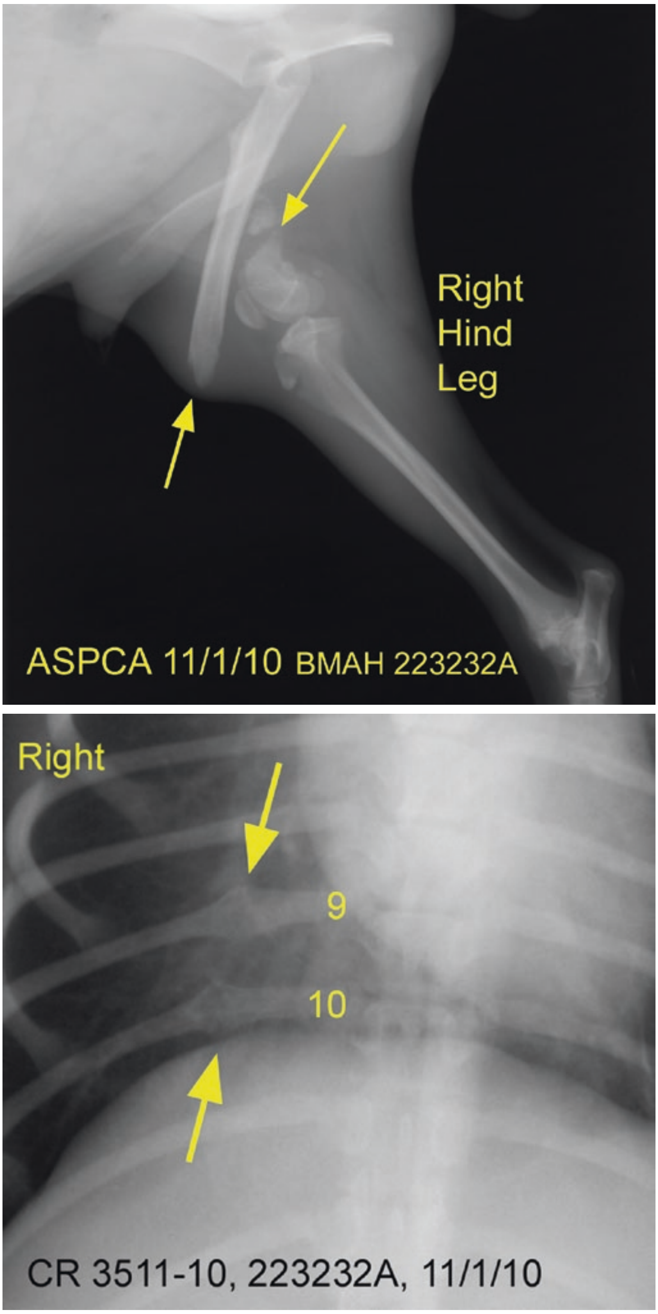

Fig. 5.33 and 5.34 The right distal femoral fracture occurred on October 25, 2016 when the dog was a relinquished to the city shelter. Chest radiographs showed healing rib fractures that could have come from a single impact at the time when either of the back legs were fractured

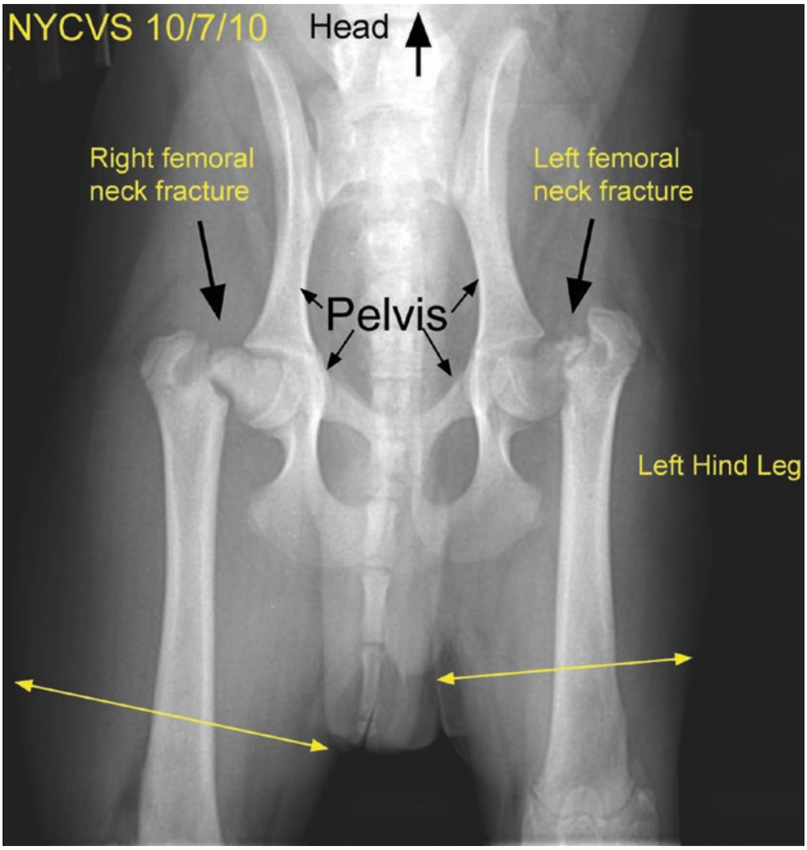

Fig. 5.35 Radiograph on October 7, 2010. Bilateral femoral neck fractures at different stages of healing (left older than right—see text) and left hind muscle atrophy

and the right femoral neck fracture occurred on October 8, 2010. A call was made to law enforcement, but the case hit a dead end. The puppy would surface again on October 25, 2016, at the city shelter.

Timeline: This puppy had bone fractures during at least three separate traumatic events: September 18, 2010, October 07, 2010, and October 25, 2010. A law enforcement investigation occurred, and it was determined that the perpetrator was an Iraq war veteran suffering from PTSD. There was no arrest and the individual responsible got the therapy she needed.

5.34 "Failure to Treat"

Not providing an injured animal with medical care is sometimes called a "failure to treat" which can be the basis for a criminal prosecution. If the animal's injuries are accidental, can it be shown that there was a failure to provide veterinary medical care for the injured animal? As mentioned previously, determining the duration of an injury can be forensically important. In the case of a badly injured animal, there is value in the determination of the age of the injury/injuries. Some state laws mandate veterinary care for medically compromised animals. In some states although the words "veterinary care," or a similar phrase, is not present in the animal cruelty statute, however, interpretation and application of the law at trial (case law) over many years has led to the conclusion that an injured or diseased animal should be provided with veterinary care, and the failure to do so may be considered a crime. State anti-cruelty laws and other laws pertaining to animals can be found at the Michigan State University Law School, Animal Legal and Historical Center [34], and the website of the Animal Legal Defense Fund [35].

5.35 Pain

The forensic evaluation should determine if the animal is in pain. Pain assessment as part of the forensic examination of a live animal is mandatory. In a noncriminal case, it would not be unusual for medical notes to have record of analgesic use with very little information in the exam notes describing the behavior an animal exhibited that has been interpreted as pain. In a deceased animal, a statement about the pain an animal experienced during life, as a result of its injuries or disease processes, can be provided in a statement of findings or in a comments section of the necropsy findings. This is important. It will be asked at trial and will be of significant value during a plea negotiation. Pain has been described as mild, moderate, and severe [36]. This classification is most appropriate in a live animal in which there behavior interpreted as pain can be observed, and a pain assessment tool such as the Glasgow composite pain scale can be used [37]. In a deceased animal, it is certainly possible to comment that a particular injury caused moderate to severe pain and/or chronic pain (see Table 5.1).

5.36 When Did a Traumatic Injury Occur Relative to the Animal's Death? Terminology

Traumatic events can be described as occurring antemortem, perimortem, and postmortem. Antemortem trauma occurs before death. It is recognized by active healing of injured tissue, associated hemorrhage, and the presence of hemosiderin. Perimortem indicates an event occurred around the time of death. There is no tissue healing or hemosiderin present. The implication is that perimortem traumatic injuries are directly associated with the manner of death or the handling of the remains. Postmortem fractures occur after death and are usually considered to be associated with taphonomic processes [38].

5.37 The Case of Delilah, a Dog Who Was Reported to Be Injured by a Cordless Drill that Fell from the Top of a Five Foot Refrigerator (Fig. 5.36 and 5.37)

An injured pit bull dog was brought to the animal hospital. The dog named Delilah presented with a fractured left front humerus (Fig. 5.37). The owner reported that a 6-lb.-cordless drill fell from the top of a refrigerator, struck the dog, and fractured the left humerus.

Delilah is a 4-year-old, 56-lb.-brindle-coated, female pit bull dog. She has a severe fracture of the left humerus. The fracture is located approximately one-third of the way from the shoulder joint to the elbow joint. This is a transverse, comminuted, compound fracture of the left humerus. Radiographs show that there are fracture lines that extend from the main part of the fracture toward the shoulder joint and toward the elbow joint. These fracture lines are parallel to the long axis of the bone.

There is extensive soft tissue swelling that extends almost the entire length of the upper leg from the shoulder joint to the elbow joint (Fig. 5.36). There is a 1-cm-diameter wound in the middle of the swollen soft tissue. There is a skin wound located on the craniolateral surface of the upper left leg. Delilah is unable to bear weight on the leg. She is in excruciating pain and resents any manipulation of the leg.

This fracture represents a catastrophic failure of the left humerus. This is a blunt force trauma injury that resulted from a very high-energy impact. The comminution (bone in pieces) of the fracture site with fracture lines extending toward the should joint and elbow joint is evidence of an impact equivalent to that expected from a motor vehicle accident or a high-rise fall from a window or roof. The extensive soft tissue trauma is also evidence of a very high-energy impact. This injury is not consistent with an impact from a 6–7 lb. object that fell 5 ft. (see following calculations).

Considering how a dog lies on the floor, an object falling from above would not strike the upper leg in a way to cause such significant damage. When a dog lies down, the upper leg is in an

Fig. 5.36 and 5.37 Delilah’s left front leg with a severe skin wound and swelling and a radiograph of the comminuted fracture

almost vertical position with the elbow and the lower leg resting on the ground. A dog does not lie down with the entire leg extended.

This is a large very heavily muscled dog. The muscle of the upper leg provides a cushion and protection of the humerus bone. A blunt force impact of the upper leg results in the transmission of energy that is absorbed and dissipated by the muscle as well as the bone. An application of blunt force to the upper leg that causes such a catastrophic failure of the bone must overwhelm the protection provided to the bone by the upper leg muscles. A 6 lb., 10 oz. object falling approximately 5 ft. would not result in an impact with this amount of energy.

Finally, this is a large dog and the humerus is a large strong bone. Causing this degree of damage to this bone would require a very high-energy impact.

Calculating the force of a falling object (protocol for calculation provided by Dr. Vincent Tranchida, Forensic Pathologist, Office of Chief Medical Examiner New York, NY). For calculating the force of a falling object, you need:

1. The mass of the object
2. The height of the drop
3. The depth of the impact

Typically, three steps are involved:

1. **Calculate the velocity of a falling object:**
   - Multiply the height of where the object is dropped by the pull of gravity (the pull of gravity on earth is 9.8 m/s).
   - Multiply this ×2.
   - Then take the square root of this number.
2. **Find the kinetic energy before impact:**
   - Square the velocity.
   - Multiply the squared velocity times the mass of the object.
   - ×1/2.
3. **Find the force of the impact:**
   - Divide the kinetic energy before impact by the distance the object travels during the impact.

1 m = 39.3700787 in.
1 m = 3.2800399 ft.

Convert lb. to kg and inches and feet to meters.

5 ft. = x m	$x/5 = 1/3.2800399$	$x = 5/3.2800399$	$x = 1.52$ m
1 in. = x m	$x/1 = 1/39.3700787$	$x = 0.0254$ m	

5.37.1 Case Example (CR 4211-11)

We have a 6 lb., 10 oz. (or 3 kg) object dropped from a height of 5 ft. (or 1.52 m) that travels through approximately 1 in. of bone (0.025 m)—Estimate of the width of Delilah's humerus, but you can adjust the number as needed:

1. **Velocity squared** = 1.52 × 9.8 m/s × 2 = 29.8 [the square root of 29.8 = velocity (5.46 m/s)]
2. **Kinetic energy before impact** = velocity squared × mass of the object × 1/2 = (29.8 × 3 × 0.5) = 44.7
3. **Force of the impact** = kinetic energy/distance = 44.7/0.025 = 1788

The force of the falling object is about 1788 Newtons. The force to fracture an adult human humerus is approximately 3500 Newtons. The reported scenario of the traumatic event was not adequate to fracture the Delilah's humerus. The owner when presented with this information changed his story.

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Sharp Force Trauma

6

Martha Smith-Blackmore and Nicholas Robinson

6.1 Description, Interpretation, and Documentation of Sharp Force Injuries

The assessment, documentation, and interpretation of sharp force injuries are essential tasks for the forensic veterinarian or veterinary forensic pathologist. For the purposes of this discussion, the term “veterinary examiner” will be used to refer to forensic veterinarian and veterinary forensic pathologist when appropriate. The purpose is to assist in establishing how a wound or injury has been caused. The basic elements of documenting sharp force injuries are to record the number, location, pattern, direction, length, and depth of the wounds and whether or not the injury caused was due to accidental or deliberate causes.

Sharp force injuries are penetrating injuries from the mechanical application of a sharp object to the body of an animal. Sharp injuries may be made by any implement with a sharp or cutting edge (e.g., knife, scissors, broken glass). In rare cases, the sharp injury is sustained when the animal is impaled on an implement or object.

The force required to inflict sharp injuries and the effect of such injuries are quite variable. A pointed object with a sharp edge may penetrate vital structures with minimal force, while a dull edge will require much greater forces and may, in addition, induce minor tissue tearing or even accompanying blunt trauma. The severity and lethality of any penetrating injury are related to the vicinity of the wound tract to vital organs and large vessels.

When documenting injuries inflicted by sharp forces for forensic purposes, it should be done in sufficient detail to permit precise interpretation or reconstruction but using enough lay terminology that the judicial system can decide the most appropriate interpretation of the injuries described and their relevance to a criminal case [1]. The veterinary examiner should not hesitate to express an evidence-based opinion of causality of injury or death and suggest the type of weapon that may have been used to inflict a wound based on training and experience. In criminal cases, the final rendering of opinion is performed by a judge or jury.

On the skin surface, the edges of a stab, incised, or chop wound are referred to as the wound’s margins, and the ends of the wound are called the angles. The length of the wound should be ascertained using a scale (ruler) or a pair of calipers from angle to angle and recorded in

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centimeters or millimeters. The wounds should be carefully measured and documented with photography and diagrams before manipulating or probing the wounds. The width of the wound should be measured at its widest point. The amount that the wound gapes depends on the elasticity of the skin and the orientation of the wound in relation to the Langer's lines (also known as tension or cleavage lines, the lines of skin tension corresponding to the natural orientation of collagen fibers in the dermis, generally parallel to the orientation of the underlying muscle fibers) [2].

The depth of the wound should be gently probed and measured at the deepest point. Care should be used when inserting probes so that no additional tissue disruption is caused. Insertion of probes should only occur after careful examination, documentation, and photography of the wound. Skin is highly elastic and receives varying support from underlying structures. The depth of penetration does not necessarily reflect the length of the weapon. The depth of the wound tract is determined by the elastic recoil of the tissues and the portion of the weapon inserted; however, any amount of the weapon may be inserted, not necessarily its entire length. Stab wounds may gape and appear shorter than the blade that was used to create the wound due to the contraction of the skin.

The shape of the wound should also be noted using simple terms, such as linear, triangular, V-shaped, or crescent-shaped. The position of the injury should be described in relationship to fixed anatomical landmarks and by using anatomical diagrams and body charts.

When documenting multiple sharp force injuries, it is important to describe every wound, using an organizational scheme to keep track of the injuries. The wounds can be numbered sequentially, beginning with the most cranial wound and working caudally and then down the limbs. The wounds can be labeled in permanent marker (e.g., Sharpie®, Sanford L.P., Oak Brook, IL) directly on the skin after it has been shaved. When there are numerous, similar sharp force injuries within a relatively small area, it is appropriate to describe the wounds together as a cluster (Fig. 6.1).

Fig. 6.1 Multiple stab wounds, numbered

The skin, soft tissue, organs, cartilage, and bone are materials with different properties, each capable of capturing different characteristics of sharp instruments. These properties must be considered when making estimates of knife orientation, type of edge (sharp, dull, straight, or serrated), and shape of the blade [3]. The edge of a cutting instrument may produce smooth, roughened, or irregular margins, depending on the blade and motion. Sharp blades and a slicing motion will produce the smoothest margins. The wounds will become progressively rougher with blade dullness and width. Blunt trauma including bone fractures may be adjacent to sharp force trauma when there are significant impacts to the knife guard or in cases of the cutting instrument being thick and heavy.

A tool mark is any impression, cut, gouge, or abrasion caused by a tool coming into contact with another object [4]. Tool marks may be left on tissues. Tool marks are divided into class and type. Class includes the general design such as a knife, single edged or double edged and serrated or straight, and the length, width, thickness, spine features, and hilt shape. Because these features vary along the length of a blade, the mark or shape of the wound inflicted depends on the angle and depth of penetration. Tool mark type characteristics are features limited to a single implement such as a blade defect acquired after manufacture.

Because of malleability and compacting properties, the cartilage can retain tool marks [3]. When bone is impacted with sharp trauma, it may conserve tool marks, if the impact is across the grain of the bone. When the impact runs with the grain of the bone, splitting occurs, deforming the mark. Precise understanding of bone conformity to impact and its interpretation is best made by a forensic physical anthropologist. Human forensic pathologists are also an invaluable resource as they are exposed on a daily basis to lethal injuries; many features of non-accidental injury have diagnostic features that are independent of the victim's species [5].

Photographs should be taken of each injury site, from an overall perspective, medium distance perspective, and close-up, with and without a scale in the image. The digital image evidence should be supported by written notes and hand-drawn sketches. In the absence of robust chain of custody protocols, sequential digital numbering (usually imparted by the camera) adds weight that all photographs are from the same specimen. The injuries should first be examined and documented before and again after shaving the fur coat from the region. The wound should be photographed gaping and again with the margins apposed. The angles will be easier to be appreciated as sharp or blunt after the margins are apposed.

6.2 Incised Wounds

Incised wounds are cuts ("slash" or "gash" wounds) inflicted by sharp instruments in which the cutting edge runs perpendicular or tangential to the skin surface cutting through the skin and deeper anatomical structures (Fig. 6.2). Incised wounds create a defect that is longer than its deep. Incisions are usually caused by sharp cutting implements, usually bladed weapons, such as knives and razors. The margins of an incisional injury tend to be straight, unbruised, unabraded, and not inverted and lacking in tissue bridges. Tissue bridges are strands of soft tissue (nerves, vessels, and other soft tissues) that extend across the wound gap seen in lacerations caused by blunt force trauma; these structures are typically severed in sharp force trauma incisions.

If the skin is loose where the blade is drawn across it, the incision may be notched. The deeper end of the incised wound is usually the starting end of the wound as the weapon will be withdrawn toward the end of the wound, but that isn't always the case.

It is important to remember that altercations are not static activities, and both the assailant and the victim may be moving during the application of an instrument that causes injury. Any relative angles and depths of contact between the victim and the weapon can therefore occur, and over-interpretation and simplification should be avoided (Fig. 6.3) [6]. If the cutting instrument

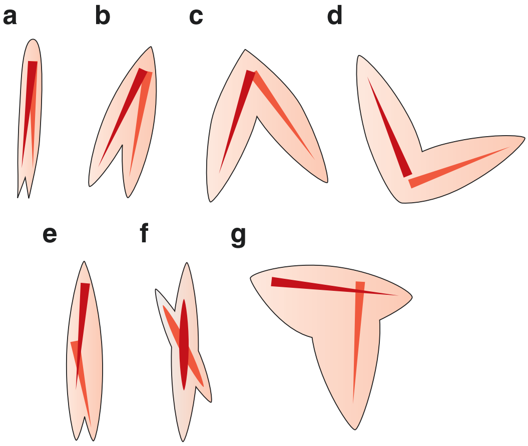

Fig. 6.2 Mechanism of formation of dovetailed incised wounds: (**a–d**), the knife is rotated or the victim animal moved against the knife; (**e–g**), simultaneous course incising motions; (**f**), double-dovetail injury inflicted by a double-edged knife. Source: Dettmeyer, Reinhard, Marcel A. Verhoff, and Harald SchuÌtz. "9: Pointed, Sharpand, Semi-sharp Force Trauma." Forensic Medicine: Fundamentals and Perspectives. Heidelberg: Springer, 2014

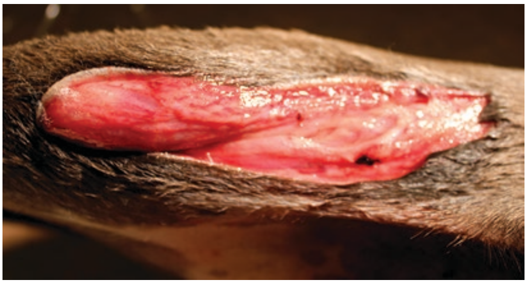

Fig. 6.3 Incised wound to the front of the right thigh on a puppy. Note the "M" shape to the top of the wound

Fig. 6.4 Stab wound with motion to the medial left elbow. The deepest aspect of this wound is obscured by the dorsal skin flap

changes direction or if the animal moves while being cut, the shape of the wound may reflect those movements (Fig. 6.4).

6.3 Stab Wounds

Stab wounds are cuts inflicted by sharp instruments in which the defect created is deeper than its long. Stab wounds are caused by sharp or pointed implements like knives, forks, ice picks, screwdrivers, or scissors. Where there are multiple stab wounds with a variety of forces, the impression of the tool may be apparent on soft tissue.

Stab wounds tend to be more dangerous than slash wounds due to the penetration of body cavities and deeper, larger blood vessels (Fig. 6.5). The appearance of the external injury may not correlate with the seriousness or lethality of internal damage to vital structures, such as the heart, liver, or major blood vessels, which can lead to death, usually from hemorrhage, air embolism, cardiac tamponade, or tension pneumothorax. If the original injury is survived, delayed complications from stab wounds such as peritonitis, pyothorax, or even sepsis may ensue. The sharper the implement used, the less tissue disruption will occur, even to the extent to which the external region of hemorrhage is the only indicator that a stab wound has been inflicted. Lethal wounds will primarily have acute tissue damage of the major organs or structures that were affected, while nonlethal wounds may have varying degrees of healing. If a body cavity is penetrated and the wound is survived, then fibrous adhesions may form between the area of injury and the viscera of the subjacent cavity.

Stab wounds to the thorax can cause V-notched wounds to the ribs, and small particles of bone can be carried along the wound tract. When the head is stabbed, punctures to the skullcap are possible, and brain-heart syndrome may ensue with neurogenic shock and subsequent myocardial necrosis (Figs. 6.6 and 6.7).

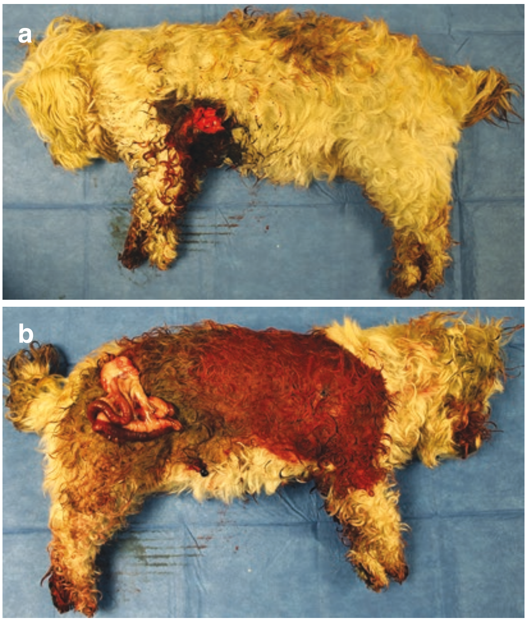

Fig. 6.5 Multiple stab wounds, numbered. In this case, the injuries were alleged to have been caused by the puppy being caught under a fence. The distribution of injuries is not consistent with that presentation. (**a**) Small breed dog with lung tissue herniated out of a stab wound to the left lateral chest. (**b**) Herniated small intestines out of a stab wound to the right flank

Fig. 6.6 Stab wound to the back of the skull in a small breed dog

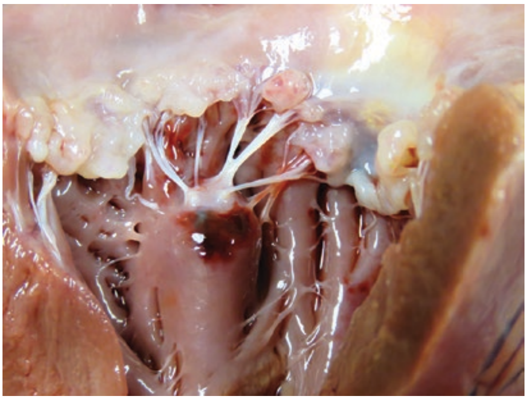

Fig. 6.7 (Heart from a dog in Fig. 6.6) Subendocardial hemorrhage and acute myocardial necrosis secondary to acute brain trauma

6.4 Chop Wounds

Edged weapons may produce sharp trauma, blunt trauma, or a combination of both. Chop (or *hack*) wounds are created with a combination of sharp and blunt force trauma, and the resulting trauma is a combination of cutting and crushing. Chop wounds may have contused or abraded margins, and the underlying bones may be fractured. Machetes, axes, lawn mower blades, and boat propellers are examples of implements that will inflict chop wounds.

Axes, and other bulky instruments, although capable of cutting, may cause lacerations because the injury caused by the size of the instrument (e.g., axe head) overrides the cutting effect of the tool. Mixed wounds are common, with some incised element, and some laceration, bruising, and swelling, and abrasion also present. Each element of the injury must be documented. Microscopically, the soft tissue injury will be largely reflected by the age of the wound with more hemorrhage and tissue disruption in the acute wounds and fibrosis in the chronic wounds. If the weapon comes in contact with bone, a strike pattern may result; however, strike patterns may differ with varying weapon characteristics. A machete creates distinct thin striations and tends not to fracture the bone, while a heavier implement such as a meat cleaver will have more pronounced striation [7, 8].

6.5 Chainsaw Injuries

Chainsaw injuries, while rare, may be encountered as accidental, intentional, or postmortem injuries to animals. Chainsaws are hazardous to operate, and injuries to human operators are caused by kickback, pushback, and pull-in. Kickback is the most common and poses the greatest hazard. Kickback occurs when the rotating chain is stopped suddenly by contact with a more solid area throwing the saw rapidly backward toward the operator [9]. It is conceivable that an operator using a chainsaw near an animal may cause accidental trauma to that animal. Accidental chainsaw injuries have been described in dogs biting a running chainsaw causing cuts to bilateral oral commissures and other oral trauma [10, 11]. Chainsaws have been used as a dismemberment tool, and postmortem dismemberment using chainsaws has been studied in pigs [12, 13].

Chainsaw injuries are complex and involve both soft tissue and skeleton [14]. Chainsaw teeth produce a characteristic defect given the serrated nature of the blade, causing avulsion with tissue loss coincident with chain width of 8–10 mm at a

Fig. 6.8 Cutting chain of chainsaw showing individual flat broad vertical and horizontal cutting teeth (Photo courtesy of Dr. Jason Brooks, Penn State University)

minimum (Fig. 6.8) [15]. The size of the saw and type of skin/fur/hide will contribute to the variation of lesion seen.

The characteristic striations created on the surface of wood as it is cut by a chainsaw can also be found on bony surfaces cut by a chainsaw [12]. The striae pattern of the chainsaw has been described as having a lack of fineness and an irregularity to the surface of cut bone. Often only portions of the tool pattern may be visible on the initial or terminal aspects of the saw cut. When an incomplete cut is made into bone with a chainsaw, the kerf is described as wide, the floor is flat to convex with a line in the middle, and the kerf walls are ridged [16]. These characteristics are retained even in cremated remains [13].

6.6 Goring Injuries

Horned animals may inflict goring injuries on other animals. These injuries can be of various shapes, sizes, and directions, and they can mimic sharp or chop injuries with penetration of the skin, underlying structures, penetration of body cavities, and accompanying contusions and rarely fractures [17]. Ventral herniation of subjacent viscera may occur and is most frequent in the flank where the muscle is thin. Goring can induce significant tissue disruption in a number of organs all of which will produce hemorrhage and attempted hemostasis with fibrin accumulation. The severity of the goring and whether vital organs were involved will determine whether any of the wounds progress to chronic wounds and show evidence of blood resorption and healing (scarring/fibrosis).

6.7 Claw Marks and Bite Wounds

Bite wounds are covered elsewhere in this text; however, it is important to note that bite wounds and deep claw marks can result in trauma that mimics sharp force injury slash wounds (Fig. 6.9). Animal fights are very dynamic, and tearing of flesh will occur as the victim of the bite pulls against the biter and vice versa. Bite wounds differ from sharp force trauma as they tend to be accompanied by significant crushing and avulsion injuries. Postmortem scavenging may produce artifact which must be noted to be different from antemortem wounds.

6.8 Trap Injuries

There are many different types of traps available for a variety of species. Iossa et al. [18] defined 34 different pathological outcomes from animal encounters with different types of traps. Some examples of commonly used traps are box trap, body-gripping trap, leghold snare, neck snare, and various types of leghold traps (Fig. 6.10). If a

Fig. 6.9 Bite wounds mimicking sharp force trauma

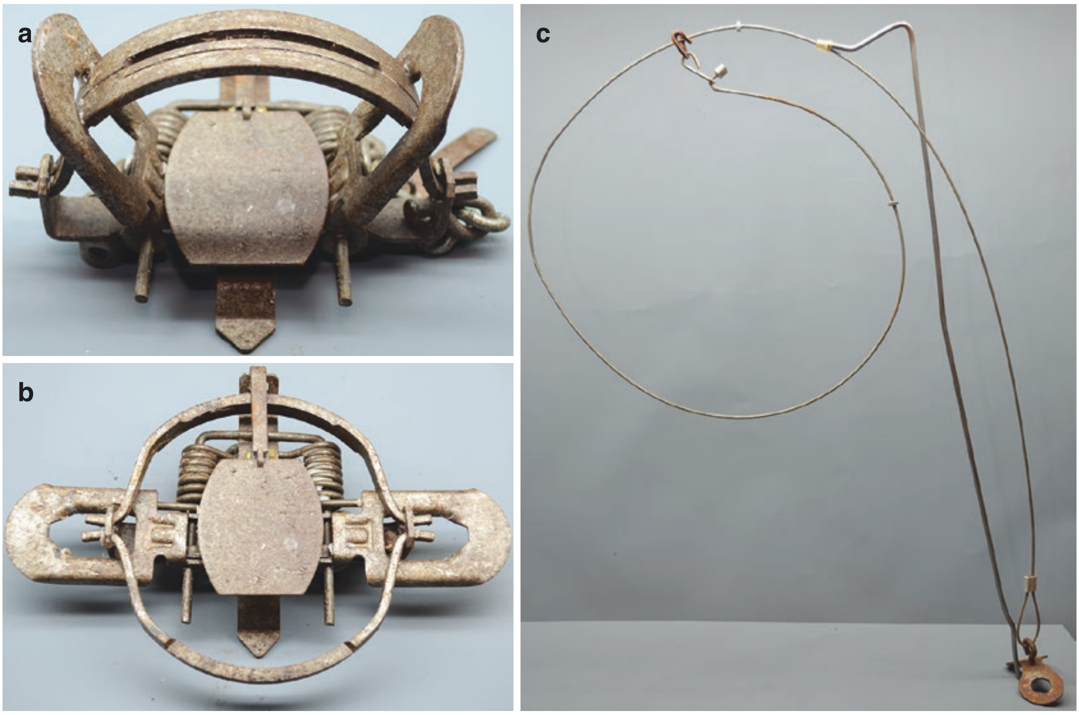

Fig. 6.10 Common types of animal traps: (**a**) Foothold spring trap (*closed*). (**b**) Foothold spring trap (*open*). (**c**) Neck snare (Photo courtesy of Dr. Jason Brooks, Penn State University)

trap injury is suspected, it is more likely to be located in a focal area on an extremity or around the head and neck. Depending on the type of device, injuries can range from minor skin lacerations through to major compound fractures of the appendicular as well as axial skeleton. If an animal is presented for examination with the trap in place, then the most difficult part of the investigation is identifying the trap type. If there is no trap available for examination, then description of the distribution pattern of wound may make matching a known trap possible. For example, there may be focal possibly linear crushing injuries to a distal limb combined with multiple puncture wounds that may indicate that an injury was caused by a leghold trap with curved spring-powered jaws containing metal teeth (traps with metal teeth have long been outlawed in the US although some antique or homemade specimens maybe encountered). The microscopic appearance of trap injuries will depend on the location and type of trap; snare-type traps will induce more skin abrasions and subcutaneous bruising with potential tissue necrosis if the snare is tight enough. Leghold traps will induce some localized tissue disruption with hemorrhage and fibrin before necrosis may occur following crushing from the trap jaws.

6.9 Bow and Arrow Injuries

Broadhead arrows are sharp-edged arrowheads used in hunting which will produce sharp trauma. Arrow injuries differ from gunshot injuries in that they lack the cavitation and energy transfer forces that create significant tissue disruption. Broadhead arrows will slice through skin and organs, sometimes in a wound tract that runs through and through an animal producing a perforating injury.

Various bow constructions exist, including traditional bows (straight or recurve), compound bows, and crossbows. A bow is a flexible arc that shoots aerodynamic arrow projectiles from the stored energy in a drawn string. The mechanics of the different designs result in a variation of bow strength or draw and the resulting kinetic energy of the arrow. Arrows may have either conical tips or bladed cutting tips known as broadheads.

A traditional bow consists of an arc and a string that is drawn back and released manually. A compound bow has mechanical aids to help with drawing the bowstring. Usually, these aids are pulleys at the tips of the limbs. A crossbow consists of a bow fixed transversely on a stock, having a trigger mechanism to release the bowstring and often incorporating or accompanied by a mechanism for bending the bow. All styles of bows may be used for hunting or archery (target sports).

The arrowhead is the projectile point of the arrow. Arrowheads that inflict cutting injuries include the fixed and mechanical broadheads (Fig. 6.11). Fixed-blade broadheads will have three or four triangular-shaped blades permanently affixed in an open, cutting configuration. Mechanical or expanding broadheads will have two or more blades that will expand upon impact. Conical-tipped arrows (field tips) produce small diameter circular or slit-like lesions which may be difficult to differentiate from firearm injuries [19]. The field tip can closely simulate a bullet wound by causing a circular entrance hole with abraded margins. The injuries inflicted by bows with cutting arrowheads have unusually clean, radiating incised wounds. The wound shapes inflicted by bladed arrowheads will reflect the shape and array of the cutting blades. Triple- or quadruple-bladed arrowheads will produce stellate or radial cuts in the same geometry as the blades causing the wound. The length of the wound will vary depending on the angle of entry or exit, the fur coat, and the thickness of the skin. The microscopic appearance of the wounds will depend on the organs through which it passes and the type of edge of the arrowhead. The sharper the arrowhead, the cleaner the cut with the resulting injury reflecting an incision with localized tissue disruption and hemorrhage for an acute injury. Variation will occur depending on the type of the organ and its response to injury and age of the wound from fibrosis and scarring in the skin to almost complete healing with little trace of previous injury in the liver.

Fig. 6.11 Types of arrowheads: conical field point (*left*), fixed broadhead (*center*), mechanical broadhead (*right*) (Photo courtesy of Dr. Jason Brooks, Penn State University)

When an arrow has passed completely through an animal, it can be difficult to differentiate the entry wound from the exit wound. Careful examination of the fur at the wound's edge can help discern the difference; the cutting edge of the arrowhead slices through the fur and then the skin and tissues during entry. During exit from the body, the skin is cut from the inside out, everting the skin edges and resulting in less cut fur at the wound edges.

The wound track of the arrow injury will be less disrupted than the wound track of a firearm projectile. This is due to the lower energy transference and a lack of associated wound cavitation. Human victims suffering from fatal crossbow injuries can maintain consciousness and capability of action longer than they would with similar wound tracks from a firearm. Depending on the degree of injury and organs involved, survival times of hours seem to be possible, even in cases with severe penetration of the body [19].

The path of the arrow track may help to provide evidence of directionality of the shooter relative to the victim; however, the direction of the arrow may change path within the body. In humans, it has been demonstrated an arrow may change direction during body passage up to as much as 90° [20].

6.10 Internal Examination

On internal examination, the examiner should describe the pathway of each sharp force injury. In cases of multiple stab wounds, wounds may remain relatively superficial, without penetration of body cavities or internal organs. Occasionally, multiple wound pathways may arise from a single skin injury. In such instances, it can be inferred that the weapon was thrust in and out of the same site.

It is entirely possible for a knife with a 3-in.-long blade to produce a wound that is 4 or 5 in. deep. When "hilt mark" injuries surround a stab wound, their presence indicates that the blade was inserted to its maximum possible depth; however, as just stated, because of the elasticity of skin, subcutaneous tissues, and internal tissues, the depth measurement of the wound can still be greater than the blade length. Obviously, it is also possible for a 3-in.-long blade to penetrate less than 3 in.

The pathway for each wound should be documented, taking note of all organs injured, as well as the direction of the wound (Fig. 6.12). As noted earlier, it is common for many pathologists to provide three directions for each wound. For example, a stab wound may have traveled from right to left, from upward, and from front to back. Another stab wound might have gone from back to front, slightly downward, without any significant right/left deviation. Other veterinary examiners choose to measure or estimate the angle of each wound, in reference to various anatomic planes. As always, the complete examination should be assess for the presence of natural disease which may be the primary cause of death, a contributing factor, or incidental finding.

6.11 Aging of Wounds

The aging of wounds requires multiple pieces of evidence. The time of injury may be quite different from the time of death, and particular attention needs to be paid to both if the correct sequence of events is to be estimated. Gross evaluation of wounds is the primary means by which wounds are aged, this coupled with the evaluation of postmortem interval. Histology is used to confirm or refute diagnoses made macroscopically as well as being used as an ancillary method when the gross examination does not yield a definitive answer. The time of death or postmortem interval is a complicated process that becomes more difficult to determine with increasing time postmortem. The details are outside the scope of this chapter, but unless the death is witnessed, a detailed and complex series of assessments are required. The age of

Fig. 6.12 Broadhead arrow wounds in a cat. (**a**) The wound is on the right shoulder. (**b**) Note cut fur at wound edges. (**c**) Wound clipped of fur and edges apposed. (**d**) Closer view of the wound. (**e**) Arrow trajectory. (**f**) Arrow path. (**g**) Arrowhead shape conserved through the liver lobe

the wound can be classified by using categories of inflammation because an inflammatory response occurs in an attempt to stem blood flow, close dead space, and ultimately heal the wound. *Acute wounds* are characterized by tissue disruption and abundant hemorrhage with variable amounts of fibrin being present for attempted hemostasis. There may be tissue necrosis depending on the wound type, but it should be minimal; these wounds are typically 0–8 h old. *Subacute wounds* can have hemorrhage, fibrin, tissue disruption, and +/− necrosis with neutrophils appearing with variable numbers of macrophages; these wounds are around 16–36 h old. *Chronic wounds* can be quite variable from the wound filling with granulation tissue and evidence of blood resorption to complete healing with a residual scar. These can be quite difficult to classify but generally range from 3–7 days and over 2 weeks depending on any residual presence of the acute/subacute process.

Complicating factors to the attempted aging of wounds include the onset of postmortem autolysis and the bacteria-mediated putrefaction. The decay of tissues can obscure many of the factors listed above. A body that has been immersed in water for a prolonged period may have had most of the blood leech away from the wound making it appear to have been postmortem in the case of an acute wound; chronic wounds would have other evidence not removed by water. Exsanguination may occur in the event of a wound to a vital organ or major vessel. When multiple wounds are sustained, this may result in wounds appearing to have been sustained postmortem due to the lack of blood around many of the wounds; this would be compounded if the body had been moved and the pool of blood from exsanguination was no longer associated with the body. Attention should be paid to the rest of the body to investigate the possibility of blood loss-related acute anemia.

There are instances in which some localized tissue hemorrhage can be induced as well as reports of fibrin clotting postmortem, but these are extremely small and usually isolated, and with other evidence, the determination of ante- or postmortem wound should be able to be ascertained.

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7 Firearm Injuries

N. Bradley-Siemens, A.I. Brower, and R. Kagan

7.1 Introduction

Veterinary forensics is a relatively new discipline within the forensic sciences. The importance of veterinary pathology to the discipline cannot be overstated, particularly in cases involving projectile injury. Projectile injuries are often lethal, or result in euthanasia, and they tend to result in litigation. Veterinary pathologists have advanced training in postmortem examination and access to purpose-built facilities and specialized equipment, often including digital radiography and computed tomography. Access to these resources can be essential in recovery, interpretation, and preservation of projectile evidence obtained from an animal. While veterinary pathologists are in the best position to conduct forensic postmortem examinations, cases involving gunshot wounds typically require a broad range of additional expertise. Thus, it is important that veterinary pathologists who engage in forensic casework integrate themselves into a team that can supply the expertise needed. In cases of projectile injury, such teams might include forensically trained veterinarians, law enforcement personnel, scene investigators, and ballistic and toolmark experts.

This chapter provides veterinary pathologists with a useful projectile injury resource. It includes an overview of the ammunition used to produce gunshot projectile injury in animals. Ballistics theory, patterns of tissue injury, methods to determine and interpret projectile trajectory, recovery and preservation of ballistic evidence, and additional techniques used in projectile case investigations are also provided. Throughout this chapter the various facets of a projectile case investigation are related to the role of the veterinary pathologist and relevance to the postmortem examination.

7.2 General Considerations

While a projectile injury may be a relatively straightforward problem to solve in terms of a medical diagnosis, these cases can be extremely complex and should not be undertaken without a full understanding of the case circumstances and the expectations of the submitter. There is currently no minimum standard of documentation to be completed if the legal status of a case is unknown. In this chapter, the authors recommend steps that mirror human forensic protocols and suggest that these be performed for every case involving a projectile injury. First, not all projectile injuries in animals are

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The views expressed by the author do not necessarily represent those of the US Fish and Wildlife Service.

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illegal. A shooting must be determined to have been done without the owner's permission, beyond the extent of a legal hunting season, without statutory protection, or must be the cause of unnecessary suffering before being investigated as a crime [1]. Additional federal and/or state laws may come into play in wildlife shootings depending on the species involved, the season, and the location in which the shooting took place. Projectile injuries to animals may be accidental or intentional, they may involve complex crime scenes and investigations beyond the injured animal, and the perpetrators can range from juveniles to adults. Cases may include public endangerment or property damage, and they may involve legal or illegal firearms.

To understand and interpret projectile injuries, veterinarians and veterinary pathologists need to understand wound ballistics, and they need to be aware that these types of cases may require analysis and documentation of large quantities of evidence in preparation for prosecution. In a postmortem examination, the pathologist must be able to distinguish entrance from exit wounds and understand factors that can affect or alter their appearance. The pathologist may need to assist in the determination of the approximate distance from which a projectile was fired based on wound examination and should determine the trajectory of the wound path into and through the body. Proper collection and preservation of ballistic evidence and assisting with other aspects of an investigation may also be necessary. Finally, proper documentation and preparation of a forensic report involves an awareness of medicolegal needs and requirements [2].

7.3 Projectile Injury in Companion Animals in the United States

There is limited data available on the number of veterinary forensic projectile cases investigated or prosecuted annually. A national database for entry of these types of crimes does not currently exist, and profile data on suspects who commit animal cruelty using firearms is also scant. A study to establish the Factors in the Assessment of Dangerousness of Perpetrators of Animal Cruelty (FADPAC) was developed by Dr. Randall Lockwood of the American Society for the Prevention of Cruelty to Animals (ASPCA). It is intended to evaluate the significance of an individual's involvement in a particular act of animal cruelty as an indicator of dangerousness or possible risk for involvement in future acts of violence against others [3]. In this assessment, 33 factors were established based on threat assessment criteria used by the National Center for the Analysis of Violent Crime and on studies of animal cruelty offenders and habitual violent offenders. The perpetrator risk assessments consider victim vulnerability (small, harmless, or nonthreatening animals), number of victims, severity of injury inflicted, close proximity to the victim at the time of injury, duration of abuse, degree of premeditation, risk of exposure when performing the act of abuse, past history of positive interactions with an animal victim, and documentation of the act through photographs or video recording. The presence of five or more of these factors is considered cause for concern about future acts [3].

Projectile injury profile features that have been reported indicate that shooting incidents almost invariably involve male perpetrators, that animal shooting from a vehicle typically involves two suspects (one to operate the vehicle and the other to shoot), and that the type of weapon used may reflect the age of the suspect [3–5]. For example, preteen and teen shooters are more likely to use air- or gas-powered weapons that use BB or lead pellet projectiles, and adults are more likely to use higher-power weapons and shotguns. Data on the types of weapons used was presented in a study of 121 projectile injuries treated at Boston's Angell Memorial Animal Hospital over an 11-year period. In this study, the vast majority of wounds were caused by handguns, with a small distribution of cases involving rifles, shotguns, and BB or pellet guns. Tendencies in weapon type have also been reported, with handgun injury predominating in urban areas, rifles and shotguns in rural areas, and air- or gas-powered guns (including pellet and BB guns) in suburban areas [6]. Historical and signalment data reported on dogs with projectile injuries in a University of Pennsylvania study indicated that the majority are young and sexually intact. These included German shepherds (18%), pit bull mixes (13%), Rottweilers (13%), Doberman pinschers (10%), and Mastiffs (2%) [4]. Companion animals shot with firearms also tend to be "outside and unsupervised," with most shootings occurring in animals roaming at large [2, 6]. Pet-Abuse.com (http://www.pet-abuse.com) is an online resource that gathers data on animal cruelty investigations in the United States. According to this source, in 2013, projectile injuries accounted for over 14% of recorded animal cruelty cases, and from 1998 to 2010, 10.3% of recorded animal cruelty cases involved shootings of companion animals (dogs, cats, and horses).

7.4 Wildlife Projectile Injury

Though the principles of case investigation are largely the same, wildlife cases have a different focus than domestic animal cases. In wildlife cases, shooting deaths may be motivated by the illegal wildlife trade, poaching, killing of animals perceived to be nuisances, failure to understand or acquire appropriate permits, and mistaken identity (such as confusing a red wolf for a coyote). In this respect, the profile differs somewhat from domestic animal cases.

In the United States, wildlife is covered under a number of federal and state laws designed to protect target species and regulate hunting. Applicable federal protections include the Migratory Bird Treaty Act, the Endangered Species Act, the Marine Mammal Protection Act, and the Bald and Golden Eagle Protection Act. When investigating wildlife deaths, species identification becomes important as this often dictates which law has been violated. If the species is not obvious to the investigator, genetic analysis or consultation with an expert capable of determining taxonomy may be necessary.

7.5 Weapons and Projectiles

To effectively evaluate projectile injuries, a basic knowledge of firearms and the ammunition they carry is necessary. There are five categories of small arms: handguns, rifles, shotguns, submachine guns, and machine guns. Projectile injuries in veterinary species are commonly caused by handguns, rifles, and shotguns; thus, the following discussion will be limited to factors involving these types of firearms.

Handguns, rifles, and air rifles typically have rifled barrels. In rifling a barrel, spiral grooves are cut the length of the inside or bore of the barrel. The metal left between the grooves is called "the lands" [2, 7] (Fig. 7.1a). The purpose of rifling is to cause

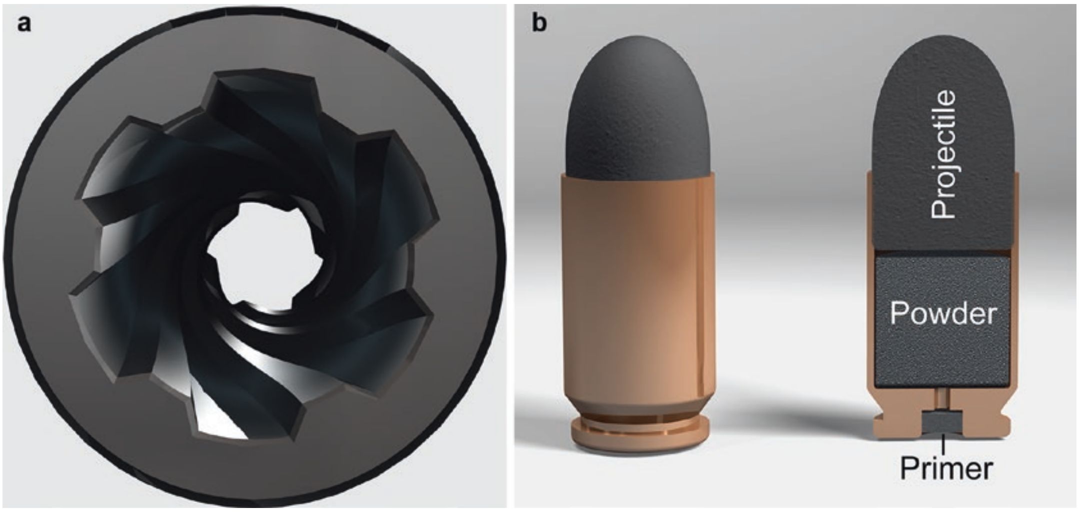

Fig. 7.1 (**a**) Rifling: illustrating lands and grooves that imprint on projectiles (**b**). Bullet cartridge: labeled segments of a bullet before being fired

a bullet to rotationally spin along its longitudinal axis, which helps to stabilize a projectile's flight through the air, improving accuracy. While rifling increases accuracy, it also slows the projectile down by decreasing velocity as the energy of the projectile is deviated from a forward direction [7].

The caliber of a weapon is determined based on the diameter of the bore of the weapon measured from land to land, so it is the diameter of a barrel before the rifling is cut. Caliber specifications can be given in inches or as metric units. Examples of the former using inches include the 0.38 special, 0.45 ACP, or a 0.357 Magnum and of the later metric units include 9 × 19 mm Parabellum or 5.56 × 45 mm cartridges.

The speed at which a projectile leaves the barrel of a weapon is called the muzzle velocity. This velocity is dependent on the type and caliber of ammunition, the length of the barrel, the propellant (gunpowder) burn rate, and the gas produced after initial ignition of the primer. Handguns are generally considered low-velocity weapons. The most common types of handguns currently in use are autoloading pistols (automatics and semiautomatics) and revolvers. A pistol has a magazine containing multiple cartridges. The magazine is placed in the handgrip of the pistol. A revolver has a revolving cylinder containing multiple chambers, and each chamber contains one cartridge. The cylinder rotates mechanically to align each chamber with the barrel and the firing pin.

A rifle is a type of weapon designed to be fired from the shoulder. Rifles are used for longer distances and have greater precision and accuracy than handguns. In the United States, rifles are required to have a minimum barrel length of 16 in. (40.6 cm). Rifles are generally considered to be a high-velocity weapon. There are multiple types of rifles: hinged or single shot, lever action, bolt-action, pump-action, and autoloading [7]. Rifles can either be semiautomatic or automatic. In a semiauto rifle, the weapon will fire one round each time the trigger is pulled back. In an auto rifle, the weapon will continue to fire rounds as long as the trigger is pulled back or until the weapon runs out of ammunition.

Handguns and rifles can have devices called suppressors, commonly referred to as silencers, attached to the end of their barrels. These attached devices are used to suppress the sound of the weapon when it is fired. Essentially, the suppressor is a metal tube with a series of baffles within it that divert gases/particles expelled from the barrel, effectively decreasing the sound created when the weapon is fired. This feature can also narrow the spread and minimize the amount of gunshot residue leaving the weapon. Suppressors not aligned correctly when attached to the barrel or homemade suppressors can alter the projectile as it leaves the barrel by causing nicks or indentations. These alterations to the projectile are referred to as a "baffle strike" [8].

A shotgun is fired from the shoulder and the barrel is smooth-bored, so no barrel rifling is present. Shotgun barrel diameter is referred to as gauge, most commonly either 10, 12, 16, 20, or 28 gauge, although other sizes also exist. Gauge more accurately refers to shotgun ammunition. A gauge is the weight of a solid metal sphere that fits into the barrel of a firearm, and this weight is given as a fraction of a pound, for example, a one-twelfth pound ball fits a 12 gauge bore. Shotguns are used for dispersion shots on moving targets at relatively short distances. They can fire multiple types of shells (ammunition), including pellets, buckshot, and slugs. In the United States, the federal law mandates a minimum barrel length of 18 in. (45.7 cm) for shotguns. The barrel of a shotgun may have a device known as a choke that can influence the dispersion of shotgun pellets. This can alter interpretation of the range of discharge in a shotgun injury [7]. Air- or gas-powered weapons range from toys to highly sophisticated pistols and rifles. An air rifle uses the expanding force of compressed air or gas to move a projectile down a rifled barrel. An air gun is differentiated from an air rifle by its smooth-bored barrel. The same projectiles may be used in either type of weapon.

7.6 Non-firearm Projectiles and Other Devices

Arrows are shafted projectiles shot with a drawn bow or crossbow. Arrow velocities do not approach those of firearms, but hunting arrows are capable of passing through a large mammal. The main type of arrowhead used for hunting large game is the broadhead tip. Broadhead tips have two to four razor-sharp blades that may be fixed in position, or designed to swing out on contact with the target ("mechanical" blades). Other types of arrowheads such as field points or blunt points are used for small game and target shooting. Field-tipped arrowheads are conical, whereas blunt points are blunt and rounded. They are designed to kill small game by "shock" (focused blunt force trauma). Judo points have wires extending from the sides of the head to facilitate the retrieval of arrows fired into vegetation.

A nail gun is a construction tool that uses blank cartridges or compressed air to fire a metal nail into an object such as wood, concrete, or steel. The caliber range of the blank cartridges is 0.22–0.38. The cartridges are loaded with a quick-burning propellant that can attain pressures higher than a firearm. While the nail is intended to penetrate the surface into which it is being discharged, it may ricochet off of a hard surface. Injuries sustained are usually accidental; however, nail guns have been used to inflict intentional injuries on victims [2].

The captive bolt gun uses compressed air or a blank cartridge ignited by a firing pin. The bolt device is usually a 7–12 cm metal rod, which is driven out of the barrel of the captive bolt gun and then recoils back into the barrel via the use of spring tension. The end of the bolt is circular, 7–12 mm in diameter, with sharpened edges, resulting in a sharp-edged, circular penetration in the skin and the bone. This device is designed for slaughtering cattle or other large animals, although there have been reported cases of captive bolt gun use in companion animals [2, 7].

A bang stick is a pipe-shaped device used to kill sharks, large fish, and alligators. The weapon will fire when in direct contact with the target/subject. The bang stick is a metal cylinder acting in the capacity of a cartridge chamber, with a firing pin device. Most bang sticks use standard handgun ammunition such as 0.357 Magnum or 0.44 Magnum. The force of the bang stick does not result from the bullet, but from the high-pressure gas that is forced into the target subject [7].

7.7 Ammunition

Ammunition is a combination of a propellant and projectile developed to achieve specific parameters of distance, target penetration, and wounding capacity. To accomplish this, ammunition design must take into consideration the caliber of the weapon in which it will be used, composition of the projectile, and the amount and burn rate of the propellant.

A cartridge consists of a cartridge case, a primer, a propellant (gunpowder), and a bullet (projectile). The cartridge case is usually composed of some type of metal, commonly brass or steel. The purpose of the cartridge case is to expand and isolate the chamber of the weapon against a backward escape of gases when the cartridge is fired. The primer is the cartridge ignition component, igniting the propellant (gunpowder) in the cartridge when it is struck by the firing pin of the firearm. In the United States, primer compounds may consist of lead styphnate, barium nitrate, and antimony sulfide. Primers are classified as a center fire or a rim fire, with the primer in the center or in the rim of the cartridge base, respectively. The propellant is the gunpowder within the cartridge that is ignited to propel the projectile forward. The bullet (projectile) is the segment of the cartridge that exits the barrel of the weapon when it discharges (Fig. 7.1b).

Modern bullets generally consist of two categories: unjacketed and jacketed. The latter category can be further subdivided into full-jacketed and semi-jacketed bullets. There are also a variety of bullet shapes including round nose, wadcutter, semi-wadcutter, and hollow-point. Round nose bullets consist of a partially blunt, conical shape, and a flat or beveled base. The wadcutter bullet resembles a cylinder with a base that is either beveled or hollow. The semi-wadcutter bullet is a truncated cone with a flat surface at the tip. At the base of the cone, there is a shoulder lip the diameter of the bore. The hollow-point bullet is similar to the semi-wadcutter with a cavity in the nose portion of the bullet used to enhance expansion (mushrooming) of the bullet when it impacts the target (Fig. 7.2). At high velocities,

Fig. 7.2 Fired projectile: projectile and semimetal jacket: these may separate in the body both may have evidence of rifling and should be recovered for evidence

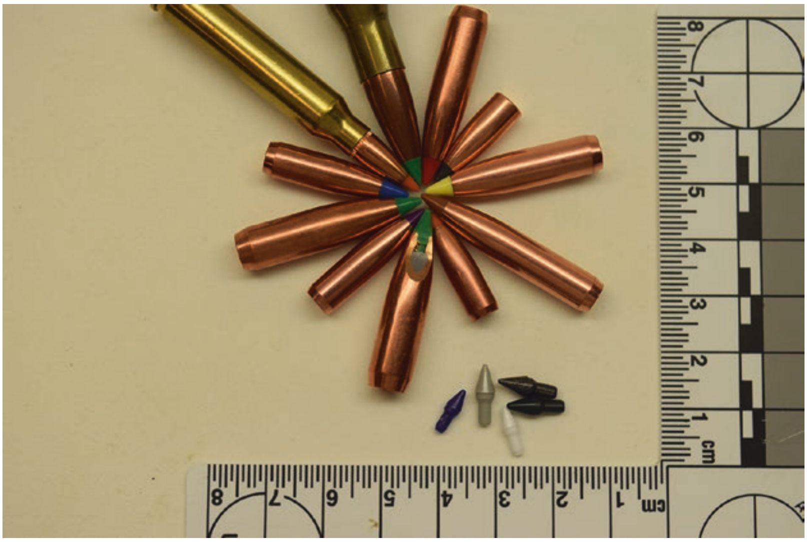

Fig. 7.3 Examples of plastic-tipped bullets. Note the range of colors, which can be used to determine bullet make and sometimes caliber

lead bullets may melt or fragment, so jacketing provides a protective metal casing around the bullet. Thus, jacketed bullets may be used in higher-velocity weapons. After entry in the body of an animal, the metal jacketing can separate from the lead bullet. The jacketed segment is essential for ballistics. This portion of the projectile may retain rifling marks that can be used to identify the weapon.

Some rifle bullets and air rifle pellets are designed with polymer tips. The purpose of the polymer tip is to fill the otherwise hollow point, stabilizing the projectile in flight but still allowing for mushrooming. The tips tend to fall out on impact with the body and may be found along the wound tract, whole or in pieces. These plastic tips come in a variety of colors, which can be used to identify bullet manufacturer and caliber [9], and though small, may stand out during necropsy due to the coloration. Bullet tips consist of a cone and stem that may break into two pieces on impact (Fig. 7.3). Air rifle tips are diamond-shaped (Fig. 7.4).

Shotgun shells are comprised of a primer in the base, powder, wadding, and shot enclosed in a cardboard or plastic casing (Fig. 7.5). Terminology to describe shells will vary in different parts of the world. In the United States, there are three standard sizes of shells based on length, 2¾, 3, and 3½ in., and the most popular

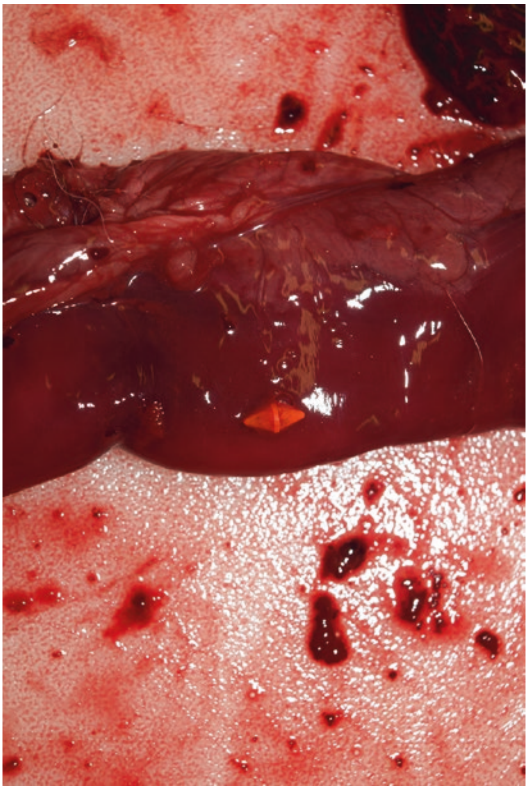

Fig. 7.4 Domestic dog, polymer tip from an air rifle pellet retrieved from a wound tract through the abdomen

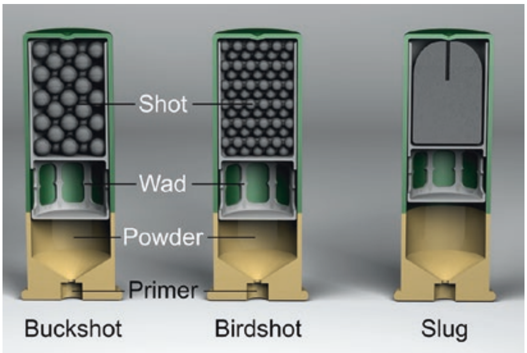

Fig. 7.5 Intact and cutaway views of 12 gauge shotgun shells, birdshot, buckshot, and slug

caliber sizes are 12 (approximately equal to an 18.5 mm bore diameter), 16 (16.8 mm bore), and 20 (15.7 mm bore). The length of the shell will determine the amount of powder, and the volume of shot varies depending on the size and number of shot pellets. Pellets are spherical metallic projectiles generally ranging in diameter from 1 to 9 mm. Shotgun pellets in the smaller range are often called birdshot pellets and range in size from 0.05 to 0.16 in. (1.3 to 4 mm), also known as size 12–1 shot using the American shot size standards. Larger shotgun pellets ranging from 0.24 to 0.36 in. (6.1 to 9.1 mm), or #4 to #000 shot, are commonly known as buckshot. Shotguns shells may also contain a slug, which is a solid piece of metal similar to a bullet [7, 8]. Depending on the manufacturer, shotgun shells may contain pellets of more than one size and/or shape. Home-loaded shotgun shells could contain any combination of materials.

Air guns or air-/gas-powered guns fire pellets or BBs. Pellets come in various shapes, including round nose, pointed, wadcutter, or flat. The most common pellet has a "wasp waist," also called diablo style (Fig. 7.6). BBs are round and resemble large shot pellets. Pellets and BBs are made from a variety of materials depending on the brand. Materials include, but are not limited to, copper, lead, steel, and zinc.

7.8 The Process of Firing a Gun

The discharge of a firearm is initiated by pulling the trigger, releasing the firing pin within the gun. When the pin strikes the primer of the cartridge case, it ignites, causing a flame. The flame enters the chamber of the cartridge case, igniting the powder and producing large quantities of gas and heat. One gram of propellant produces 1 L of gas under high temperature and pressure. The intense heat of the gas exerts pressure on the projectile and the sides of the cartridge. The gas pressure on the base of the projectile propels it down the barrel of the weapon. After the bullet exits the barrel, a burst of flame, gas and unburnt powder, soot, primer residue, and vaporized metal from the projectile and cartridge case all follow the projectile. This material is referred to as gunshot residue (GSR). In revolvers a similar material will emerge from both the cylinder-barrel gap and the barrel [7]. The GSR may also travel rearward from a weapon and onto a shooter.

Fig. 7.6 Round and pointed tipped air rifle pellets

7.9 Ballistics and Wounding Capacity

Ballistics is the science of projectile travel. The flight path of a projectile can be broken down into three stages: internal ballistics, the path of a projectile within the weapon; external ballistics, the projectile's flight through the air; and terminal ballistics, the projectile pathway through an object. When a projectile enters the body of a victim, terminal ballistics may also be referred to as "wound ballistics" [10].

The physics underlying wound ballistics is partially expressed in the equation: kinetic energy = ½ × mass × velocity^2. The equation describes the influences mass and velocity have on the amount of kinetic energy that can be delivered to tissues by a projectile. When the mass of a projectile is doubled, the kinetic energy is doubled; however, if the velocity is doubled, the kinetic energy is quadrupled. In addition to the variables described in the kinetic energy equation, the effects of distance traveled and changes in media along the path will influence the capacity of a projectile to cause tissue damage. Projectile velocity changes with the distance traveled before contact, affecting the amount of kinetic energy transferred to tissue. The resistance of tissues to the projectile will also influence the amount of energy delivered. For example, skin provides enough resistance that a minimum bullet velocity of 50–60 m/s is required for penetration. All of these factors influence the final kinetic energy transferred to tissues, and it is the kinetic energy transfer, in combination with weapon type, that best predicts the wounding capacity of a projectile [7, 11].

7.10 Examination and Necropsy: General Considerations

Determination of the cause and manner of death in projectile injury cases may be insufficient to address all of the questions these cases raise. In addition to standard necropsy procedures and reporting, the pathologist must provide information on entry and exit wounds, projectile trajectory, and related forms of tissue injury. The pathologist must also be aware of external evidence that needs to be collected *prior to* the postmortem examination and must, whenever possible, retrieve ballistics evidence. Before manipulating the carcass, a plan should be developed that takes into account the best sequence for gathering evidence. Figure 7.7 provides a suggested stepwise procedure that will address most case scenarios. Prior to receiving the case, communicate with the law enforcement agency crime scene investigation (CSI) unit or state crime laboratory, and be familiar with the types of evidence they can process and how they want evidence submitted. These units can offer a great deal of expertise in evidence handling and collection and may be able to provide trained personnel to obtain and analyze evidence such as GSR. Image analysis (see specific examples below) should also be completed before in-depth physical

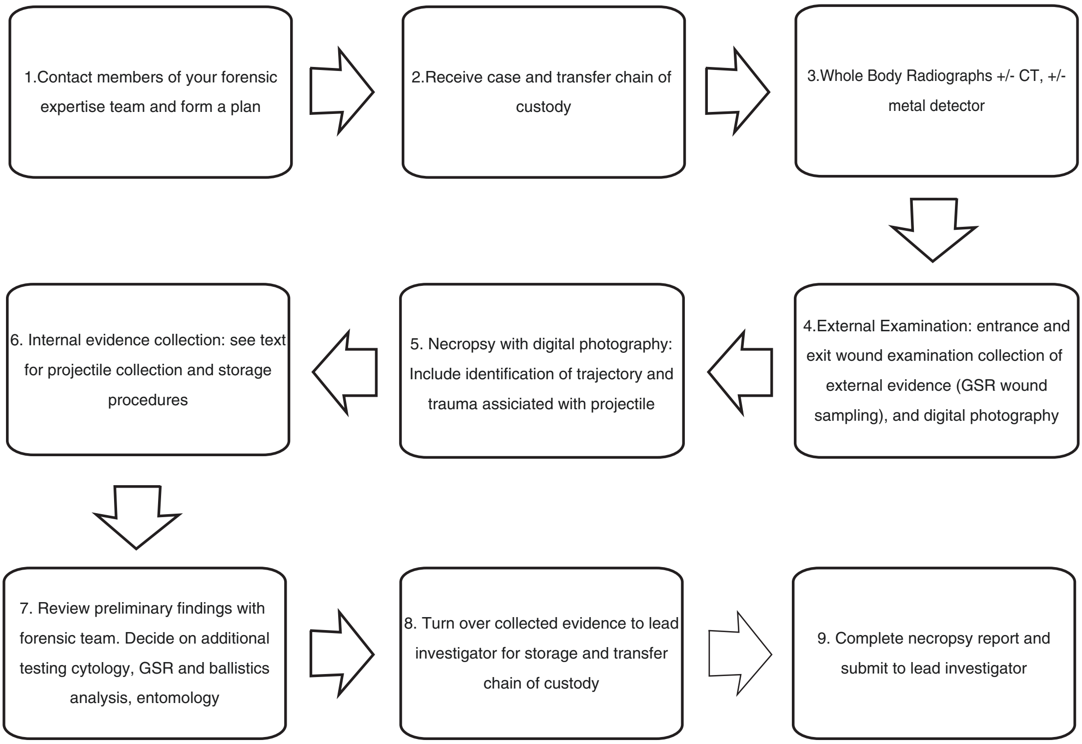

Fig. 7.7 Case process for forensic pathology projectile injury

manipulation of the body is conducted. Manipulation of the body during removal from a body bag or during the course of the external exam should be minimized until the presence and positioning of a projectile are established and external evidence has been collected. Investigation teams may check a body with a metal detector prior to turning it over to a pathologist, but pathologists may also consider having this piece of equipment in their necropsy facility. If a metal detector is to be used, be mindful of metal on the table or the surface the body is on that may complicate analysis and interpretation.

Following image analysis, additional planning should occur for the recovery of each projectile and its components, or for identifying a projectile's path in the event of complete perforation through the body. The pathway (trajectory) within the body needs to be photographed and documented. Documentation will describe any organs or structures involved in the path of the projectile and provide measurements of any injuries sustained. Pathway descriptions should be kept simple and succinct, listing organs or structures involved using anatomically correct terms, and using the directional terms up, down, left, right, forward, and backwards. In cases with multiple projectile wounds, it may be helpful to number the wound tracts on a diagram. Additionally, projectile wounds may be numbered, beginning in the cranial region of the body working caudally. Identifying each tract in this way can help keep things organized in the mind of the investigator between the necropsy and report writing. A good diagram can also be used as a visual aid for trial.

7.11 Photography

A complete set of digital images should be taken starting with photographs of the body as received, including any packaging or seals. A minimum database of external digital photography should include full-body right and left lateral images of sufficient quality that the animal can be identified. If the body is still in rigor mortis, the photographs should capture this body position. Images should also include all external evidence, such as blood and other fluids on the body, collars and tags, ropes, chains, leaves, or other environmental or foreign material that might be trapped in the fur or received with the body. Closer images of external lesions and environmental evidence should also be taken at this time. Photographs should follow best practices for forensic imaging (see Chap. 1).

7.12 Radiology

In any forensic case, but especially when projectile injury is suspected, whole-body radiographs prior to postmortem examination are strongly advised. Therefore, if undertaking forensic projectile case works, one should either have radiographic equipment available on-site or should have a working relationship with facility that does. Radiographic images need to be taken with a minimum of two views with standard markers and identification. If the body is received in a body bag or other packaging, it is recommended that the first images be taken of the body while still in the bag to document any superficially located pellets or bullet fragments that may fall off of the body during unpackaging and manipulation. Refer to Chap. 1 for imaging and imaging documentation guidelines.

Radiographs can show the shape and density characteristics of the projectile, location of the projectile within the body, and often will better show bone fractures than gross postmortem examination (Fig. 7.8). Radiographs can also help to define the trajectory of the projectile within the body. Examination of radiographs should include identification and localization of the projectile and any related fragments in the body. Wound tracts may also be identified based on the presence of gas, hemorrhage, bone or metal opacities. The direction of fragments or beveling of bone in images may help determine the direction of the projectile and contribute to entry and exit wound identification (Fig. 7.9). If a projectile hits a bone, the majority of the bone fragments will be found between the altered bone and any exit wound, indicating the direction of travel of the projectile. Radiographs should not be used to draw conclusions regarding caliber.

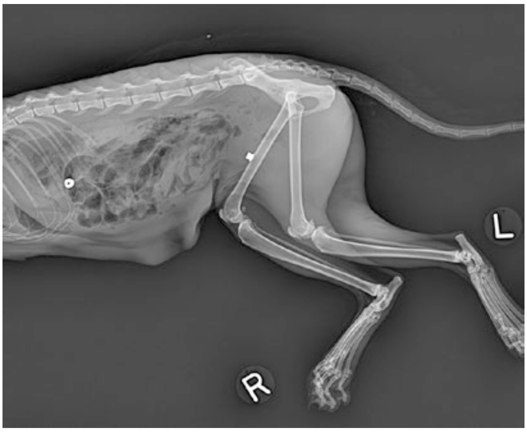

Fig. 7.8 Domestic cat, this radiograph shows both an end-on and lengthwise view of plastic-tripped air rifle pellets. Note the central area of radiolucency visible in the end-on pellet where the plastic tip is located

Fig. 7.9 Gray wolf (*Canis lupus*), this radiograph of the thorax shows the classic “lead snowstorm” consistent with fragmentation of a bullet fired from a high-velocity rifle. The bullet traveled across the body in a ventral to dorsal and slightly cranial to caudal direction

This is considered inaccurate as angle and distance from the film can affect measurements taken from the image.

When attempting to retrieve small fragments obscured by traumatized tissue, fur or feathers, taking additional radiographs of the area overlain by a metal grid (hardware cloth works well) can help the investigator localize the fragment.

7.13 Computed Axial Tomography

Computerized axial tomography (CT) operates on the same principle as radiography but offers improved full-body scanning and visualization for projectile retrieval and fracture description [12]. CT images differ from radiographic images in that the imaging is repeated as the body moves through a rotating beam of radiation. Data from all angles around the body, equal to hundreds of individual X-rays, are then compiled through computer software to form images that are constructed as slices. Because the slices of the object are taken in sequence as the object is moved through the rotating beam, the compilation can create a 3-D view of the entire body. CT imaging is a rapid and increasingly affordable technology that allows postmortem examination without disturbing anatomical structures. While CT imaging has similar soft tissue limitations to radiography, it greatly improves three-dimensional localization of projectiles and projectile paths prior to necropsy [13]. In general, digital imaging is conducive to storage and use of images for legal proceedings, and the comprehensive nature of CT scans offers in-depth, full-body medicolegal documentation of trauma that is superior to standard radiography.

7.14 Collecting External Evidence

Because protocols may differ between laboratories, collection of external evidence should be discussed with the responsible forensic laboratory before proceeding. Some procedures include excision of the entry wound and surrounding tissue (to a specified diameter) untouched and as a whole. Skin and the adjacent underlying soft tissues can be placed on a piece of cork or Styrofoam to keep the shape, size, and anatomical orientation for further analysis. These types of samples can be analyzed using a scanning electron microscope and X-ray spectrometry to determine the quality and quantity of soot particles, information that can be used to help determine the range of discharge. There are several simple techniques used to collect evidence during the external examination. When powder particles associated with gunshot wounds are found, they can be scraped into a Post-it® note and placed in a sealed paper envelope. Entry and exit wounds should be shaved, and the fur placed in a cellophane or waxed paper envelope. Clear tape can be used to retrieve stippling from the skin and then placed on a glass slide. Sections of skin containing contact wounds with soot or stippling can also be taken at necropsy and either forwarded to a ballistics expert or photographed, sampled for residue, and fixed in formalin [2, 14].

7.15 Necropsy Findings: Wound Analysis

Necropsy findings may be straightforward, as in the case of a single gunshot wound with a retained projectile, or they may be more complex. Multiple gunshot wounds and/or projectiles (such as shotgun pellets), through-and-through wounds with little to no projectile fragmentation, and the generation of secondary projectiles from bone fragments can all complicate analysis.

Projectile injury needs to be differentiated from other forms of trauma, including wounds from captive bolt stunners, motor vehicles, animal bites, and lacerations. Whole-body radiographs will be one of the best ways to diagnose gunshot injury, but determination of wound characteristics is also critical in the assessment of any projectile injury.

Determination of entrance and exit wounds and identification of any intermediary wounds are necessary early steps when conducting a complete projectile injury postmortem examination. This determination is critical for crime scene recreation and trajectory analysis. Projectile wounds may be penetrating or perforating. A penetrating wound occurs when a projectile enters a body and does not exit, in which case the projectile may be referred to as a "retained missile." A perforating wound occurs when the projectile passes completely through the body or organ. Additionally, projectile wounds can be considered graze or tangential. A graze wound involves the projectile striking the skin at a shallow angle, causing an abrasion. In a tangential wound, the skin is torn to the level of the subcutaneous tissue [2, 10].

Projectiles "punch in" and "explode out" of the body, resulting in entrance wounds that are classically smaller and tidier than exit wounds (Fig. 7.10). Entrance wounds are typically round to ovoid with a thin rim of abrasion (abrasion ring) where the projectile pushed into the tissue. Fur or feathers may protrude into the wound (Table 7.1). An abrasion ring is present in most entrance wounds, no matter the range, and consists of a reddish to reddish-brown zone of abraded skin caused by the heat of the projectile entering the tissue.

Depending on how close the shooter was to the victims, there may be soot marks, gunpowder, and/or burns at the entry wound. During the discharge of a weapon, in addition to the powder, soot from the ignition of the powder emerges from the muzzle. The soot is carbon and contains vaporized metals from the primer, projectile, and cartridge case. If the muzzle of the barrel is held close to the body of the animal, soot can be deposited on the animal. These marks are typically found on exposed skin; thus, long or dense hair coats may obscure these findings. The "flame," also called the muzzle flash, produced when firing a gun, is composed of superheated incandescent gases. These gases can cause searing of skin or fur around contact and near-contact gunshot entrance wounds. Soot or burned hair may still be present in these cases, thus it is

Table 7.1 Characteristics of entry and exit wounds

Entry wound	Exit wound
Punched-out circular hole, usually small diameter (may be less than the projectile)	Slit-shaped, stellate, irregular, small, or large diameter
Abrasion ring (collar) around periphery	Usually no abrasion ring
Inward indented skin or fur	Maybe protruding underlying tissue
GSR (gunshot residue), stippling, or soot dependent on distance the weapon was fired	Usually GSR absent

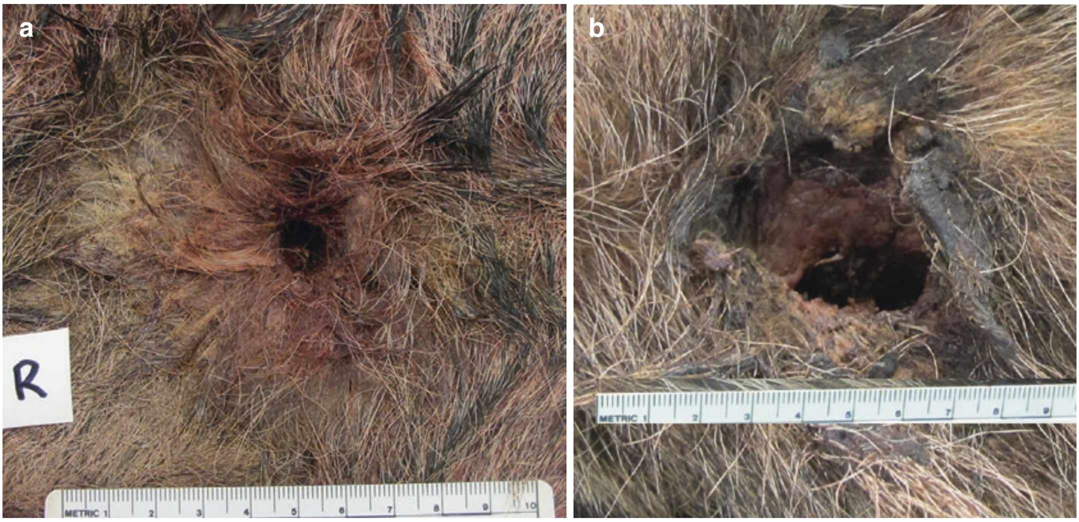

Fig. 7.10 (**a**, **b**) Gray wolf, comparison of an entrance (**a**) and exit (**b**) wound made by a high-velocity rifle. Note the smaller size and rounder shape of the entrance wound as compared to the larger, more irregular exit wound

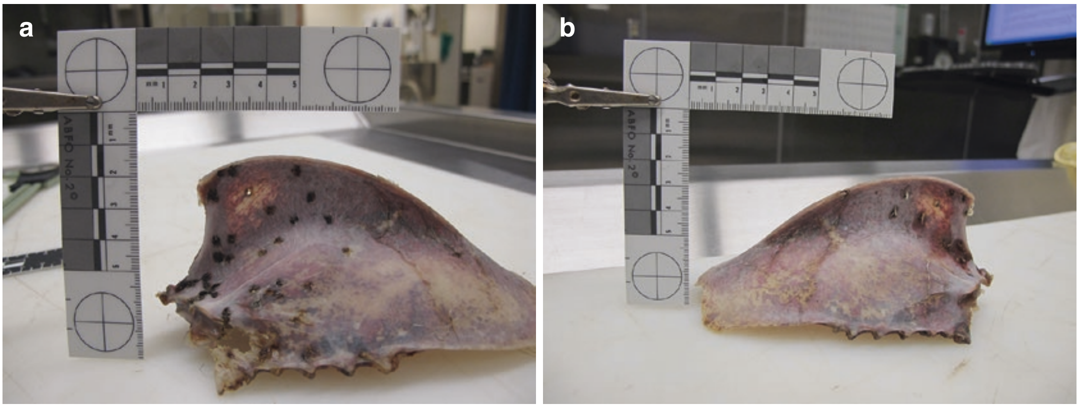

Fig. 7.11 (**a**, **b**) Sternum from a bald eagle (*Haliaeetus leucocephalus*), comparison of entrance (**a**) and exit (**b**) shot pellet wounds. On the side of impact, the holes are well delineated with edges pushed inward (medially). On the exit side, the holes are splintered with fragments pushed outward (laterally)

important to sample hair over projectile wounds prior to further examination of the entrance wound. The size, intensity, and appearance of the soot pattern and the range at which it occurs will depend on multiple variables, including range of fire, type of gunpowder, angle of the muzzle to the target, barrel length of the weapon, weapon caliber, type of weapon, and the type of target. Additional factors influencing the soot pattern include weather conditions such as wind, air pressure, or rainfall. Because most areas of the animals that will be examined are haired, it will be necessary to sample hair for powder and soot. It will then be necessary to shave the area around wounds to identify any evidence on the skin.

It is relatively common to receive projectile wound cases for which there is no intact entrance wound in the skin. This may be due to predation, postmortem decomposition, or removal of tissue under some other circumstances. In the absence of this critical evidence, interpretation of fractures in bones, with the aid of previously described imaging tools, can be very important in identifying projectile injury. For example, identifying beveling of fractured bone edges in the direction of the projectile's path (Fig. 7.11) and retention of hair or skin driven into fracture sites by the projectile can be very helpful in directing an investigation of a poorly preserved carcass. Funnel-like bone loss caused by a projectile passing through a flat bone can assist with the determination of the trajectory direction of a projectile (Fig. 7.12). Similar findings are regularly encountered in captive bolt gun lesions of slaughtered cattle.

Irregular entrance wounds can occur if the shot was made with the muzzle of the gun contacting the body surface or if the bullet ricocheted or passed through a barrier or other intermediate target prior to entering the body. Intermediary wounds occur when a projectile passes through one part of the body, and then reenters another part, causing a reentry wound. In perching birds, the folded wing may be an intermediate target, resulting in what is essentially a reentry wound to the body cavity. A reentry wound normally does not have an abrasion ring which can help differentiate it from an entry wound. An intermediary wound may also be caused by a projectile passing through an object such as a door, wall or window, before hitting the animal's body. This results in a larger or more irregular entry wound due to deformation and/or tumbling of the projectile. If the intermediary object fragments it can cause a wider dispersal of shotgun pellets, thus altering the gunshot range determination. If one or more fragments become embedded in the projectile this may, cause further deformation and loss of stability [2, 7].

Exit wounds can be of any size and shape; however, they will lack the abrasion ring of the entrance wound. Exit wounds typically have jag-

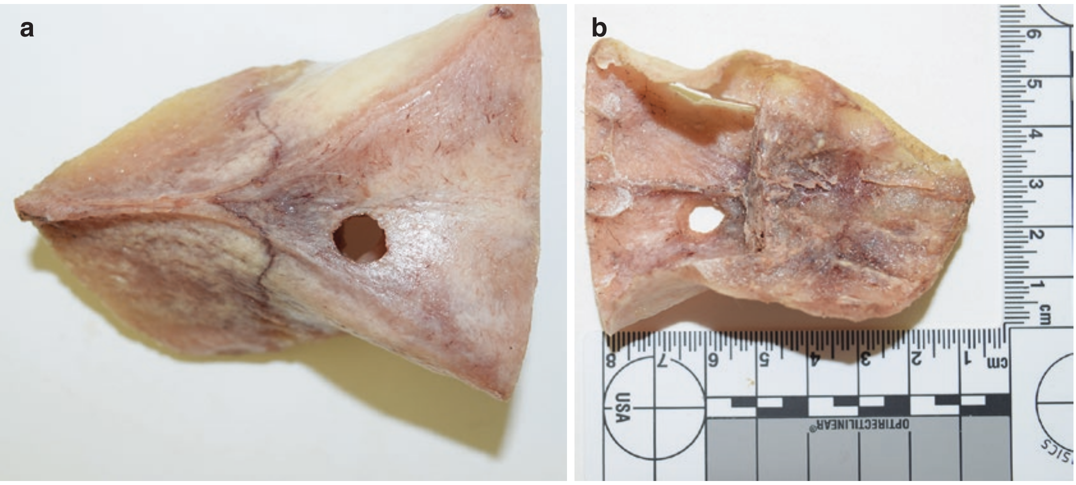

Fig. 7.12 (**a**, **b**) Domestic dog skull (frontal bone), comparison of entrance (**a**) and exit (**b**) side of a wound made by a 0.22 air rifle pellet (cadaver study). Note the smooth edges around the entrance as compared to the internally beveled exit side

ged margins and a larger diameter than that of the entrance wound (Fig. 7.10). Low velocity projectiles can cause a slit-like exit wound resembling a stab wound. Exit and intermediary wounds are unlike entrance wounds in that their appearance will be less dependent on the size of the projectile, and their surface will hold less or none of the evidence related to the firing of the weapon. A summary of the features of entry and exit wounds is provided in Table 7.1.

7.16 Distance

Projectile wounds can be classified as contact, near contact, intermediate, or distant/indeterminate, based on the distance from which they were fired. Contact wounds are caused by placing the end of the muzzle in direct contact with the skin at the time a gun is fired. These may be further subclassified as hard, loose, angled, or incomplete contact wounds. In hard-contact wounds, the barrel is pushed hard against the skin, so that the skin rises up around the end of the barrel. The edges of the wound are seared by the hot gases exiting the barrel. In this type of wound, soot is forced into the skin. In skin areas supported by bone, a separation of the skin from the subcutis may occur forming a “soot cavity.” In loose-contact wounds, the barrel is in contact with the skin but held lightly against the skin. Any gas preceding the projectile pushes the skin inward, creating a gap between the skin and the end of the barrel allowing the gas to escape. Soot is deposited in a zone around the entrance. Angled-contact wounds occur when the barrel is placed at an acute angle to the skin; the circumference of the end of the barrel (muzzle) is not in complete contact with the skin. Gas and soot radiate outward from the muzzle where contact is not complete. This causes an eccentric pattern of soot with two zones. The inner zone, most distinguishable, is a blackened seared area of the skin or fur having a pear, circular, or oval shape. The larger outside zone is shaped like a fan of light gray soot. All or the majority of the inner zone will be on the opposite side of the wound from the muzzle, pointing in the direction the gun was fired. Incomplete-contact wounds are similar to angled-contact wounds. The muzzle of the gun is in contact with the skin; however, the surface of the body may not be completely flat, causing a space between the end of the barrel and the skin. Soot and gas escape from this space causing an area of seared skin or fur [7].

As a weapon moves from contact to varying distances away from a target, the deposition of soot or powder residue will change, but accurate interpretation of these differences may require additional analysis using scanning electron microscopy or spectrometry or other techniques. A ballistics expert will consider the appearance of an entrance wound in light of known variations in types of weapons and ammunition, and may also need to test fire a specific weapon to determine the distance from which a weapon was fired.

Near-contact wounds are created when the firearm is not in contact with the skin but is held a very short distance away. The distance is so small that most of the particles escaping cannot disperse and they mark the skin. In near-contact wounds, the entrance wound is surrounded by a zone of powder soot covering seared and blackened skin or fur, lacking muzzle imprint. The zone of searing is larger than that observed in a loose-contact wound. The soot is embedded in the skin of the seared zone. In near-contact angled wounds, soot radiates outward from the end of the barrel creating two zones similar to angled-contact wounds. In a near-contact angled wound, the majority of the blackened seared zone is on the same side of the barrel pointing toward the weapon. The direction of fire is in the direction opposite of the searing.

The transition from near-contact to intermediate-distance wounds is subtle. Intermediate-range wounds occur when the muzzle of the weapon is held at a distance away from the body when fired yet is close enough that the powder grains discharged with the projectile cause “powder tattooing or stippling” of the skin. Powder tattooing involves both embedding of gunpowder particles in the skin and hemorrhage. The hemorrhage associated with tattooing appears as numerous reddish-brown to orange-red punctate lesions surrounding an entry wound. The distribution around the wound is either symmetrical or eccentric depending on the angle that the gun was fired. Powder tattooing is an antemortem event, thus its presence indicates that an animal was alive when it was shot. The distance for an intermediate range wound from a handgun is generally greater than 1.0 cm and can be over 1.0 m depending on the weapon fired [7]. These wounds can assist in determining direction of fire based on tissue beveling and angle of an abrasion ring or soot marks.

At ranges greater than intermediate range, neither soot nor powder tattooing is present near the entrance wound, and the only marks are those produced by the projectile perforating the skin. Wounds inflicted from these longer ranges may be referred to as distance-range wounds or, more recently, as “indeterminate range.”

With shotgun wounds, as the muzzle-to-animal separation distance increases, the diameter of the pellet pattern will increase. At extreme distances, 41–100 m, pellets separate creating individual injuries or striking surrounding inanimate objects. If the angle of a shotgun discharge is perpendicular to the body, the pellet pattern is usually circular, while an angled discharge will cause an eccentric pattern. In both instances, the diameter of the pattern is range dependent [7, 8].

7.17 Examination of the Wound Tract

There are two main mechanisms by which projectiles damage tissue: laceration and/or crushing from direct contact with the projectile or projectile fragment and cavitation. By the first mechanism, tissue damage results from physical contact with the projectile or fragments and occurs directly along the wound path. Cavitation results from the transfer of kinetic energy along the path of the projectile. This energy transfer forms a larger temporary wound cavity by outward expansion with stretching and tearing of tissue along the trajectory. Pulsating collapse of the temporary cavity results in the formation of a permanent cavity that, depending on the elasticity of the tissue involved, may be considerably wider than the projectile. Projectile velocity has the greatest influence on the cavity formed, but the varying ability or tendency of a given projectile to fragment, or to partially or completely flatten (mushroom) on impact, in combination with caliber and the presence and design of an outer jacket, also significantly affect wound capacity [15, 16].

When projectiles have passed completely through the body or have been fragmented, the

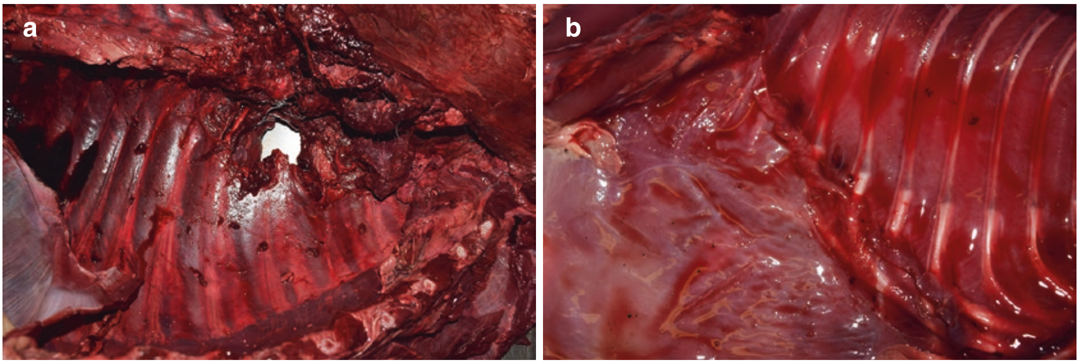

Fig. 7.13 (**a**, **b**) Ribcage from a gray wolf with a high-velocity rifle wound (**a**) and a domestic cat with a low-velocity (air rifle) wound (**b**). Note the large, cavernous wound tract resulting from the high-velocity firearm as compared to the small holes caused by the air rifle

character of the permanent wound tract can still be used to determine the general type of weapon involved. High velocity gunshot, due to the amount of kinetic energy involved and fragmentation of the bullet, tends to result in larger paths of destruction. Comminuted bone fractures and/or pulpifaction of soft tissues along the bullet path are suggestive of a high-velocity firearm such as a rifle. Conversely, damage limited directly along the path of the projectile is more consistent with low-velocity weapons such as handguns, air rifles, and arrows (Fig. 7.13). A notable exception is a shotgun fired at close range, which creates a wide swath of damage due to the impact of the dense group of shot pellets.

Tissue characteristics influence the degree of damage a projectile causes. Thicker soft tissue, such as the liver and brain, and dense rigid tissue such as bone offer greater resistance, increasing the energy transfer. Elastic tissues that can deform on impact, such as the lung or muscle, are more resistant to damage, particularly that caused by cavitation. Therefore, tissue such as the intestine may display only contusions, whereas organs such as the liver may be severely lacerated [11, 15]. Bone fractures are further dependent on the type of bone and the angle of impact. The cancellous bone is softer, enhancing its ability to absorb energy, resulting in less fragmentation. The cortical bone, due to its greater density, tends to fracture and fragment.

If a projectile hits the skull, intracranial increase of pressure may contribute to concentric fractures that form a target-shaped arrangement of fracture lines. When a bullet makes contact with bone, there are deceleration, deformation, and potential fragmentation or tumbling effects on the projectile and the bone. Bone fragments can act as additional projectiles and can influence the direction and range of the original projectile [7, 15]. The nature of the tissue also influences drag on the projectile's terminal ballistics, altering the velocity it travels through the tissue. While the shape of the frontal surface of a projectile largely determines the size of the cavity, current measurement techniques are too variable to allow postmortem caliber derivation [7]. Advances in the use and evaluation of computed tomography to characterize cavity dimensions have the potential to improve measurement accuracy such that cavity dimension measurement may contribute data useful for weapon and projectile identification in the future [17].

Histopathology of gunshot cases is useful to identify or rule out complicating factors such as disease but is of little value in diagnosis of the actual gunshot injury. At this time, histologic evaluation for the purposes of determining range of fire or trajectory is not recommended. Soot and gunpowder may be visible on H&E-stained sections of the wound, but findings are not specific and other wound tract debris may appear similar [18]. The actual wounds are microscopically consistent with mechanical thermal injury but, again, are not specific. Embolization, particularly of shot pellets and other small projectiles, can cause death in the absence of an otherwise fatal wound. Emboli of bone or soft tissue may also be noted on histologic examination.

If the victim survives the initial injury, only to succumb later to secondary infection or debilitation, the cause of death is the underlying (also known as proximate) injury. In cases where the animal is received injured with no history, radiographic evidence of metal fragments can be invaluable in determining the underlying cause of the wounds.

Lead poisoning secondary to retention of lead fragments or shot pellets has not been demonstrated in wildlife [19, 20]. Ingestion of shot pellets licked off of superficial injuries (or conceivably from another nonfatal shot to the stomach) could potentially cause lead toxicity.

7.18 Comparison of Gunshot and Arrow Wounds

Broadhead arrows have a classic y-shaped or star-shaped entry wound (Fig. 7.14) that may only be appreciated by reopposing the skin edges. Field tips are conical and can be difficult to differentiate from other low-velocity projectiles [21]. The absence of metal in the wound tract is suggestive of arrow injury; however, a through-and-through bullet wound could not be ruled out. Chemical spot tests of the wound surface have reportedly been a helpful diagnostic aid as would other residue tests that test for the presence of GSR and metal material present in ammunition on the surface in question [22]. If an animal is presented with an arrow present in the wound, the investigator should not assume that the cause of death was an arrow wound. A thorough necropsy is still warranted as perpetrators claiming legal harvest of game during arrow season may place an arrow in a gunshot wound to obfuscate.

7.19 Angles and Trajectories

Defining a projectile path or trajectory is a critical component in the reconstruction of crime scenes. Trajectories can give information on the location of the shooter, including distance from the victim, identify intermediate objects in the path, aid in the interpretation of the order of shots fired, and help to determine the weapon and ammunition used. Trajectory can also be used to corroborate or refute testimony provided by an assailant or witnesses. To establish a trajectory, the pathologist must first find the entrance wound

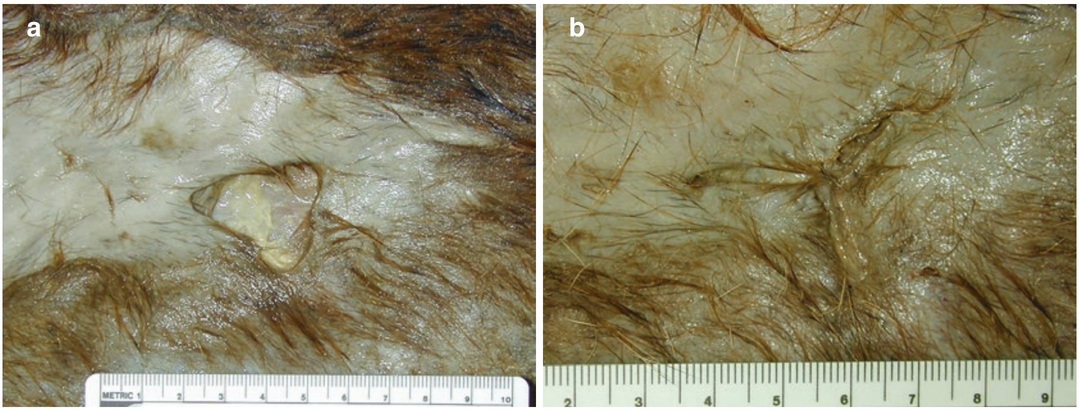

Fig. 7.14 (**a**, **b**) Florida panther (*Puma concolor coryi*), entrance wound from a broadhead arrow. (**a**) is the wound as presented. (**b**) shows the wound with the edges pushed together, revealing the "Y" shape of the arrowhead. In this case, a lack of hemorrhage around the wound is due to marked decomposition. Photo credit: Rhoda Ralston

or wounds. Measurements of the length and width of the bullet entrance wound are taken to establish the degree of the angle of the wound.

Trajectory rods can help in the visualization and characterization of projectile trajectories in some cases. Rods must be straight and can be made of wood, fiberglass, or plastic. Metal rods are also available, but they may cause scatter if being used with CT imaging. The animal can be placed in sternal recumbency or in the position in which it was found at the crime scene. The rods are meant to simulate the path of the projectile into and potentially through a body. Trajectory rods should be used with caution. If used incorrectly, a rod can manipulate valuable and relevant forensic evidence located in the wound canal and may cause an artificial canal. Placement of a trajectory rod and/or probing of a wound canal should only be done after exploration of the wound canal has taken place with thorough documentation. In addition, not all entry wounds lend themselves to placement of trajectory rods. For example, entry wounds in bone can be rigid enough to prevent placement of rods without physically altering the wound, and thus rods should not be used in these cases.

Once placed, photographs should be taken from the front, back, left, right, or bottom and from above the body. These directional photos contribute to the development of theories based on investigative findings and should be discussed with the rest of the forensic team. Additional techniques used with trajectory rods include the addition of a centering piece that can be placed first to ensure that the weight of the trajectory rod does not alter the angle of the wound. Once placed into the centering piece or directly into the wound, angles can be measured from the rods by centering a protractor against the middle of the entry wound. An angle finder is used to insure the protractors' level, and a plumb line is hung from the rod and bullet hole to determine horizontal and vertical angles. There are several types of trajectory kits that provide these tools (Kaleidoscope System Fusion Trajectory Kit®, Evi-Paq® Trajectory Kit).

Lasers can be used in conjunction with rods to improve the examiner's understanding of trajectory. Once a trajectory rod has been placed, and measurements taken if so desired, a laser can be attached to the rod and the light followed to an area of probability for the origin of the shot. Photographic fog can be sprayed over the laser beam to assist in visualizing the light beam and to improve photographic evidence. Lasers can also be mounted on a tripod in the area of probability and shown into transparent trajectory rods to establish the likely location of the shooter.

The trajectory techniques described above can be very helpful, but they are not uniformly applicable. Physical proportions, normal range of motion, and differences in locomotion for the species under investigation can influence likely paths of trajectory and should also be considered. Current concepts in trajectory analysis are largely drawn from two sources, individual case analysis in humans and research studies, many of which were conducted by the military in pigs and dogs [15, 23]. The studies conducted on dogs are most applicable to veterinary forensic trajectory analysis, but often the weapons and ammunition used in these studies are not consistent with that encountered in a nonmilitary context [24]. Ballistics data available from human civilian case studies tends to better represent the types of weapons and ammunition seen in veterinary forensic pathology. While data on projectile trajectory analysis in veterinary species is limited, there are some physical commonalities that support extrapolation of data across species. For example, during steady motion, there is universality to animal patterns and the extent to which anatomical parts bend [25]. For this reason, one should be able to factor in relative proportionality and predict rough bending patterns and maximum angles using data across species.

Repeatability and reliability of a technique should be considered when producing discoverable evidence in a legal case, and the production of trajectory angles may be the most controversial technique introduced in this review. Angles obtained using rods are dependent on positioning of the animal. The exact position of the victim prior to death is often unknown and can be particularly difficult to determine if a projectile penetrates a victim while the victim is in motion, if there is evidence of intermediary wounds, or fragmentation of the projectile. Additionally, angles may be inaccurate or differ depending on who acquired them. The practicality and relevance of the information that could be gained from trajectory rods and angles should be considered on a case-by-case basis and in consultation with the forensic team.

7.20 The Necropsy Report

The postmortem report should include the number, appearance, and locations of all entrance and exit wounds, as well as some description of the path of the projectile (trajectory), the injuries produced, and the site of lodgment if a projectile failed to exit the body. It should also include the description of any retrieved projectile. Retrieval of all projectile segments should be done with gloved hands, plastic instruments, or forceps with rubber tubing to prevent damage of forensic evidence.

7.21 Projectiles as Evidence: Collection and Storage

The following are best practices for handling projectile evidence. Once a projectile is recovered, it should be examined for the presence of tissue or markings. The projectile can be gently cleaned for examination and photography and prepared for cytological analysis by placing it in a test tube with sterile saline. Once the debris has been rinsed off, place it on a surgical towel to dry. The saline from the container can then be forwarded to law enforcement agency CSI personnel for cytological analysis. Projectile cytology may be necessary if the projectile went through something or someone prior to entry into the animal under examination [7]. Once cleaned and dried, the projectile should be photographed. Identification, date, measurement, police report number, and location of recovery should be included in these images. Finally, the projectile should be measured and weighed before storing in wax paper in a dry match box-type container with identifying information.

Projectiles are considered evidence; thus, they require an intact chain of custody. If a projectile, or any other evidence obtained during a postmortem investigation is given directly to an officer or investigator, a date and signature in an evidence log will maintain this chain. The movement of evidence should also be documented in your report, including the name and identity number of the officer or investigator who received the evidence. While evidence is in the possession of the pathologist, it must be stored in a secure location.

7.22 Advanced Techniques in Gunpowder Analysis

The main component of modern gunpowder is nitrated cellulose. When a weapon is fired, particles of unburned, partially burnt, and burnt gunpowder containing nitrite and nitrate compounds are expelled from the barrel. With the multiple textures and colors of animal fur, it can be difficult to visualize a residue pattern with the naked eye. Using a wavelength of 455 nm or the crime scene search (CSS) range (485–530 nm), an alternative light source can be used to examine the area around the wound for any adhering particles. It is crucial to document any observed patterns before an area is shaved for examination.

Infrared photography can be beneficial in demonstrating the nitric acid present in ammunition on the fur of an animal. This may help distinguish a residue pattern, which can assist in determining the distance from which a projectile has been fired. Digital cameras can be enhanced with an infrared lens attachment to facilitate taking these types of entrance wound pictures. Ballistics experts can also use microscopy of fur from entrance wounds to distinguish particles of burned and unburned gunpowder residue, or use chemical analysis, called a Griess test, to detect organic nitrite residue left on hair or other materials [8, 26].

Two additional tests may help to identify GSR. A presumptive, rapid test for GSR identifies the presence of nitrocellulose surrounding a gunshot entrance wound (Dr. Rachel Touroo, Forensic

Director of ASPCA, University of Florida). This test uses a spray or a sponge device applied to the area around the suspected entry wound and, if positive, results in an immediate color change. This may be particularly helpful when a projectile wound is suspected, based on the wounds observed, but is not supported with radiologic or physical projectile evidence. If a positive result is obtained, more aggressive confirmatory testing can be pursued in the lab. The sodium rhodizonate test is used to detect the presence of lead around the wound. Lead can be found in the smoke, primer, and projectiles themselves. The presence of particles of lead is a random occurrence involving a variety of variables such as leading, metal fouling, or a dirty gun barrel [8]. A possible disadvantage to both tests is that they result in a color change to the surrounding fur and tissue, which may not be desirable. Additional nondestructive methods are currently being developed for the detection of nitrites and lead on haired skin [27]. These tests should be discussed with local law enforcement for possible application and use, especially with through-and-through wounds.

Conclusion

Deciding whether or not to accept any form of forensic pathology case, projectile injury or otherwise, can be challenging for veterinarians and veterinary pathologists. Understanding what additional tasks this form of postmortem examination may require, and what expectations exist beyond the typical diagnostic postmortem report, is an important part of the decision-making process. This chapter was intended to provide background information on the nature of projectile injury and describe the examination technique to clarify the additional steps and expertise required by medicolegal cases involving projectile injury. We hope that it has also conveyed the necessity for establishing relationships with an extended team of forensic experts and that it will spark interest among veterinary anatomic pathologists to look deeper into the subject and to begin taking on these challenging and rewarding cases.

Legal Note The findings and conclusions in this chapter are those of the author and do not necessarily represent the views of the US Fish and Wildlife Service.

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8

Strangulation, Suffocation, and Asphyxia

Beverly J. McEwen

8.1 Introduction

Asphyxia, derived from anciet Greek “asphuxia,” literally translates as “stopping of the pulse,” yet, since the mid-eighteenth century, the term was transformed to imply a lack of oxygen resulting in death [1]. In the forensic context, asphyxia refers to death by rapid cerebral anoxia or hypoxia due to accidental or non-accidental causes [2, 3]. Accidental or non-accidental suffocation, strangulation, and mechanical asphyxia in animals are described in a small number of textbooks [4–7], case reports [8–10], and reviews [3]. In contrast, there is a plethora of literature on asphyxia in the human medical forensic literature in textbooks [11–16], reviews, case series, and case reports. In people, the incidence of various types of asphyxial deaths are well documented in registers such as the Centers for Disease Control (CDC) [17]. At present, the true frequency of deaths in animals due to drowning and non-drowning asphyxia is not known, although statistics from one website (pet-abuse.com) indicates they comprise about 2.8% of their reported cases of animal cruelty [18]. In 2016, the Federal Bureau of Investigation (FBI) began collecting data on acts of animal cruelty that should eventually provide detailed and accurate data on the frequency of asphyxia-related deaths in animals reported to police in the National Incident-Based Reporting System (NIBRS) [19].

Deprivation of oxidative respiration results in cellular injury or death and asphyxia is fundamentally an acute lack of sufficient oxygen delivery to the brain [2]. Oxygenation of blood requires that there is adequate [20, 21]:

- Environmental oxygen (21% oxygen in air)
- Effective ventilation
- Oxygen and carbon dioxide exchange across the alveolar capillaries
- Percentage of hemoglobin in an arterial blood is occupied by oxygen molecules (SaO_2)
- Partial pressure of dissolved oxygen in the blood (PaO_2)
- Hemoglobin concentration and integrity
- Delivery of oxygen to tissues
- Release of oxygen into the tissues
- Utilization of oxygen by cells

Mechanisms that interfere with any individual or combinations of the above oxygen uptake, transport, or delivery functions, if severe enough, will be lethal [20]. Hypoxia indicates reduced oxygen and anoxia designates oxygen depletion [14, 20]. Hypoxemia causes reduced oxygen delivery to tissues and therefore cellular hypoxia, degeneration, and death. Glycolytic energy production can occur in hypoxic states, but is abrogated with ischemia as the substrates for

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glycolysis do not reach the tissues [22] and waste products are not removed [23].

In companion animals, an SaO_2 of 91–100% reflects a normal PaO_2 of 80–100 mmHg [23, 24]. By definition, hypoxemia is an SaO_2 of less than 95% or a PaO_2 of less than 80 mmHg [25]. Serious hypoxemia occurs at an SaO_2 less than 90%, or a PaO_2 less than than 60 mmHg [26, 27]. According to Swann, when the SaO_2 concentration is 12% or less, dogs survive only 2–5 min [28]. Irreversible cerebral damage occurs in dogs when the arterial oxygen drops below 20–23 mmHg and the cerebral blood flow is below 10 mL/100 g/min [23, 29]. In contrast, equine hemoglobin has a higher affinity for oxygen, so that 90% SaO_2 saturation is achieved at a PaO_2 of only 54 mmHg, indicating that horses are more tolerant of low PaO_2 values [23, 27].

8.2 Classification and Terminology

Asphyxia is a mechanism of death rather than a cause of death [2, 3, 16] although most classifications of asphyxia are structured on the initial circumstances rather than the mechanism. Different classification schemes of asphyxia abound in medical pathology [11–14, 30–32] and any scheme may be used, although efforts to standardize the classification of asphyxia are proposed [30, 31]. Figure 8.1 provides a classification of asphyxia [3] based upon that proposed by Sauvageau and Boghossian [31] supplemented by associated pathophysiologic mechanisms [30, 33] and underlying reasons for cerebral anoxia or hypoxia [2, 30, 33].

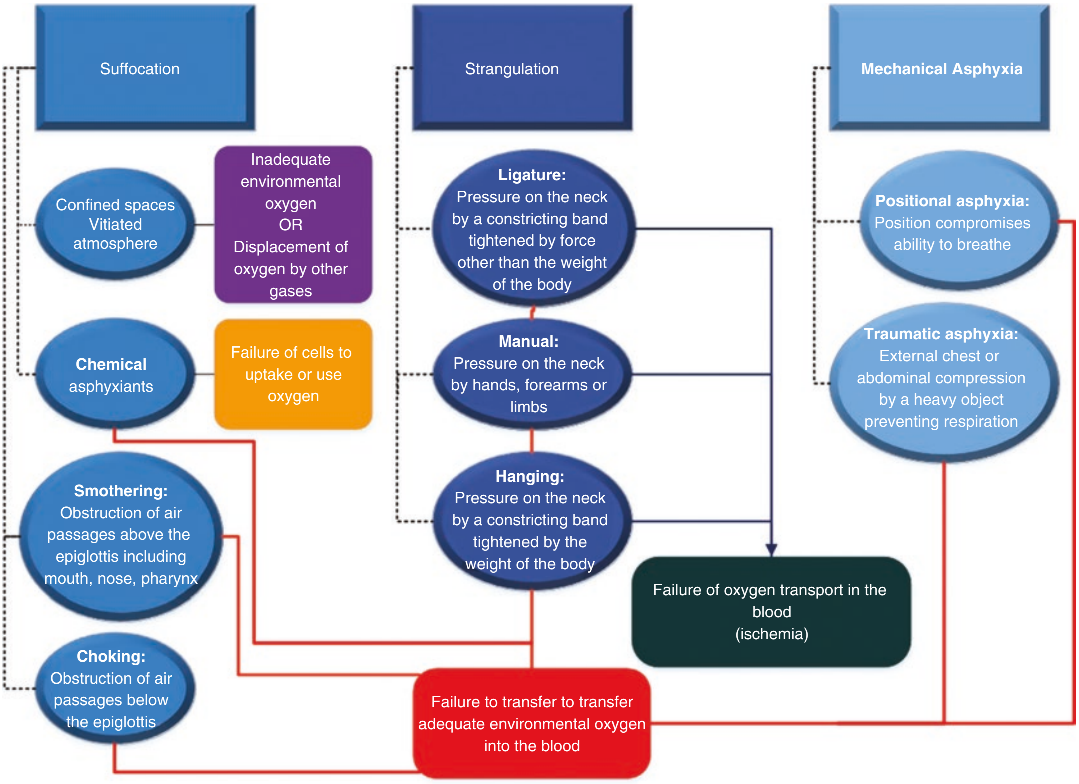

Fig. 8.1 Classification and definition of asphyxia

Table 8.1 Additional descriptive terms for specific trauma associated with asphyxia used in human forensic pathology but recommended to avoid in veterinary forensic pathology

Term	Description
Garroting	Historically, a form of Spanish judicial execution ligature strangulation where a stick, metal, or similar object is placed under the ligature and twisted
Burking	A combination of chest compression and smothering
Chokehold	Application of pressure to the neck by forearm or other cylindrical object and pulled back by the other hand
Carotid sleeper hold	Compression of the carotid arteries with an open airway
Throttling	Strangulation by hand or rarely by ligature
Gagging	Material fixed over the face or in the oral cavity to prevent breathing
Incaprettamento	Ligature strangulation with a person in a prone position, feet and wrists are bound together and then tied to a ligature around the neck

Definitions and descriptions of other terms relating to asphyxia used in medical forensic pathology are given in Table 8.1, but it is recommended to avoid many of them in veterinary and medical [31] forensic pathology as their usage is derived from specific contexts and potentially creates confusion.

8.3 Asphyxia: Traditional Misconceptions and Myths

A number of myths and misconceptions regarding the mechanisms and lesions of asphyxia propagated in the medical literature need to be addressed.

Myth 1: Cyanosis, fluidity of the blood, engorgement of the right ventricle, visceral congestion, and petechiae are classic tenets of death due to asphyxia [32, 34].

These five characteristics are now often referred to as the “obsolescent quintet” [32, 34]. Although neither the presence or absence of petechiae can confirm death due to asphyxia, of the five lesions, petechiae are the most compelling indicator of fatal neck compression [2, 32, 34, 35] because they indicate impaired venous return from the head. Compression of neck veins with a patent arterial supply ruptures postcapillary venules, producing petechial hemorrhages [2, 30, 34–36]. The development of petechiae in fatal neck compression depends on the duration, consistency, and intensity of the force applied to arteries and veins [3, 14, 32]. Petechiae are most commonly seen in the skin, conjunctiva, sclera, larynx, and oral mucosa [34, 37–41], but their presence is not pathognomonic for strangulation as they can develop with strenuous sneezing, coughing, vasculopathies, and clotting disorders [14, 20, 35, 42]. Knowing the disposition of the body at the death scene is critical to determining the significance of petechiae: if an animal was found in an inverted position, lividity will result in congestion and vibices which may mimic antemortem petechiae [35, 43]. If the arterial supply is completely occluded, then petechiae will not develop as the blood is prevented from entering the tissues. Therefore, the absence of petechiae does not preclude strangulation as a cause of death if both arterial and venous vascular supplies are obstructed. *Tardieu spots* are foci of extravased blood that develop during the perimortem or postmortem period and resemble antemortem petechiae or purpura [35, 43, 44]; the term is often used to describe scleral petechiae and to infer asphyxial death in medical forensic pathology [13, 35]. This phrase should be avoided in veterinary forensic pathology due to the inaccurate use in medicine and the implied causal inference [3].

Myth 2: Pulmonary edema is a pathognomonic feature of asphyxia.

Pulmonary edema with or without hemorrhage, atelectasis, or emphysema is described in animal and human victims of strangulation and suffocation [20, 28, 45–52] but also occurs with many other conditions. Some studies propose that pulmonary lesions in lungs from people that were strangled, drowned, aspirated, and/or suffocated or to people dying due to non-asphyxial causes can be discriminated by morphometric and semiquantitative histological parameters [52, 53]. These studies were done on relatively fresh specimens, and for such techniques to be a useful adjunct to histologic diagnosis of asphyxia in animals, additional research and validation are required.

Myth 3: Cardioinhibitory reflex cardiac arrest and vasovagal inhibition are confirmed mechanisms of death due to asphyxia.

According to Pollanen, vasovagal inhibition and cardioinhibitory reflex cardiac arrest (CiRCA) are "probably better viewed as part of the folklore of forensic pathology rather than a robust concept based on scientific evidence." [2] Notwithstanding, this is still a hotly debated topic in medical forensic pathology, as these mechanisms are often invoked especially when anatomic lesions are absent [2, 13, 41, 54–58]. Stimulation of the carotid sinus with activation of a baroreflex and subsequent circulatory collapse caused by a blow to the neck in people was not confirmed as a sole cause of death, in a systematic review of cases [57], nor was it observed in experiments on animals [59]. Clinically significant conditions such as sympathetic stimulation, drug abuse, or underlying cardiac disease were present in cases of suspected CiRCA, save one [57]. Similarly, stimulation of the vagus nerve by strangulation or foreign body esophageal occlusion causing bradycardia and cardiac asystole was invoked over 120 years ago as a mechanism of death; however, it was only hypothesized to occur [60] and lacks supporting scientific proof [2, 14].

There is, however, substantial evidence for significant involvement of the concurrent and physiologically opposing sympathetic and parasympathetic systems in the pathophysiology of asphyxia. Current theories of autonomic conflict between the sympathetic and parasympathetic responses, described as a "brainstorm which accelerates premature death of the heart and brain," are supported in experimental carbon dioxide-induced asphyxia in rats [61]. Bidirectional signals between the heart and brain intensify as cardiac function deteriorates resulting in autonomic cardiac toxicity. The autonomic effects are mitigated by removing the effects of the sympathetic but not the parasympathetic system [61]. A similar theory of autonomic conflict between parasympathetic and sympathetic responses in individuals with predisposing conditions and immersed in cold water is postulated to occur in some cases of drowning [62, 63].

8.4 Categories of Asphyxia

8.4.1 Strangulation

All forms of strangulation cause neck compression and death may be due to partial or complete vascular and/or airway obstruction, although the exact mechanism is still not completely understood [60, 64, 65]. Airway obstruction may occur by direct compression of the trachea or by dorsal displacement of the tongue or epiglottis to the posterior pharynx [58, 66] but death can occur without tracheal occlusion in people [31] and dogs [44, 67]. There is no consensus on the number and types of lesions with strangulation in people [68] as the duration, consistency, and type of applied force, the anatomic site of compression, and the structures that are occluded influence the physiologic responses, the time until death, and the lesions produced [58, 60, 64, 65]. The difference between ligature strangulation and hanging is that in hanging, the ligature is tightened by the weight of the body and, with ligature strangulation, the constricting band around the neck is tightened by force other than the body weight [31]. In hanging, the body can be suspended, partially suspended, or not suspended, and, with the head acting as a fulcrum, the ligature placement and angle of the head will affect the force needed to partially or totally occlude the vessels [58]. This distinction is significant in people as most (not all) hangings are suicidal or are due to autoerotic asphyxia, whereas non-accidental ligature strangulations and all manual strangulations are homicides [14]. Obviously in animals, hanging and ligature strangulations will be accidental or intentional.

During strangulation the larynx, trachea, jugular veins, common carotid, and vertebral arteries and their branches may be partially or totally occluded [31]. The pressures cited to occlude the neck vessels and trachea in people are 2 kg for the jugular veins, 5 kg for the carotid arteries, 15 kg for the trachea, and 30 kg for the vertebral arteries [14, 49]. These values were established over a 100 years ago on human cadavers [49, 60], and it is not known if they can be applied to living animals, but the relative pressures required to obstruct the trachea and vessels are likely applicable. More recent experiments on human cadavers reported that if the ligature knot is placed submentally, the pressure required to occlude the vertebral arteries is 7 kg [58]. Changes in the pressure applied to the neck by ligature or manual strangulation often results in intermittent blood flow to and from the brain, whereas complete obstruction of the arterial vascular supply is thought to occur with hanging in people [32].

Veterinary pathologists must be aware that anatomic variations in the blood supply to the brain in animals affect the degree and duration of physiologic responses to various types of asphyxia, especially strangulation, compared to people [3, 69]. Because dogs and cats have abundant collateral circulation to the brain, complete cerebral ischemia with hanging is unlikely [31, 60], and it is likely for this reason that they remain conscious longer and develop cardiac arrest later than that described in people. In the nineteenth century, Tardieu stated that: "*la resistance des animaux, aux diver genres d'asphyxie est plus grande que celle de l'homme*" (i.e., "the resistance of animals to various kinds of asphyxia is greater than that of man") [44].

In people the brain is supplied by the internal carotid and vertebral-basilar arterial systems [70, 71], whereas internal carotid arteries are absent or vestigial in cats and are small in dogs [71]. The numerous extracranial and intracranial anastomoses in dogs and cats create an extensive collateral cerebral circulation that makes them less susceptible to cerebral ischemia than people [3, 69]. Compression of the carotid arteries in people causes rapid unconsciousness [12], whereas ligation of both common carotid arteries in dogs, pigs, goats, and calves did not produce neurological deficits or even a behavioral change [72]. Dogs will even survive simultaneous ligation of the common carotid and vertebral arteries, and cerebral global ischemia is not produced [73–77] unless there is concurrent severe hypotension [75]. For example, permanent brain damage in people occurs within 4 min of total cerebral arterial occlusion [35] but takes 8 min in dogs [78]. Even after 6 min of cerebral ischemia, dogs eventually recovered to full functionality in one experiment [78].

Early experiments on non-anesthetized animals, while disturbing, provide some indication of the reaction of animals to strangulation [3, 5, 69]. Tardieu described experiments in which dogs died 12–20 min after total suspension, whereas a dog partially suspended, with paws able to touch the ground, was relatively quiet for the first 5 min at which time convulsions began and were followed by unconsciousness at 10 min and death at 28 min [44]. Tardieu described a tracheotomized dog that survived after 3 h of hanging, further supporting the difference between dogs and humans in their response to hanging [44]. A Mastiff experimentally strangled with a ligature did not become unconscious until 3.5 min: it was passive for the first 55 s, after which is behaved violently, throwing itself against a wall rolling, defecating, and urinating [44]. In anesthetized dogs, if the trachea is occluded regardless of vascular compromise, cardiac arrest occurs within 4–6 min [74, 78–84]. If only the vascular supply is occluded and the trachea is patent, cardiac arrest in animals takes up to twice as long as with concurrent tracheal obstruction [79, 83].

Pathologists may be asked about the physiologic, behavioral reactions and suffering or pain that occurs in asphyxiated animals. Physiological responses in anesthetized or non-anesthetized animals with experimental occlusion of the cerebral blood supply with or without tracheal occlusion are available in the literature [74–76, 79, 80, 83–85] and are recently reviewed [3, 69]. It is suggested that the literature on animal welfare, particularly on breathlessness or "air hunger" in animals, be consulted should such questions arise [86, 87].

8.4.1.1 Lesions of Strangulation

While specific lesions may be characteristic of strangulation, none are pathognomonic Table 8.2 [3, 5, 6, 9, 37, 40]. Pulmonary edema, congestion, emphysema, and atelectasis are reported, but these lesions are commonly observed in animals dying due to natural causes. Congestion of the cervix is reported in a dog due to lividity during vertical suspension of the body [37]. The presence or absence of inflammation and hemorrhage in the lungs, trachea, larynx, skin, and eyes should be documented in all cases of suspected strangulation [3, 5]. Lesions of acute respiratory distress syndrome are reported following strangulation [50, 88, 89] in those animals that do not die immediately. The temporal appearance of the histological lesions in the brain of animals with ischemic-hypoxic damage are thoroughly reviewed by Finnie [90, 91].

Reports of intentional and accidental strangulation including punitive training methods known as "helicoptering" where the dog is grabbed by the collar and is swung or lifted are described in the veterinary literature [5, 7, 9, 40]. Other than the ligature, the most relevant lesion in ligature strangulation is the ligature mark [92, 93]; however, the absence of a ligature mark does not rule out strangulation. The type and placement of the ligature, the duration of strangulation, and the characteristics of the animal's fur will impact the presence and appearance of lesions [5]. If the animal is submitted for necropsy with a ligature, in situ photographs at the scene are essential. Transport and handling of the body prior to the necropsy may displace the ligature, and the postmortem documentation may differ from that described at the scene. Swabs for DNA from the ligature, the oral cavity, and claws of the animal should be taken prior to its removal. If the ligature cannot be slipped over the head, then it should be cut, leaving the knot intact [13], placed in a paper evidence bag, and appropriately sealed. Ligatures can be towels, fabric, wire, cords, collars, leashes, ropes, chains, or other types of material that are used to encircle and constrict the neck. Shaving the neck often reveals ligature marks and abrasions that were not apparent due to the protective layer of the animals fur (Figs. 8.2 and 8.3). The ligature mark should be described as single or multiple, complete or incomplete, and the location on the neck and relationship to other lesions such as petechiae, congestion, or abrasions should be documented. The entire skin should be reflected

Table 8.2 Lesions of strangulation described in animals

Tissues or organs	Lesion
Skin and subcutaneous tissue	Ligature mark or furrow
	Subcutaneous ligature mark
	Contusions, abrasions
	Congestion
	Petechiae
Muscles of the neck	Contusions, hemorrhage, congestion
Laryngeal-hyoid apparatus	Laryngeal-hyoid fractures
Lungs	Congestion, edema, hemorrhage
	Multifocal atelectasis
	Emphysema
	Lesions of acute respiratory distress syndrome
Eyes	Scleral congestion
	Conjunctival petechiae

Fig. 8.2 Rope ligature around neck indenting the skin. Photograph courtesy of Dr. J. DeLay

in all cases of suspected traumatic animal abuse. The ligature mark may be more clearly visible in the subcutis than on the skin due to compression of the tissue (Figs. 8.4 and 8.5). Histologically, flattening of the epidermis and adnexal epithelium is described in ligature mark of rats killed by hanging, and those hanged following euthanasia, indicating that these lesions are not pathognomonic for an antemortem reaction [94].

Fig. 8.3 The ventral neck of the dog shown in Fig. 8.2 following ligature removal and shaving of the skin. The ligature created a deep furrow. Several small abrasions and a contusion were apparent after shaving the skin. Photograph courtesy of Dr. J. DeLay

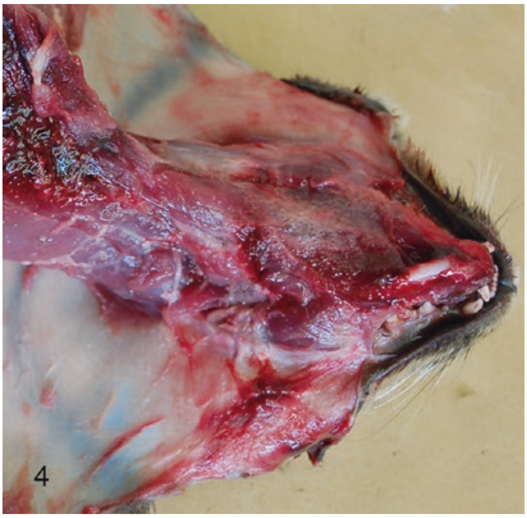

Fig. 8.5 The ventral neck of the same cat shown in Fig. 8.4. A circumferential discrete dark band caused by compression by the ligature is evident in the subcutis. The hemorrhage of the ventral neck muscles and mandible is due to blunt force trauma

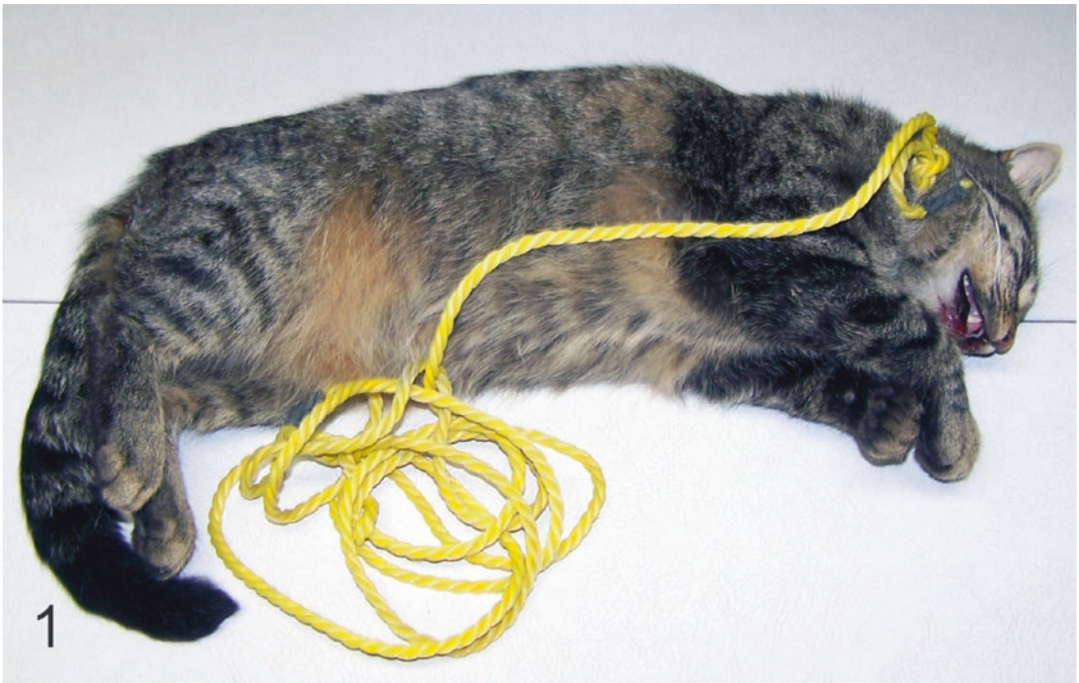

Fig. 8.4 A cat found with a knotted rope ligature

Positive immunohistochemical staining of the skin for proinflammatory cytokines such as interleukin-1β may eventually aid in determining the vitality of the changes at the ligature mark and wound age estimation [94].

External lesions and internal contusions or hemorrhage should be examined with reference to the significant anatomic structures of the neck including the trachea, laryngeal-hyoid apparatus, carotid arteries, and jugular veins. Detailed examples of neck dissection are available in the medical literature [95]. In animals, a layered dissection with careful removal of ventral neck muscles, exposing the larynx, trachea, carotid arteries, especially the carotid bifurcation, and jugular veins should be attempted in any suspected case of strangulation [3]. Intimal tears of carotid arteries are reported with strangulation [41, 66, 96, 97] (Fig. 8.6). Fractures of the laryngeal-hyoid bones and cartilages are reported in animals [9, 10, 37, 39, 98], and some may have a callus indicating prior injury [98]. Careful palpation, removal, and fixation of the larynx and proximal trachea en bloc enable additional examination by serial sections of these structures [3]. Microscopic hemorrhages and fractures may be present in the laryngeal-hyoid apparatus even if gross lesions are not present [99]. In dogs, the stylohyoid, thyrohyoid, and epihyoid bones ossify in utero, and the basihyoid and ceratohyoid bones ossify 1 and 2 months, respectively, following birth [77]. Considerable variation of the mineralization of the thyroid and cricoid cartilages is reported in dogs [100] occurring as early as 6–12 months of age [77].

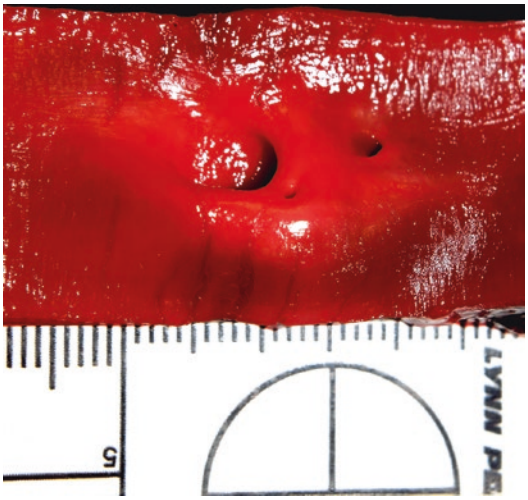

Fig. 8.6 Three horizontal intimal lacerations of the carotid artery from a horse that was moved following death by a wire neck ligature. Histologically, a vital reaction was not present in the artery

Pressure and release during manual and some types of ligature strangulation increases the likelihood of petechiae because the arterial blood supply may be intermittent and the extensive cerebral collateral circulation in dogs and cats likely mitigates complete arterial occlusion with complete venous occlusion [3]. Strangled animals may have localized or distant abrasions, contusions or other lesions of blunt force trauma, sexual abuse, and/or projectile wounds ([3, 37, 98]). Abrasions, lacerations, or contusions may be self-inflicted while struggling or may be caused by the perpetrator.

Vertebral fractures (hangman's fracture) frequently occur with judicial hanging as a result of the submental placement of the ligature, sudden drop, and suspension of the body [101]. Vertebral fractures are rare in suicidal hanging in people unless there are concurrent risk factors such as obesity, degenerative disease of the cervical spine, a sudden drop, and complete suspension of the body [14, 38]. The frequency of vertebral fractures in animals that are hanged is not known.

8.4.1.2 Case Report: Manual Strangulation [102][1]

During a violent domestic dispute, a Cairn/Wire terrier mixed breed, 13-week-old male dog was witnessed to be manually strangled and swung around multiple times. The witness observed the puppy gasping for breath for "several hours" before it died. It was buried in a shallow grave and was exhumed by a law enforcement officer after 2 days. Fractures, luxations, or subluxations were not present on orthogonal whole body radiographs. A 2.0 cm partially circumferential area of pallor on the neck was evident after the skin was shaved. A 7 × 3 mm laceration was

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[1] Case material courtesy of Dr. Nancy Bradley-Siemens, Midwestern University

present on the left dorsal aspect of the head. Irregular contusions were caudal to the left ear and left lateral neck at the ramus of the mandible. The dorsal lungs were diffusely red and sank in formalin. A small focal wedge shaped area of hemorrhage was present in the ventral caudal right lung lobe. Histologically, lesions in the lungs were moderately severe fibrinosuppurative interstitial pneumonia with hyaline membranes consistent with acute respiratory distress syndrome.

8.4.2 Suffocation

8.4.2.1 Obstructive Suffocation

Choking, smothering, inadequate environmental oxygen, and chemical asphyxiants are collectively classified as forms of suffocation [31]. Obstruction of the air passages above the epiglottis is smothering, whereas choking is defined as obstruction of air passages below the epiglottis (Fig. 8.1). Diagnosis of accidental obstructive asphyxia caused by inhalation or swallowing of objects such as food or toys that lodge in the trachea, in the larynx, or in the proximal esophagus is straightforward. Animals may have foreign objects inserted into their mouth or oral cavity (Fig. 8.7) or may have their muzzle taped shut. Objects in the oral cavity may obstruct the airway or displace the tongue which results in airway obstruction. Those made of absorbent material may become saturated with fluid or vomitus and become impermeable to air. This not only affects the ability to breathe, but may also decrease heat dissipation in animals that cannot sweat [24]. Obligate nasal breathers such as horses, rabbits, and small rodents [103] will suffocate if their nasal cavity is obstructed by foreign objects. One researcher has postulated that in horses, the bit of a bridle causes nasopharyngeal obstruction and asphyxia in racehorses by a variety of mechanisms; however, this hypothesis has not been proven and requires additional research [104].

Fig. 8.7 A cloth gag containing many sharp pins inserted into the oral cavity of a cat. Photograph courtesy of Dr. M. Spinato

Following experimental obstruction of the trachea in anesthetized dogs, cardiac arrest may occur within 4–6 min [79] but may take as long as 9–10 min [83]. In one experiment, all dogs with cardiac arrest could be resuscitated after 5 min of obstructive asphyxia, some between 5 and 10 min, and none after 10 min [105]. In 6–8-month-old anesthetized miniature pigs, cardiac arrest occurred between 13 and 20 min (16.8 ± 1.3 min) following clamping of an endotracheal tube [106].

8.4.2.2 Nonobstructive Suffocation

Nonobstructive, nonchemical suffocation may occur with an absolute decrease in environmental oxygen, replacement of oxygen by other gases such as carbon dioxide (CO_2), methane, or inert gases such as helium, nitrogen, or argon [107, 108]. Inert gases, classified as simple asphyxiants displace environmental oxygen resulting in hypoxia. Chemical asphyxiants such as carbon monoxide (CO), cyanide (CN), and hydrogen sulfide (H_2S) interfere with oxygen transport in mitochondria [108]. The physiologic effects of inhaled simple and chemical asphyxiants, currently or previously used for euthanasia of animals, are described in the American Veterinary Medical Association (AVMA) guidelines for euthanasia [87].

Nonobstructive Suffocation: Oxygen Depletion and Vitiated Atmosphere

Experiments to determine the effects of oxygen depletion on non-anesthetized dogs by breathing pure nitrogen [28] gradual oxygen depletion [109] or rebreathing air with CO_2 removed [110] resulted in death within 3.5–4 min, 11–35 min, and 16–26 min, respectively. Gasping or a tenfold increase in respiratory rate was reported in the dogs breathing pure nitrogen or rebreathing air [28, 110]. Dogs, however, are less susceptible to very low oxygen concentrations than people: dogs develop cardiac arrest between 9 and 21 min breathing oxygen concentrations less than 2.43%, whereas people will die in less than a minute if the oxygen concentration is 2–3% [111]. For euthanasia, pigs require at least 7 min of exposure to oxygen concentrations less than 2% [87].

Animals confined in airtight spaces will die due to depletion of environmental oxygen, and death may be accelerated by hyperthermia and heat stroke [3]. In contrast to obstructive asphyxia, cardiac arrest in dogs takes at least twice as long and up to 30 min with nonobstructive asphyxia by plastic bag suffocation [79] or in experiments where the inspired oxygen concentration is 2.43% [28, 110]. Animals may become entrapped in airtight containers or may have their heads lodged into cans [8] or cups and, if unable to extricate themselves, will suffocate.

Case Report: Death Due to Entrapment in Confined Space [3]

A young, pregnant cat, presumed to be a stray animal by the person who found it, was transported to an animal shelter by placing it in a 15 L airtight plastic container during the 60 min drive. Upon arrival at the shelter, the cat was dead and rigor mortis had not started to develop. The animal was received for postmortem the next day. Externally, the cat's claws were frayed. The lungs were diffusely congested, mildly edematous with focally extensive emphysema of the cranial lobes (Fig. 8.8).

Based upon data for oxygen consumption in the domestic cat [112], it was determined that the available oxygen would have been depleted within 70 min (Table 8.3). The calculations do not account for the increased physical exertion, increase in CO_2, and possible increased environmental temperature or hyperthermia, all or some of which would have likely decreased the survival time.

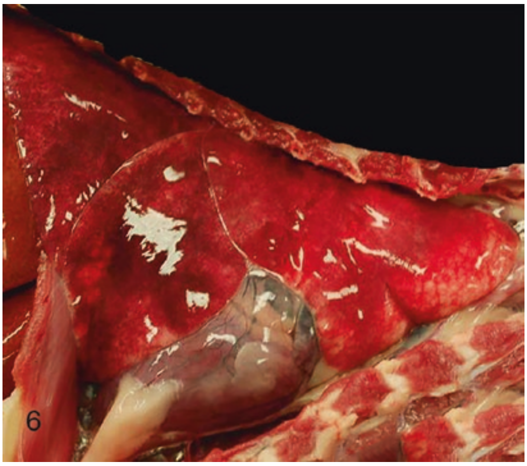

Fig. 8.8 Cat, thorax. The lungs are diffusely edematous, multifocally congested and emphysematous. This cat was placed in a 15-L airtight container and was dead when removed after an hour

Table 8.3 Calculation for oxygen consumption by a cat entrapped in a 15 L airtight plastic pail [3]

Parameter	Measurement
Volume of air	15,000 mL
Container air volume displaced by the cat	3000 mL
Air available in container	12,000 mL
Oxygen available in container (air = 21% O2)	2520 mL
Weight of cat	4900 g
Oxygen consumption, cat [112]	0.44 mL/g/h
Oxygen consumption/minute by entrapped cat	36 mL/min
Time to consume available oxygen	70 min

Carbon Dioxide Toxicity

Carbon dioxide (CO_2) is a product of tissue metabolism and acts as a simple asphyxiant by replacing environmental oxygen. CO_2 is a stable, odorless, colorless gas, normally present indoors at concentrations of less than 0.1% [113]. Toxic exposure often occurs in situations where it is released from combustion, fermentation, putrefaction, or dry ice [113], and nonfatal hypercarbia due to malfunction of an anesthetic machine is reported in dogs and cats [114]. The mechanism of CO_2 toxicity is thoroughly reviewed by Guais [113].

CO_2 is used for intentional euthanasia, and the maximum acceptable concentration of 0.08–3% depends upon the duration of exposure and rate of displacement of oxygen [87]. Above 80% CO_2, inhalation results in an isoelectric EEG within 30 s and respiratory arrest within 1 min in anesthetized dogs, but it takes 7–8 min to develop cardiac arrest with 80% CO_2 compared to 1 min with 100% CO_2 [115]. Inhaled CO_2 causes respiratory acidosis and is painful due to the formation of carbonic acid on mucous membranes of the respiratory tract and conjunctiva [87, 116]. It also causes breathlessness (air hunger) and induces a fear response due to its effect on the amygdala [87, 117]. If concentrations are gradually increased during euthanasia, the distress and aversive behavior may be diminished [87]. Animals and people will adapt to chronic, low-level continuous but not intermittent exposure to CO_2 [113, 116].

Chemical Asphyxiants

Asphyxiants may be toxic gases, liquids, or solids that can be inhaled, ingested, injected, or percutaneously absorbed inhalation [107]. Chemical asphyxiants either impair the transport of oxygen by hemoglobin or interferes with oxygen use at the tissue level by inhibiting cytochrome c oxidase and oxidative phosphorylation (azides, CO, cyanides, hydrogen sulfide, nitrous oxide [107, 118]). Due to their high metabolic rate, the brain and heart are the most frequently affected organs. Most azide toxicity occurs in people as a result of industrial or laboratory exposure or accidents [107] (Table 8.4).

Table 8.4 Systemic asphyxiants [107, 108]

Asphyxiant	Source
Azides (sodium azide, lead azide, hydrazoic azide, hydrazoic acid)	Car airbags
	Primers
	Shell detonators
	Broad spectrum biocides
Carbon monoxide	Car exhaust
	Propane and butane heaters
	Propane generators
	Methylene chloride (a paint stripper that is metabolized to CO)
	Produced when desflurane, enflurane, isoflurane are passed through dry absorbents containing a strong alkali (potassium or sodium hydroxide) [119]
Cyanides and cyanogens	Hydrogen cyanide
	Seeds of *Prunus* species (apricots, bitter almonds, choke cherries, peaches)
	Laetrile (sold in some health food stores as vitamin B17)
	Industrial solvents (acetonitrile, propionitrile)
	Fumigants (cyanogen bromide)
Smoke inhalation	Fire smoke contains many toxic products of combustion: CO, cyanide, CO_2, irritant gases, soot
Hydrogen sulfide	Decomposition of sulfur-containing organic material (compost pits, manure pits, sewers)
	Mixture of household chemicals and toilet bowl cleaners
Methemoglobin-inducing substances	Prescription and non-prescription drugs
	Chemicals (e.g., cocaine, copper sulfate, nitrous oxide, zinc phosphide etc.)

Carbon Monoxide

Carbon monoxide (CO) is an odorless, colorless gas produced by incomplete combustion of hydrocarbon fuels such as coal, peat, gasoline, propane, butane in engines, heaters, or generators. Other sources of CO include anesthetic gases desflurane, enflurane, and isoflurane when passed through dry absorbents containing a strong alkali (potassium or sodium hydroxide) [119], and inhaled fumes from the paint stripper, methylene chloride are metabolized in the liver to CO [120]. Smoke inhalation, one of the more common sources of CO and cyanide toxicity in animals, is reviewed in volume 2 Chap. 2 (thermal/electrical injuries).

CO directly competes with oxygen for hemoglobin binding sites and has a 200–300 times greater affinity for hemoglobin than does oxygen

[37, 107, 121]. Because of the rapid uptake with a T ½ of 50 s [122], even low concentrations and brief exposure can produce toxic levels of carboxyhemoglobin. CO binds to a variety of heme proteins, platelet heme, myoglobin, and mitochondrial cytochrome *c* oxidase, causing toxic effects beyond displacing oxygen from binding hemoglobin by [107, 121, 123]:

- Interrupting cellular respiration with production of reactive oxygen species (ROS).
- ROS causes neuronal necrosis and apoptosis.
- Dysfunctional cellular respiration invoking stress induced activation of hypoxia-inducible factor 1α, which results in neurologic or myocardial injury if the CO concentration is high.
- Pro-inflammatory effects by promoting platelet-neutrophil aggregation leading to neutrophil degranulation and release of myeloperoxidase and proteases.

Because of these manifold actions, the carboxyhemoglobin levels may not correlate with clinical signs or eventual outcome, [123] and unlike other asphyxiants, neurological lesions may be caused without prior unconsciousness. Survival time with carbon monoxide exposure is time and concentration dependent [87]. Dogs masked with 0.6% CO in air died within 20–30 min with 75–85% carboxyhemoglobin levels in blood [109]. Following 2 h of inhaling 0.18–0.22% CO in air, dogs convulsed and became unconscious although they did not die until 7–8 h following exposure [109]. At 1% CO in air, dogs were hyperpneic at 8 min and died between 16 and 26 min [124]. The American Veterinary Medical Association recommends concentrations of 4–6% of carbon monoxide for euthanasia of dogs although there may be vocalization and agitation prior to unconsciousness [87].

Carboxyhemoglobin levels in arterial or venous blood are required for a definitive diagnosis of CO toxicity and are remarkably stable in blood samples stored for weeks or months [125, 126]. The half-life of carboxyhemoglobin in blood depends upon the duration of exposure, the saturation of hemoglobin with CO [127], and, if treated, the concentration of oxygen given to patients [127–129]. Experimentally, the variation in the half-life of carboxyhemoglobin is likely due to biological differences and experimental protocols: the half-life of carboxyhemoglobin in dogs was 114 ± 42 min ventilated with air following CO exposure [122], whereas in pigs ventilated with 30% oxygen following inhalation of 1% CO for 10 min, the half-life was 60.5 min ± 4.7 [130].

Lesions of CO toxicity are due to a combination of hypoxia and direct toxic effects, and their presence may depend upon the survival time of the animal [121, 131]. The cherry red color of soft tissues with CO and HCN toxicity must be differentiated from freezing or refrigeration artifact [34, 37] although it may also be masked by advanced postmortem changes. The cherry red color of CO toxicity is stable and may be observed even in early stages of decomposition [41], whereas it is evanescent with HCN and therefore may be absent even in a relatively fresh animal [3, 37]. Initial lesions following acute exposure to CO are non-specific and may include variably intense vascular congestion, perineuronal, and perivascular edema of the cerebral cortex, corpus striatum, sensory, and visceral efferent centers [109]. Specific histologic lesions described with experimental and accidental CO toxicity in dogs and cats include neuronal necrosis of the palladium, substantia nigra, and cerebellum [131–133]. Demyelination, necrosis, and cavitation of the cerebral deep white matter are reported to occur experimentally in cats and dogs following a period of survival [109]. Myocardial coagulation necrosis is reported in cats [132] and people [121]. The distribution of the brain lesions in acute CO toxicity is thought to be related to the cerebral blood supply, direct cytotoxicity, hypotension, and hypoxia [132–134]. Histological evidence of smoke inhalation, burns with a vital reaction, and laryngeal edema will aid in determining if the animal was alive prior to the fire.

Cyanide Toxicity

Cyanide occurs in plants, fertilizers, pesticides, rubber, components of common household goods (plastics, melamine, upholstery), and as sodium or potassium salts used in mining and industry

[135]. Hydrogen cyanide (HCN), also known as prussic acid, is a colorless gas. Inhalation, ingestion, and contact are the routes of exposure which can be acute, subacute, or chronic [136]. Plant sources of cyanide include cassava, almonds, wild cherries, pits of peaches, apricots, choke cherries, and *Sorghum bicolor* [137] although over 300 species of plants may cause acute or chronic cyanogenic glucoside toxicity [136]. Livestock exposure is usually by ingestion of plants containing high levels of cyanogenic glucosides, and wildlife in particular may be exposed to NaCN which is a by-product of mining [138]. Chronic exposure to cyanide may cause hypothyroidism and cystitis ataxia toxidromes usually in livestock [136]. CO and hydrogen cyanide are significant chemical asphyxiants among the over 400 gases released in fires responsible for acute toxic effects [139]. The acute lethal dosage for most animals is approximately 2 mg/kg, and cyanogenic levels of ≥200 ppm in plants are dangerous [136]. Ingested cyanide is detoxified to thiocyanate by rhodanese, a mitochondrial enzyme; however, because dogs have lower rhodanese levels than most other species [135, 136], they are reportedly the species most sensitive to cyanide toxicity [135].

Cyanide completely disrupts cellular respiration by blocking the transport of electrons from cytochrome oxidase a_3 (a subunit of cytochrome c oxidase) to oxygen, preventing the mitochondria from using oxygen and therefore decreases ATP needed for cellular function [140]. Cells then switch to anaerobic respiration, resulting in lactic acidosis [122]. The half-life in dogs infused with potassium cyanide in one experiment was 129 min [122] and may not be detectable with toxicological analysis of blood due to its instability [14].

The onset of clinical signs is rapid, usually less than half an hour and survival is rarely more than 2 h [136]. Pathologists must be aware that at postmortem, cyanide can be absorbed percutaneously; the gas can be inhaled from stomach contents of the animal [37]. Clinical signs reported in animals include hyperpnea, dyspnea, tachycardia, salivation, muscle fasciculations, defecation, urination, lacrimation, convulsions, and vomition ([136, 141]). At postmortem, tissues are often cherry red due to oxyhemoglobin; however, this color dissipates quickly [5]. Pulmonary edema and marked necrosis of the gray matter were present in dogs exposed to 143–633 ppm of hydrogen cyanide for 2–10 min [141].

Hydrogen Sulfide

Hydrogen sulfide (H_2S) is an asphyxiant and mucosal irritant. H_2S binds to cytochrome c oxidase and inhibits cellular respiration at high concentrations (>20 μm) [118]. It is a component of "sewer gas," produced by fermentation of organic matter [14, 107] and even a small concentration of 1000–2000 ppm (0.1–0.2%) in air can be rapidly fatal. The rapid incapacitation of victims of H_2S toxicity is referred to as "knockdown," and frequently, those coming to their aid are also rapidly incapacitated [108]. It is a highly toxic gas, that is colorless, heavier than air, and characteristically smells like rotten eggs. Reports of H_2S toxicity in swine [142], poultry [143], and cattle [144] are usually due to exposure to recently agitated manure pit gas. Recumbency, convulsions, and rapid death occur [144, 145], although animals that survive may be blind and recumbent and cattle have ruminal atony [144]. H_2S is also a strong irritant of the mucous membranes of the eyes, respiratory tract, and skin [108, 145].

In animals dying acutely, lesions are non-specific including pulmonary hemorrhage, congestion, and edema [142, 145–147]. In people, gray-green discoloration of the viscera and brain [34, 148] are reported [142, 145] with acute toxicity, although this could possibly be due in part to the formation of sulfhemoglobin by bacteria during decomposition [146]. Laminar cortical necrosis of the cerebral cortex, gray matter of the cerebellum, and hippocampus were present in cattle that survived for 2 days following exposure [144].

Lesions of Suffocation

With the exception of choking on a large foreign object, gross and microscopic lesions of nonobstructive suffocation are non-specific and variable [3, 12, 14, 37, 149]. The body should be examined for tape or adhesives from tape, fibers, or other trace evidence that may have been used

Table 8.5 Lesions in suffocation are often not specific [3, 37, 151]

Tissue or organ	Lesion
Lungs	Emphysema, hemorrhage, congestion (Tardieu), edema
	Acute respiratory distress syndrome
Trachea	Possibly foreign material if aspirated or gagged
Soft tissues	Cherry red with CO and HCN toxicity
	Possible abrasions, contusions

to smother, muzzle, or choke the animal [3, 37]. If foreign objects are not present externally, there may be trace evidence in the air passages. Histological examination for hemorrhage, ischemia, necrosis, and embedded or aspirated foreign material is recommended. Abrasions and contusions may occur during restraint of the animal during obstructive suffocation or may be self-inflicted in attempts to escape in obstructive and nonobstructive suffocation. The claws should be examined for fraying, and swabs should be taken if DNA analysis is considered. Sand, soil, and gravel in the trachea and nasal cavity indicate inhalation, but if only present in the oral cavity then passive transfer of the material following death must be considered [3, 37].

Non-cardiogenic pulmonary edema occurs in dogs with partial, total, or intermittent airway obstruction [47, 50, 81]. In most cases of suffocation, pulmonary lesions are non-specific and may be various combinations of emphysema, hemorrhage, congestion, and edema [3, 37, 147, 150]. If death is not immediately fatal and depending upon the mode of suffocation, pulmonary lesions of respiratory distress syndrome [3] and neurological or myocardial lesions as described may also be present [131–133] (Table 8.5).

8.4.3 Mechanical Asphyxia

Mechanical asphyxia occurs when respiration is physically impeded. Traumatic asphyxia is due to external pressure on the chest, whereas positional or postural asphyxia develops if the animal is in a position that inhibits the ability to breathe and inhibits venous return to the heart [3]. In animals traumatic asphyxia may occur if animals are crowded and trampled [37, 152], trapped under heavy objects, or intentionally by physical violence or in sadistic videos [4, 153, 156].

For positional asphyxia to occur, three conditions must be met: the position of the body impedes normal gas exchange, the victim cannot move to another position, and other causes of natural or traumatic death are not involved [36, 154]. Animals trapped in a posture obstructing respiration by producing pressure on the thorax or narrowing of the airways will die if they are unable to extricate themselves due to the awkward position, traumatic injury, or prior incapacitation [3]. Positional asphyxia will also occur in an animal hanging from the hind feet in an inverted position, resulting in pressure on the respiratory tract from the abdominal organs and reduced cerebral circulation [154, 155]. Rabbits hung by the hindlimbs died within 17–44 h due to a combination of impairment of respiration due to thoracic pressure on the diaphragm from abdominal viscera and circulatory failure due to subsequent hypoxia [155]. In people, death is thought to be due to hypoxia and cardiac failure due to cardiac overload [36, 156, 157] and may take hours to days [14].

8.4.4 Lesions of Mechanical Asphyxia

The history and information from the scene, especially the position of the animal when found, are often required to contextualize the postmortem findings [3, 156, 158]. Lesions of positional asphyxia may include petechiae, ecchymoses, congestion, and edema directly proportional to the degree of venous obstruction, but may be absent if there is concurrent arterial occlusion anterior to the heart [36]. Accompanying traumatic lesions may be intentional or self-inflicted during struggling. *Masque ecchymotique* is a term used in medical forensic pathology to describe discoloration of the face, neck, and upper body due to mechanical asphyxia [156], features that may be difficult to see due to the animal's coat.

The skin should be reflected in order to determine if ligatures were placed around the hindlimbs in cases of suspected inversion. Fractures, laceration, contusions, abrasions, and internal hemorrhage may be present in cases of traumatic asphyxia [3], and retinal and tympanic hemorrhages, cerebral edema, pulmonary congestion, and petechiae are described in people [156].

8.5 Summary

Despite the plethora of literature available, medical pathologists are frequently in a quandary regarding the diagnosis of asphyxia-related deaths [34, 41, 43]. Animals and people are similar in that death due to the various mechanisms of asphyxia may or may not produce lesions, and, if present, the lesions are neither sensitive nor specific indicators of asphyxiation [2, 3, 20, 34, 37, 43]. Nonetheless, the presence and absence of lesions should be documented in all forensic necropsies: this is the evidence. The interpretation or opinion that the death was due to asphyxia requires definitive and compelling evidence from the postmortem, history, death scene, and/or body [2, 12, 20, 34, 158–160]. This is clearly evident from the case examples of manual strangulation and suffocation. Unlike medical examiners, veterinary pathologists rarely attend a death scene. Veterinary pathologists are often not provided the entire information from the scene investigators [161] and therefore may lack some of the crucial information required to confirm death due to an asphyxial mechanism. In cases of strangulation, there may be evidence of a ligature, contusions, abrasions, hemorrhage, and/or fracture of the laryngeal-hyoid apparatus. Petechiae particularly above an area of neck compression, requires explanation for the venous obstruction resulting in these lesions, especially if the animal was not found in an inverted position.

Beyond the necropsy, pathologists may be faced with questions of forensic importance that revolve around the behavioral and physiological responses in animals subjected to strangulation, suffocation, or traumatic asphyxia in order to determine if the animal suffered. Aversive behavior to noxious or painful stimuli are addressed in the increasing literature on animal welfare [86, 87, 162–164], and several reviews have specifically tackled the welfare implications of breathlessness in animals [86, 163]. How long does it take to become unconscious and develop respiratory and cardiac arrest and when does irreversible cerebral ischemia occur? People become unconscious during hanging within 8–18 s and develop irreversible brain damage within 4–6 min [38, 64]. While there is no prescriptive answer to these questions in animals, the peer-reviewed literature indicates that for some mechanisms of asphyxia [9, 28, 49, 69], consciousness is maintained for longer periods and the onset of death is often longer than in people.

Experimental data offers some insight into the pathophysiology of various mechanisms of asphyxia in animals [3, 70, 165], but does not provide the veterinary pathologist with sufficient or easily applicable information when confronted with these cases. The paucity of peer-reviewed literature in veterinary forensic pathology will hopefully be remedied in the future by the publication of case series, case reports, and registries describing macroscopic and microscopic lesions in cases of confirmed asphyxial deaths.

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