RHABDOVIRIDAE

It encompasses six genera of viruses that infect a broad range of hosts

- Mammals, birds, fish, insects and plants
- some of the family members being transmitted by arthropod vectors

The family contains

- Rabies
- vesicular stomatitis virus and bovine ephemeral fever viruses
- rhabdoviruses of fish

There are also many unclassified rhabdoviruses that infect cattle, pigs, kangaroos, wallabies, birds, and reptiles; however, the pathogenic significance of most of these viruses remains uncertain

PROPERTIES OF RHABDOVIRUSES

Classification

The family *Rhabdoviridae* includes four genera that contain animal viruses: the genera *Lyssavirus*, *Vesiculovirus*, *Ephemerovirus*, and *Novirhabdovirus*

Two additional genera include rhabdoviruses that exclusively infect plants:

- **the genera *Cytorhabdovirus* and *Nucleorhabdovirus***

Individual spp of rhabdovirus are distinguished genetically and serologically

LYSSA VIRUS

Aravan lyssavirus	Aravan virus (ARAV)
Australian bat lyssavirus	Australian bat lyssavirus (ABLV)
Bokeloh bat lyssavirus	Bokeloh bat lyssavirus (BBLV)
Duvenhage lyssavirus	Duvenhage virus (DUVV)
European bat 1 lyssavirus	European bat lyssavirus 1 (EBLV-1)
European bat 2 lyssavirus	European bat lyssavirus 2 (EBLV-2)
Ikoma lyssavirus	Ikoma lyssavirus (IKOV)
Irkut lyssavirus	Irkut virus (IRKV)
Khujand lyssavirus	Khujand virus (KHUV)
Lagos bat lyssavirus	Lagos bat virus (LBV)
Mokola lyssavirus	Mokola virus (MOKV)
Rabies lyssavirus	rabies virus (RABV)
Shimoni bat lyssavirus	Shimoni bat virus (SHIBV)
West Caucasian bat lyssavirus	West Caucasian bat virus (WCBV)

NEGATIVE STAINED TRANSMISSION ELECTRON MICROSCOPE IMAGE OF RABIES VIRUS

Each of these viruses is capable of causing rabies-like disease in animals and humans

Certain terrestrial mammals are reservoir hosts of rabies virus, and bats are potential reservoirs

The genus *Vesiculovirus* includes vesicular stomatitis Indiana and vesicular stomatitis New Jersey viruses.

The genus *Ephemerovirus* contains bovine ephemeral fever virus and other serologically distinct viruses that also infect cattle but are not pathogenic

The genus *Novirhabdovirus* contains the important fish pathogens, infectious hematopoietic necrosis virus and viral hemorrhagic septicemia virus

VIRION PROPERTIES

Rhabdovirus virions are approximately 45–100nm in diameter and 100–430nm long

Consist of a helically coiled cylindrical nucleocapsid surrounded by an envelope with large (5–10nm in length) glycoprotein spikes

The precise cylindrical form of the nucleocapsid is what gives the viruses their distinctive bullet or conical shape

The genome is a single molecule of linear, negative sense, ssRNA, 11–15kb in size

Rabies virus **encode five genes in the order *3'-N-P-M-G-L-5':***

- **N is the nucleoprotein gene that encodes the major component of the viral nucleocapsid**
- **P is a cofactor of the viral polymerase**
- **M is an inner virion protein that facilitates virion budding by binding to the nucleocapsid and to the cytoplasmic domain of the glycoprotein**
- **G is the glycoprotein that forms trimers make up the virion surface spikes**
- **L is the RdRp functions in transcription and RNA replication**

The glycoprotein (G) contains neutralizing epitopes, which are targets of vaccine-induced immunity; it and the nucleoprotein include epitopes involved in cell-mediated immunity

Virions also contain lipids, their composition reflecting the composition of host-cell membranes, and carbohydrates as side chains on the glycoprotein

Rhabdoviruses are relatively stable in the environment, especially when the pH is alkaline but are thermolabile and sensitive to the UV irradiation of sunlight

Rabies and vesicular stomatitis viruses are inactivated readily by detergent-based disinfectants and iodine-containing preparations

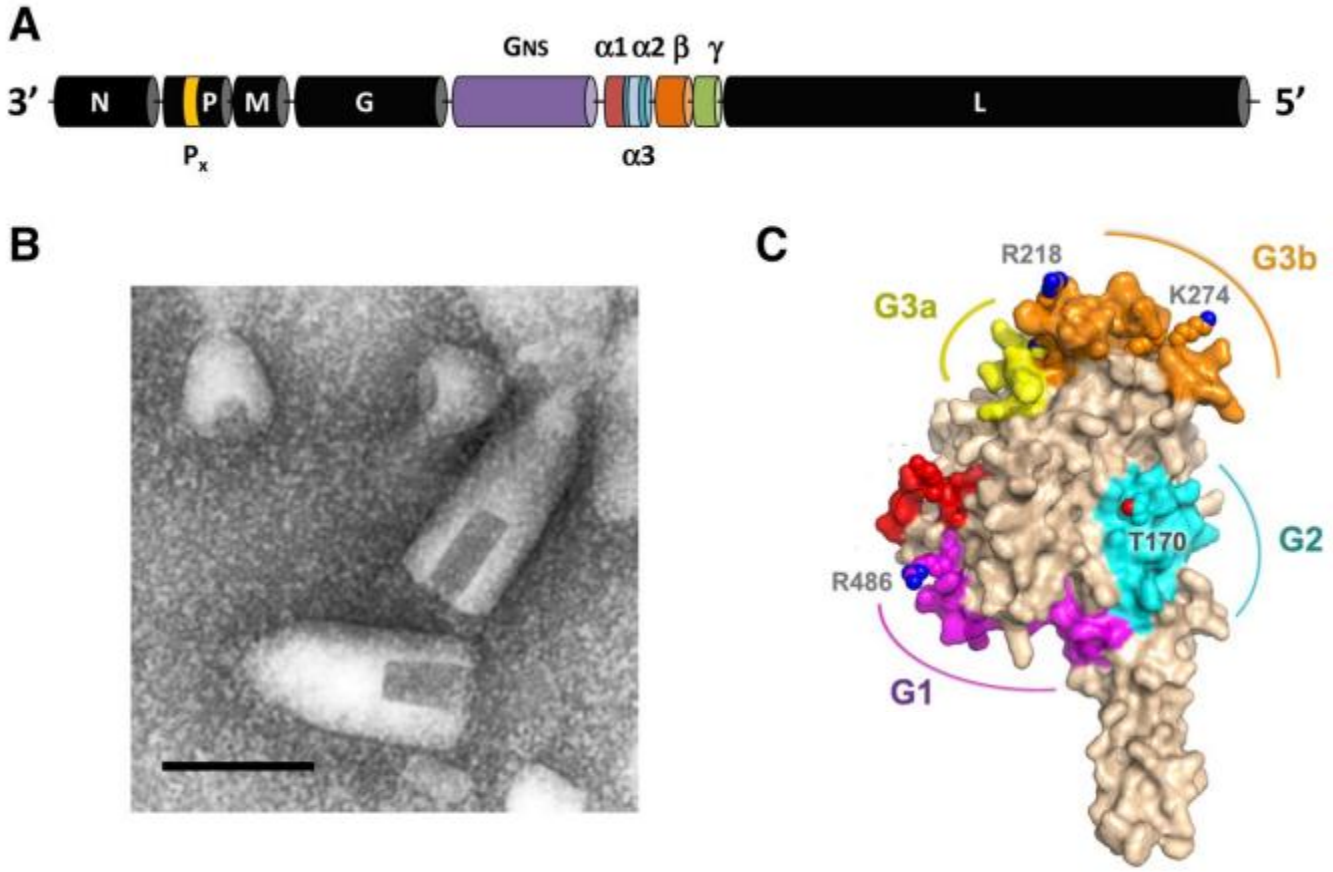


Fig: A. Structural organization of the 14.9 kb BEFV genome shown as arranged in negative sense. Structural protein genes (N, P, M, G and L) are shown in black and the various accessory genes are colored

B Transmission electron micrograph showing BEFV virions and defective-interfering (DI) particles

C. Structural model of a monomeric subunit of the BEFV G protein

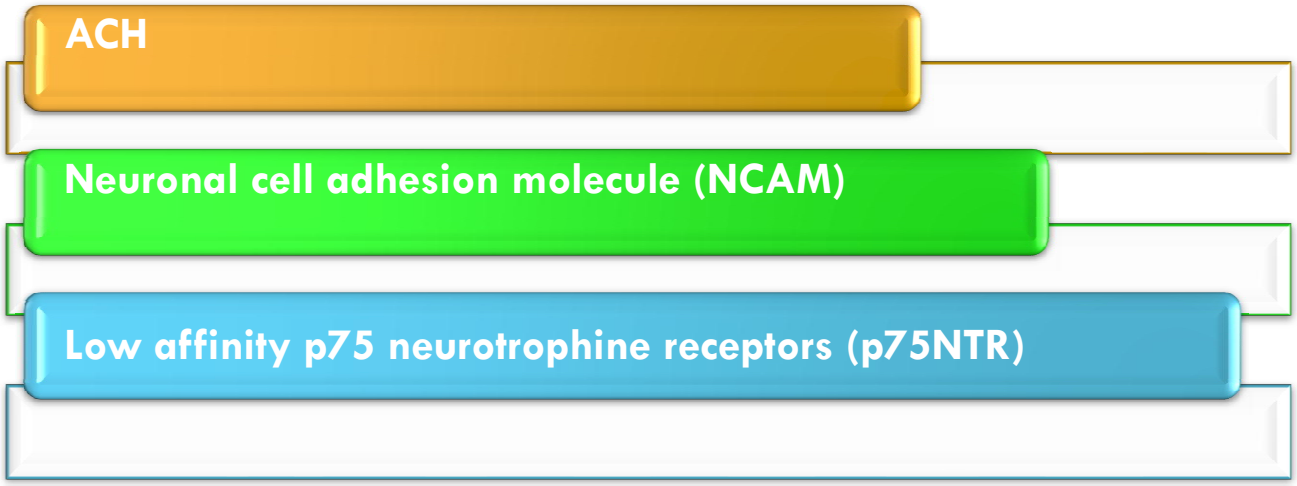
- **three major neutralization sites (G1, G2 and G3a/b) and amino acid residues**

VIRUS REPLICATION

Virus entry into host cells occurs by receptor-mediated endocytosis via coated pits and subsequent pH-dependent fusion of the viral envelope with the endosomal membrane releases the viral nucleocapsid into the cytoplasm

- replication exclusively occurs in the cytoplasm
- The viral gp-G is solely responsible for receptor recognition and cell entry
- Specific cell receptors have not clearly been identified for individual rhabdoviruses

Rabies Receptors



- ✓ **Phosphatidyl choline is a proposed receptor for vesicular stomatitis virus**

- ❖ **Fibronectin for viral hemorrhagic septicemia virus**

Replication first involves mRNA transcription from the genomic RNA via the virion polymerase

When sufficient quantities of the nucleocapsid (N) and phosphoprotein (P) have been expressed, there is a switch from transcription of mRNA to positive-sense antigenomes, which then serve as the template for synthesis of negative-stranded, genomic RNA

Using virion RNA as a template, the viral transcriptase transcribes five subgenomic mRNA species

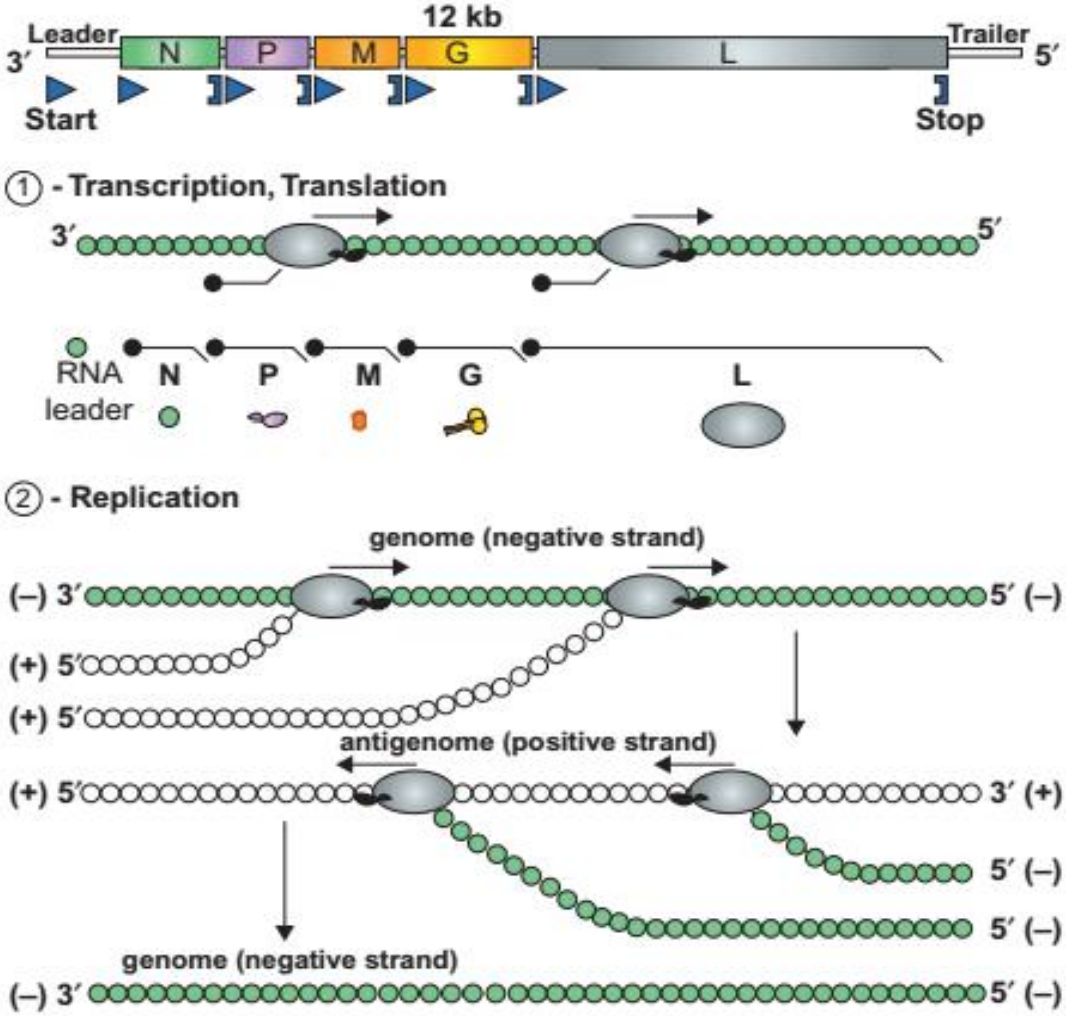


FIG. GENOME OF VESICULAR STOMATITIS VIRUS.
1. MODE OF TRANSCRIPTION
2. REPLICATION

KEY

G GLYCOPROTEIN
N NUCLEOCAPSID
P PHOSPHOPROTEIN
M MATRIX PROTEIN
L RNA POLYMERASE

RABIES VIRUS

infect all mammals and infection results in death

Death due to rabies is responsible for 1.74 million people throughout the globe

Other Lyssaviruses complicate the concept of "Rabies Free", infect human and animals in Britain and Australia)

Infection acquired from bites of rabid animals

Epidemiologic investigations shown spill-over events (cross-species transmission)

Incubation period 14-90 days (dog); Human 2-7 years

Cat upto 2 years

There are two clinical forms of rabies

1. Furious rabies
2. Dumb (Paralytic) rabies

   Hydrophobia due to pharyngeal paralysis

   Death due to paralysis of respiratory muscles

PATHOGENESIS

The course of clinical sign affected by

- dose and genotype of virus
- the location and severity of the bite
- the species of animal involved

Bite ➡ Virus multiply initially in the neuro-muscular junction (Nicotinic ACH and Neural cell adhesion Molecule NCAM) ➡ Skeletal Muscle (Virus attach to nicotinic ACH ➡ Retrograde axonal migration to CNS ➡ Replicate in the motor neuron of the SC and Dorsal root ganglia and rapid ascent to brain ➡ Infection of brain neurons ➡ Centrifugal spread along nerves to salivary glands, skin, and other organs

DIAGNOSIS, PREVENTION AND CONTROL

Negri bodies in the neurons

If rabies is suspected, the suspect animal killed and brain tissue collected for testing (medulla, cerebellum, and hippocampus)

Post-mortem diagnosis by FAT or IFAT

RT-PCR

Rabies viral proteins are highly immunogenic; numerous vaccines have been developed for human and animals

infectious rabies virus is susceptible to antibody-mediated neutralization and clearance during this early stage of infection E.g. Pasteurian post-exposure vaccination and Hyperimmune globulin

During long incubation period Hyperimmune globulin delay between the initial virus replication in muscle cells and the entry of virus into the nervous system

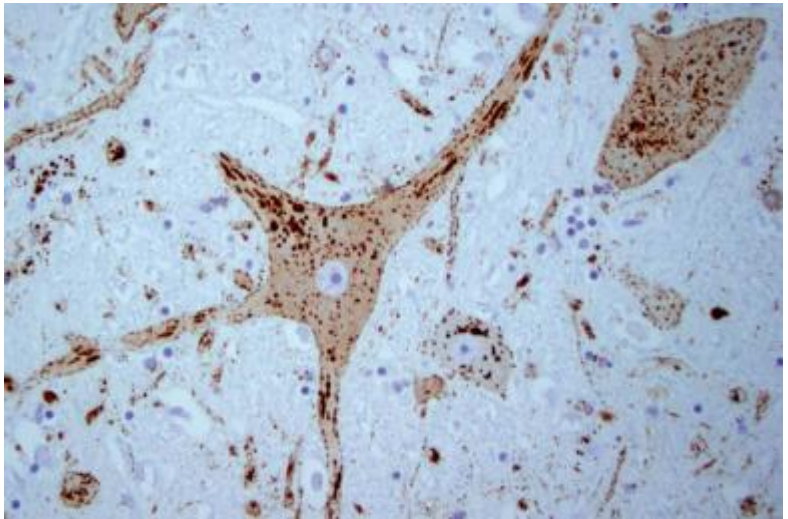
DIAG...

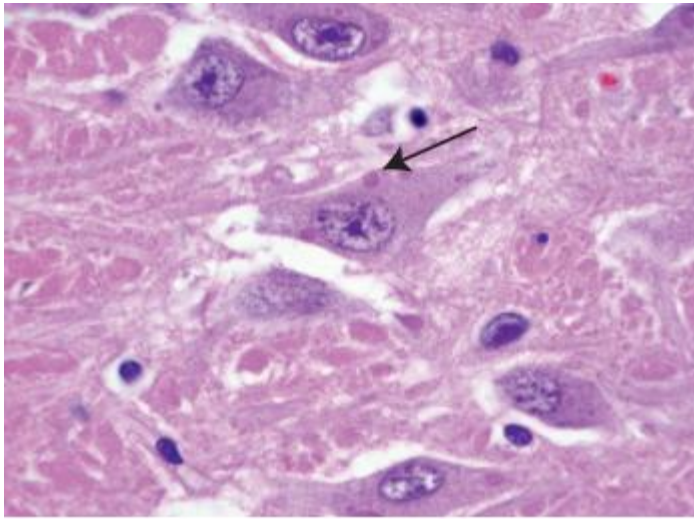
Inactivated, live-attenuated and recombinant vaccines

nervous tissue containing inactivated virus cause immunologically mediated nervous disease in some individuals

Original live-attenuated vaccines that are propagated in embryonated duck eggs also induce allergic reactions in some immunized individuals

Recombinant vaccina virus vaccine (Express G) used to immunized wild animals as a bit





OTHER LYSSAVIRUSES

Duvenhage, Lagos bat and Mokola viruses circulate in Africa

European bat lyssaviruses 1 and 2

Australian bat lyssavirus (black flying fox, fruit bat, *Pteropus alecto* and insectivorous bat (*Saccolaimus flaviventrus*)

BOVINE EPHEMERAL FEVER VIRUS

3-day stiff-sickness

An arthropod-transmitted disease of cattle and water buffalo that spans tropical and subtropical zones of Africa, Australia, the ME and Asia

Clinical signs are characteristics, but all are not seen in individual animal

Sudden onset of biphasic or polyphasic fever

Immediate drop in milk production

Other clinical signs: associated with the second and later febrile phases; these include depression, stiffness, and lameness, and less often nasal and ocular discharges, cessation of rumination, constipation, and abortion

Morbidity rates often approach 100%, and the mortality rate in an outbreak is usually very low (less than 1%), but on occasion it can reach 10–20% in mature, well-conditioned beef cattle and high-producing dairy cattle

Subclinical cases: their relative rate is unknown because antibody testing is confounded by intercurrent infections in the same areas by related but non-pathogenic rhabdoviruses

Clinical disease is restricted to domestic cattle and buffalo

Seasonal disease in surge with arthropod vector population

Potential vectors include culicine and anopheline mosquitoes, and possibly *Culicoides* midges

Both enzootic and epizootic spread is limited by the distribution of appropriate vectors

Pathogenesis is complex and probably reflects pathophysiologic and immunologic effects mediated by the release and activity of various inflammatory mediators

Injury to the endothelial lining of small blood vessels is central to the expression of bovine ephemeral fever, but there is no evidence that the virus causes widespread tissue destruction

early neutrophilia with an abnormal level of immature neutrophils in the circulation ("left shift")

There is an increase in plasma fibrinogen and a significant decrease in plasma calcium

Therapeutically, there is a dramatic response to antiinflammatory drugs, and often to calcium infusion

Gross (macroscopic) lesions include serofibrinous polyserositis and synovitis, pulmonary and lymph node edema, and focal necrosis of selected muscles

DIAGNOSIS, PREVENTION AND CONTROL

Clinical signs and Epidemiological presentation

Lab dx hampered by lack of gold standard

Virus isolation by blind passage in mosquito cell line or suckling mouse brain

RT-PCR

ELISA, IFAT, AGID

Prevention by vector control is impractical in the areas of the world where this disease is prevalent

Infection results in solid, long-lasting immunity

recombinant baculovirus-expressed G protein vaccine

PICORNAVIRIDAE

played an important role in the respective histories of virology in both human and veterinary medicine

In 1897, Loeffler and Frosch showed that FMD was caused by a filterable agent

Polioviruses, which are classified in the genus *Enterovirus*, and other picornaviruses were involved in key devp'ts of virology

- including the growth of viruses in cell culture, quantitative plaque assays, infectious clones of specific viruses, X-ray crystallographic analysis of virion structure at the atomic level, RNA replication, and viral protein synthesis

Genus	Virus	Spp affected and diseases
Aphtovirus	FMDv	Cattle, sheep, goat, swine and wild angulates
	Equine rhinitis A virus	Equine, camelides Systemic with respiratory sign
	Bovine rhinitis B virus	Cattle Mild respiratory diseas
Cardiovirus	Encephalomyocarditis virus	Rodents, swine, elephants, primates, mammals in contact with rodents Encephalomyelitis and myocarditis
	Theiler's mouse encephalomyelitis virus	Mice Murine polyomyelitis
Enterovirus	Human enteroviruses A, B, C, D	Human Aseptic meningitis, poliomyelitis, myocarditis
	Human rhinovirus A, B, C	Human Respiratory disease
	Swine vesicular disease virus	Swine Vesicular disease
	Bovine enterovirus	Cattle Mild enteric and respiratory disease
	Procine enterovirus B	Swine Asymptomatic infection
Erbovirus	Equine rhinitis B virus	Horses Mild enteritis
Teschovirus	Porcine teschovirus 1 (porcine enterovirus 1)	Swine Polioencephalomyelitis
Tremovirus	Avian encephalomyelitis virus	Chicken Emcephalomyelitis

PROPERTIES OF PICORNAVIRUSES CLASSIFICATION

The family is divided currently into eight genera: *Aphthovirus*, *Enterovirus*, *Teschovirus*, *Cardiovirus*, *Erbovirus*, *Kobuvirus*, *Hepatovirus*, and *Parechovirus* but it is likely that the number of genera will soon increase to 13

The former genus *Rhinovirus* was abolished in 2006, and the member rhinoviruses (99 serotypes of human rhinovirus and two of bovine rhinovirus) allocated to the genus *Enterovirus*

An important difference between viruses in the various genera is their stability at low pH; such differences were utilized in the classification

Specifically, the aphthoviruses are unstable below pH 7, whereas the enteroviruses, hepatoviruses, cardioviruses, and parechoviruses are stable at pH 3

All picornaviruses are positive-sense ssRNA viruses with a 5'-UTR

The RNA is uncapped, but does have a VPg covalently linked to the 5' end

There are major structural differences in the 5'-UTR among the genera: the length of the 5'-UTR in picornaviruses varies from ~500 to 1200 nucleotides and contains one of four different internal ribosome entry sites (IRES)

FMDV is also unique in having three similar, but not identical, VPg proteins that are present in equimolar amounts among the virion RNAs

Equine rhinitis A virus shares many genomic characteristics with FMDV, but its genome encodes only a single copy of the *VPg gene*

VIRION PROPERTIES

Picornavirus virions are non-enveloped, ~30nm in diameter, and have icosahedral symmetry

Virions appear smooth and round in outline in EM and in images reconstructed from X-ray crystallographic analyses

The genome consists of a single molecule of linear, positive-sense, ssRNA, 7–8.8kb in size

Both the 5' and 3' ends of the RNA contain untranslated regulatory sequences

The genomic RNA is polyadenylated at its 3' end and has a protein, VPg, linked covalently to its 5' end

Genomic RNA is infectious

The virions are constructed from 60 copies each of four capsid proteins, VP1 (1D), VP2 (1B), VP3 (1C), and VP4 (1A), and a single copy of the genome linked protein, VPg (3B)

VP1, VP2, and VP3 are structurally similar to one another, each being composed of a wedge-shaped

Amino acid substitutions correlating with antigenic variation occur in the surface-oriented loop regions

FMDV have a comparatively smooth surface, with no canyon structure

The attachment site for host-cell receptors is located on VP1, within the G–H loop

These sites have serotype and subtype antigenic specificities that differ among the various strains of FMDV

The stability of picornaviruses to environmental conditions is importnat in the epidemiology of the diseases they cause, and in the selection of methods of disinfection

- if protected by mucus or feces and shielded from strong sunlight, most picornaviruses are relatively heat stable at usual ambient temp

Some enteroviruses, for instance, may survive for several days, and often weeks, in feces

Aerosols of aphthoviruses are less stable, but under conditions of high humidity they may remain viable for several hours

Because of differences in their pH stability, only certain disinfectants are suitable for use against each virus;

- sodium carbonate (washing soda) is effective against FMDV, but is not effective against swine vesicular disease virus

VIRUS REPLICATION

Poliovirus, which in nature only infects humans and nonhuman primates, has been the principal model for studying the replication of RNA viruses

This model served as the basis for analyzing the replication pattern for all other picornaviruses, and deviations from this model have provided support for the continuing re-classification of the picornaviruses

The cellular receptors for many picornaviruses are known, and are surprisingly diverse

The receptors for polioviruses, coxsackie B viruses, and some human rhinoviruses are members of the Ig–superfamily

For other picornaviruses, many other cell surface molecules serve as receptors and co-receptors, including heparan sulfate, low-density lipoproteins, immunoglobulin superfamily and integrins

FMD virus can use two d/t receptors, depending on the passage history of the individual virus strain

- **Specifically, field strains of FMDV bind to integrins, whereas cell-culture-passaged virus can use heparan sulfate as a receptor, but this change in receptor specificity results in attenuation of the virus**

FMD viruses can also enter cells via Fc receptors if virions are complexed with non-neutralizing IgG molecules

FMD

Seven serotypes O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1

viruses occurred in most parts of the world, often causing extensive epizootics in domestic cattle and swine

Sheep and many species of wildlife are also susceptible

Mortality is typically low, but morbidity is high

In Cattle IP 2-8 days there is fever, loss of appetite, depression

marked decrease in milk production

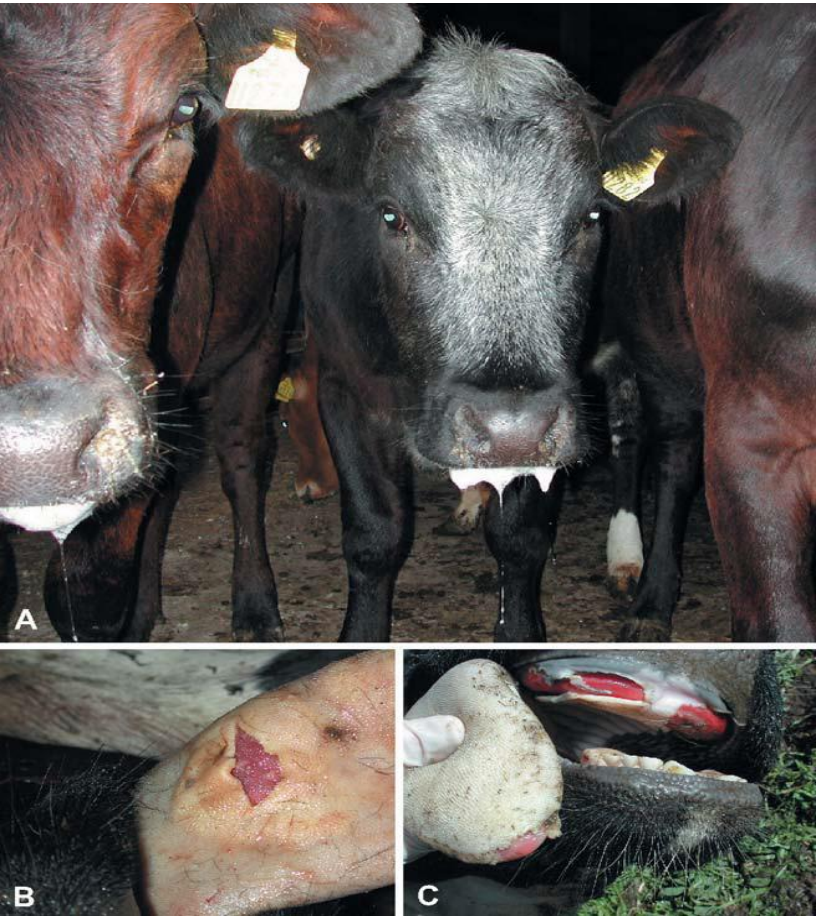
Drooling

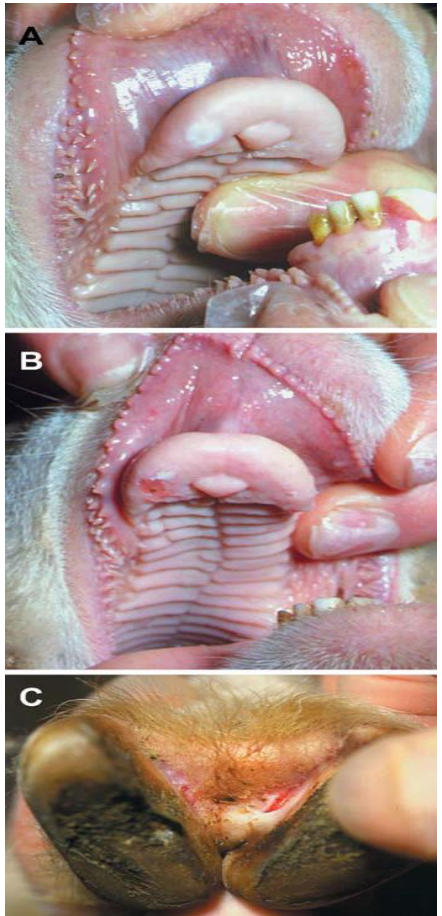
Vesicle development on the tongue and gums

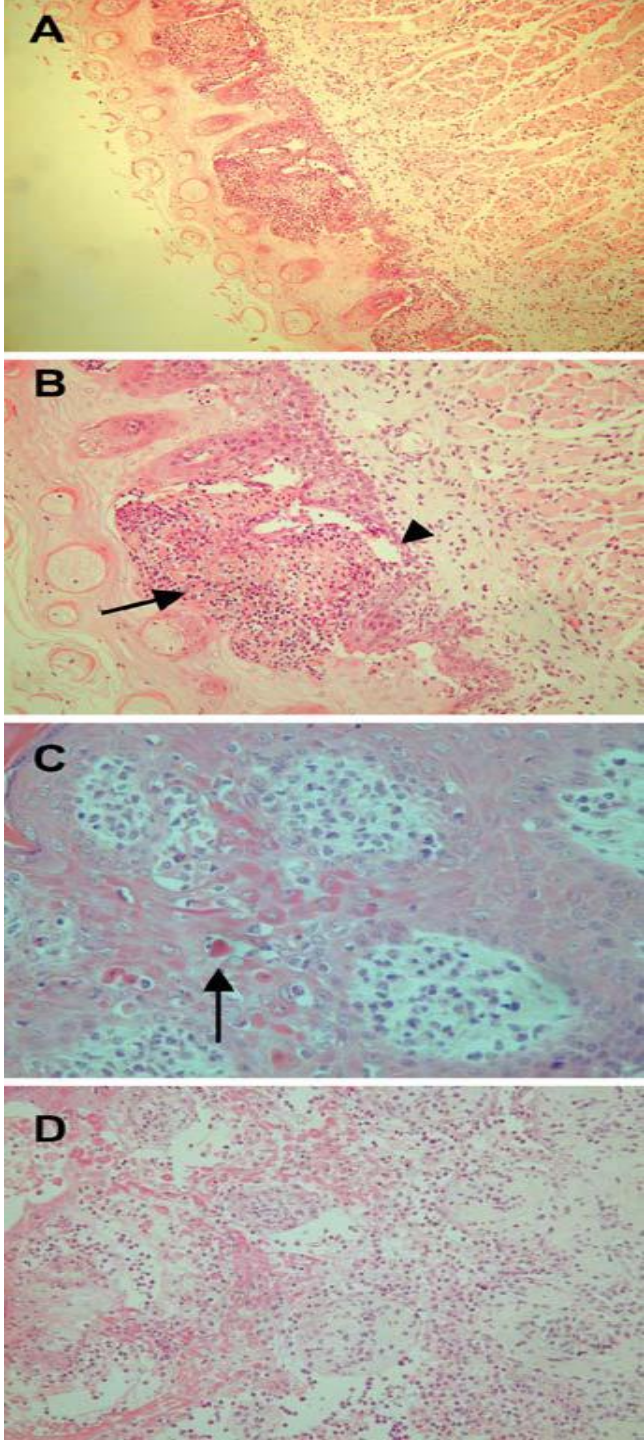
FIG.

Cattle and Sheep infected with FMDv serotype O

Dental pad and Coronary band skin after 3 Days PI







PARAMYXOVIRIDAE

Several of the most devastating diseases of animals and humans are caused by members of the family *Paramyxoviridae*.

- Rinderpest, canine distemper, Newcastle disease, measles and mumps

Other viruses in this family also cause disease in a wide variety of mammals, birds, & reptiles, amongst many examples:

- respiratory syncytial viruses in cattle, sheep, goats, and wildlife
- Sendai virus (murine parainfluenza virus 1) in mice
- avian rhinotracheitis virus (metapneumovirus) in turkeys and chickens
- phocine morbillivirus in seals
- genus *Henipavirus* that naturally infect various species of bats, but cause high mortality rates in infected humans and animals

PROPERTIES OF PARAMYXOVIRUSES

Classification

The family is subdivided into the subfamilies *Paramyxovirinae* and *Pneumovirinae*.

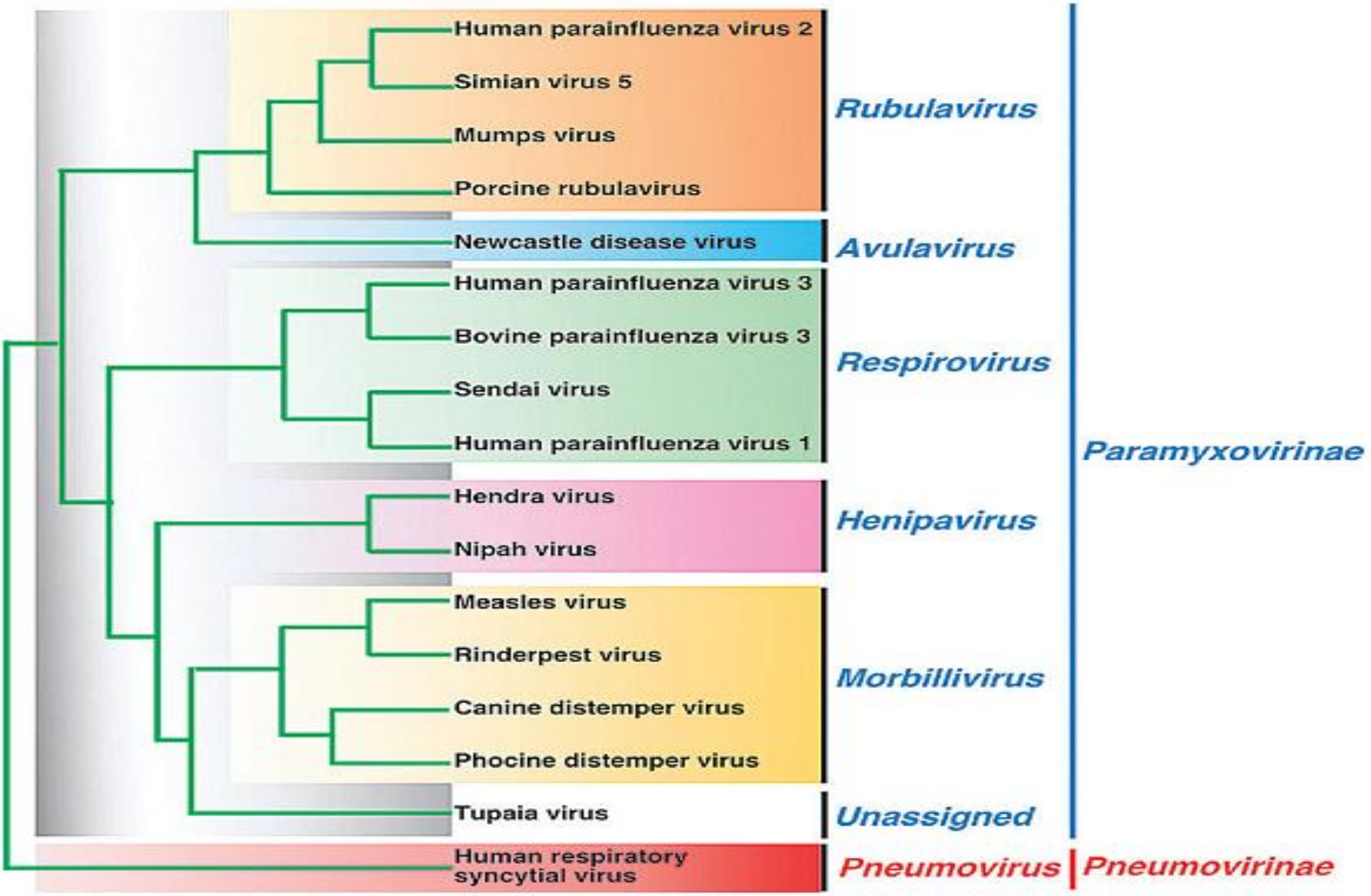
Paramyxovirinae* containing the genera *Respirovirus, Avulavirus, Henipavirus, Rubulavirus,* and *Morbillivirus

Pnemovirinae* contain the genera *Pneumovirus* and *Metapneumovirus

The family continues to expand rapidly as new viruses are discovered in wild animal populations, with a growing list of relatively uncharacterized viruses

Unassigned genera Fer-de-Lance and a variety of related ophidian paramyxoviruses of reptiles

PHYLOGENETIC RELATIONSHIPS THE FAMILY *PARAMYXOVIRIDAE*



VIRION PROPERTIES

pleomorphic (spherical as well as filamentous), 150–350nm in diameter

Enveloped, covered with large gp spikes (8–14nm in length), and contain a "herring-bone-shaped" helically symmetrical nucleocapsid, approximately 1µm in length and 18nm (*Paramyxovirinae)*

The genome consists of a single linear molecule of negative-sense, ssRNA, 13–19kb in size

The RNA does not contain a 5' cap and is not polyadenylated at the 3' end, but does have functional 5' and 3' non-coding elements

Three nucleocapsid proteins

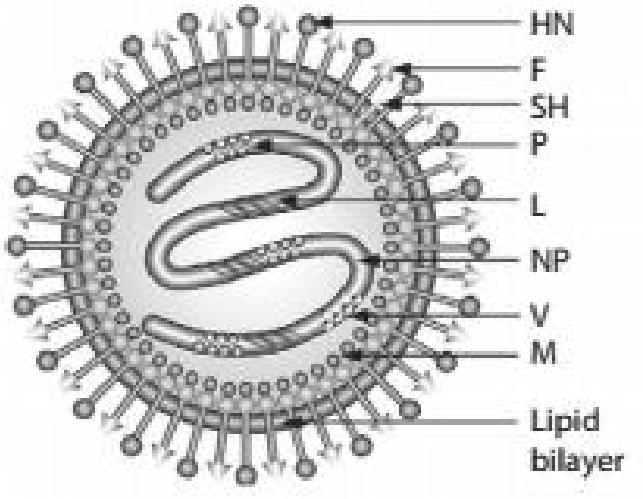
- an RNA-binding protein (N)
- a phosphoprotein (P)
- a large polymerase protein (L)

Three membrane proteins

- an unglycosylated matrix protein (M)
- two glycosylated envelope proteins a fusion protein (F) and an attachment protein (hemagglutinin (H), hemagglutinin-neuraminidase (HN) or glycoprotein G) hemagglutinating nor neuraminidase activities

Variably conserved proteins include

- non-structural proteins (C, NS1, NS2),
- a cysteine rich protein (V) that binds zinc
- a small integral membrane protein (SH)
- transcription factors M2-1 and M2-2



The envelope spikes of paramyxoviruses are composed of two glycoproteins:

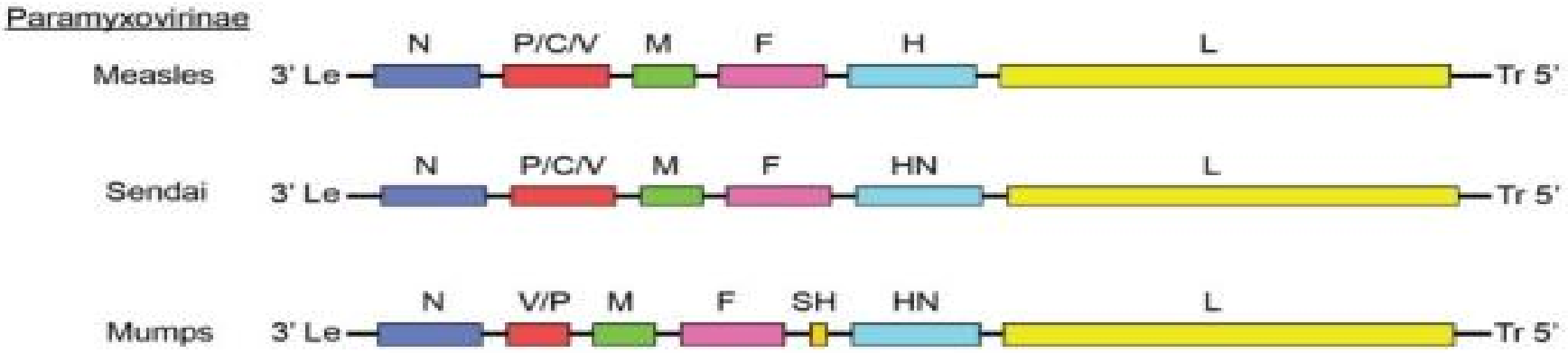
I. **Fusion protein (F) and HN (*Respirovirus*, *Avulavirus*, *Rubulavirus*), H (*Morbillivirus*), G (*Henipavirus*, *Pneumovirus*, *Metapneumovirus*)**

Envelope proteins have key roles in the pathogenesis of all paramyxovirus infections

One glycoprotein (HN, H, or G) is responsible for cell attachment

F protein mediates the fusion of the viral envelope with the plasma membrane of the host cell membrane fusion initiated by the paramyxovirus F protein not dependent upon a low pH environment

Neutralizing antibodies specific for the attachment glycoprotein (HN, H, or G) inhibit adsorption of virus to cellular receptors, but antibodies specific to F can also neutralize viral infectivity



Proteolytic cleavage by cellular proteases of Fo protein produces active F protein

Cleaved peptides remain in close proximity by virtue of linking disulfide bonds

The specific nature of the cleavage process and the characteristics of the F0 protein differ among viruses in the d/t genera

The paramyxoviruses can be crudely divided into two groups:

- those with a single basic amino acid at the cleavage site
- those with multiple basic amino acids at the cleavage site

The cleavage of F0 is essential for infectivity, and is a key determinant of pathogenicity;

- virulent strains of avian paramyxovirus 1 (NDV) have multiple basic residues at the cleavage site, which means that the F protein can be cleaved intracellularly by furin, an endopeptidase in the *trans-Golgi network*

The ubiquitous presence of this enzyme in cells facilitates the production of infectious virus in all cells capable of being infected by NCDV

Avirulent forms of the virus have a single basic residue at the cleavage site, and the F0 protein is present in mature virions

These viruses are only activated by extracellular proteases with appropriate substrate specificity or trypsin-like enzymes in epithelial cells of the respiratory and GIT

This limited "cleavability" restricts infectivity of the virus to fewer spp. of birds and significantly reduces the pathogenic potential of these viruses

After cleavage, the newly generated amino-terminal sequence of the F1 protein has a hydrophobic domain, and it is involved in fusion

The M protein is the most abundant protein in the virion and interacts with the lipid envelope, the cytoplasmic "tails" of the F and hemagglutinin–neuraminidase-like proteins, and the ribonucleoprotein

These interactions are consistent with M having a central role in the assembly of mature virions, by providing the structural link b/n the envelope gps and the ribonucleoprotein

M proteins are also implicated in controlling the levels of RNA synthesis

VIRUS REPLICATION

Paramyxoviruses usually cause lytic infection in cell cultures, but adaptation of the virus is usually necessary to achieve high-titer yields of virus

Formation of syncytia is a characteristic feature of many paramyxovirus infections in non-polarized cell cultures and infections in animals, but less so in polarized cell culture systems

Acidophilic cytoplasmic inclusions composed of Ribonucleoprotien structures are characteristic and, although their replication is entirely cytoplasmic

- Morbilliviruses also produce characteristic acidophilic intranuclear inclusions that are complexes of nuclear elements and N protein

Hemadsorption is a distinctive feature of paramyxoviruses that encode an HN protein, and of some morbilliviruses, but not of pneumoviruses

Paramyxoviruses replicate in the cytoplasm of infected cells; there is no requirement for nuclear functions

The virus attachment proteins (Hemagglutinin–Neuraminidase (NH), Hemagglutinine (H) and Glycoprotien (G)), recognize compatible ligands on the surface of host cells

The neuraminidase activity of this protein is assumed to assist the virus in release from infected cells by removing the sialic acid residues that could bind virus to an already infected cell

PESTE DES PETITS RUMINANTS VIRUS

highly contagious, systemic disease of goats and sheep

caused by a closely related morbillivirus, PPRV

The infection was first described in West Africa, but now occurs in sub-Saharan Africa, the Middle East, and the Asian subcontinent, including Nepal, Bangladesh, and Tibet

PPRV has been grouped into four distinct lineages based on the sequence of the F protein

Regardless of lineage, all strains belong to a single serogroup

1. Lineage 1 occur in West Africa
2. Linage 2 occur in West Africa
3. lineage 3 in East Africa, the Middle East, and southern India
4. lineage 4 extends from the Middle East to Tibet

Transmission of the virus is by close contact with infected animals

Virus is excreted for several days before the onset of significant clinical signs, such that spread of the virus is enhanced with the hadling of animals

Wild animals are not believed to play a major role in the spread of virus

The natural infection occurs in sheep and goats, with goats being more severely affected

Different breeds of goat show different morbidity rates, and young animals are more severely affected

Case fatality rates can be as high as 85% in goats, but rarely above 10% in sheep

cattle can be experimentally infected with both viruses; some putative cases of RP may in fact have been PPRV instead

In goats, a febrile response occurs at 2–8 days after infection

Clinical signs include fever, anorexia, nasal and ocular discharges, necrotic stomatitis and gingivitis, and diarrhea. Bronchopneumonia is a frequent complication

The course of the disease may be peracute, acute, or chronic, depending on strain of virus, age of animals and breed of host

PATHOGENESIS

Infectious aerosol → replicates within mononuclear leukocytes in the tonsils, mandibular and pharyngeal lymph nodes

Within 2–3 days, virus is transported during leukocyte-associated viremia to lymphoid tissues throughout the body, as well as the epithelium lining the gastrointestinal and respiratory tracts

CD150 (signaling lymphocyte activation molecule) as a receptor, which is consistent with the cellular and tissue tropism of PPRV
SLAM is present on immature thymocytes, activated lymphocytes, macrophages, and dendritic cells

The virus also infects and replicates in endothelial cells and some epithelial cells, presumably through a CD150-independent pathway, causing multifocal necrosis and inflammation in a variety of mucous membranes

infection triggers a rapid innate and acquired immune response, including a vigorous interferon response

viral protein or proteins, most likely the P protein, block the interferon response through inhibition of the phosphorylation and nuclear translocation of STAT (signal transducers and activator of transcription) proteins

PATHO…

Profound lymphopenia: virus-mediated destruction of lymphocytes in all lymphoid tissues, including GALT

Immune cells that support replication of PPRV also produce numerous potent immuno-regulatory cytokines after appropriate stimulation

The production and release of these cytokines, coupled with severe virus-induced lymphopenia, responsible for the profound but transient immunosuppression

marked dehydration; disseminated erosions and ulceration of the mucosal lining of the oral cavity, esophagus, and forestomachs; diffuse hemorrhage and necrosis of the mucosa of the abomasum; focal congestion and hemorrhage in the mucosa of the intestinal tract, with hemorrhagic necrosis of Peyer's patches

Segmental vascular congestion within the mucosa of the large intestine can produce characteristic "zebra stripes."

DIAGNOSIS AND CONTROL

Virus isolation in primary lamb kidney cells is still used to obtain isolates for molecular characterization and comparison.

VNT to distinguish b/n antibodies induced in animals by PPRV and RPV infections.

A live-attenuated vaccine based on a lineage 2 virus isolate (Nigeria 75/1) is now the vaccine of choice

RP vaccine is no longer recommended to prevent PPR, b/c of the RP eradication program

CANINE DISTEMPER

Highly contagious acute febrile disease of dogs

The etiology demonstrated by Carré in 1906

CDV recently emerged as a significant pathogen in family *Felidae*, also. Thousands of African lions died from CD, possibly acquired infection from free roaming Canids like Hyena, Wild dog….

Host range → All spp. of the families

1. ***Canidae*** **(Dog, Fox, Coyots, Jackels, Wolf, …)**
2. ***Procyonidae*** **(Raccon, Panda…)**
3. ***Mustelidae*** **(Ferret, Mink, baggers, Skunk, …)**
4. ***Felidae*** **(Lion, Chetahs, Leopard, …)**

CD...

canine distemper virus has also caused high mortality rates in black-footed ferrets (*Mustela nigripes*), the bat-eared fox (*Otocyon megalotis*), red pandas (*Ailurus fulgus*), hyenas (genus *Hyaena*), African wild dogs (genus *Lycaon*), raccoons (genus *Procyon*), palm civets (*Paradoxurus hermaphroditus*) and and Caspian (*Pusa caspica*)

There are 7 Linages based sequence analysis of the H gene: Asia-1, Asia-2, American-1, America-2, Arctic-like, European wildlife and Europe

The traditional vaccine strains of CDV: Snyder Hill, Onderstepoort and Lederle —> are all included in the America-1 lineage

Clinical signs depend on

1. the strain of the virus
2. the host age and immune status,
3. levels of environmental stress

DIAGNOSIS

Serological diagnosis hampered by vaccinal antigens esp. Modified live vaccines

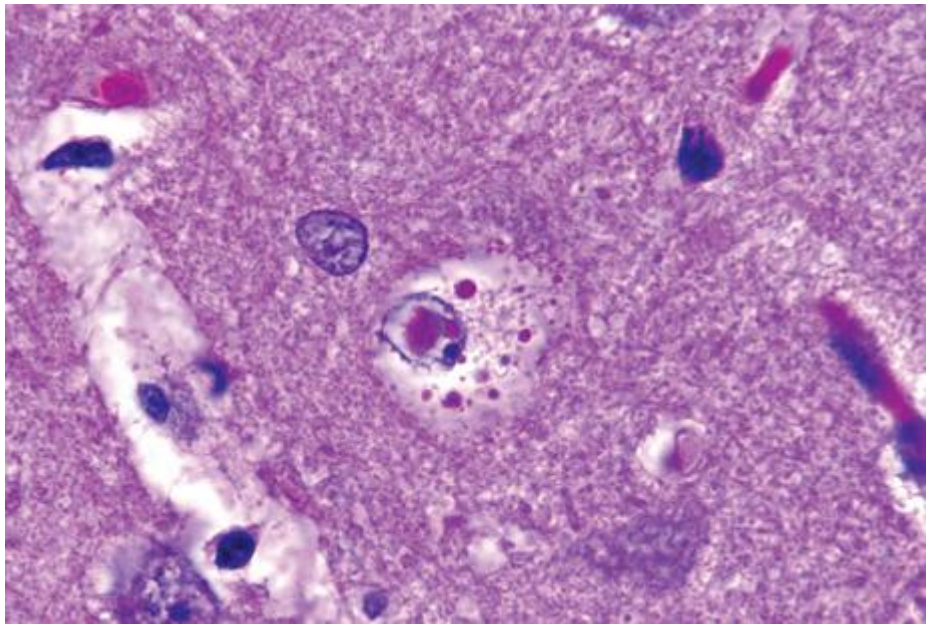
RT-PCR

Virus isolation: co-cultivation of lymphocytes from suspect animals with cell lines expressing the CD150 (SLAM) molecule

After initial isolation, the virus can adapt to grow in primary dog lung cells or MDCK or Vero

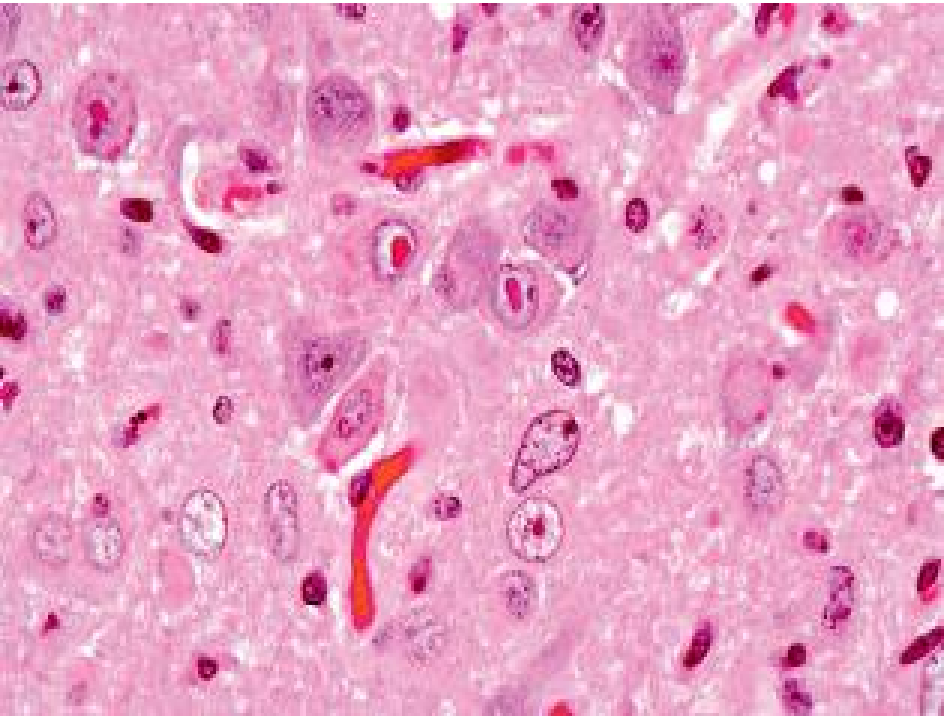
Immunohistochemistry and histopathology

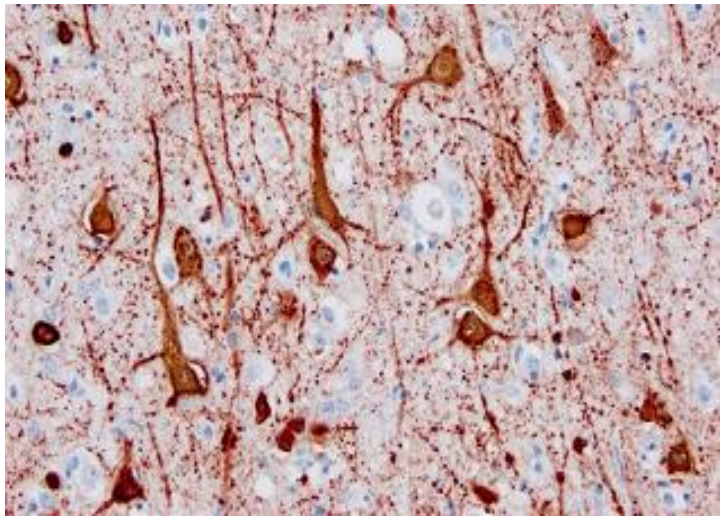
IFAT



Figs. Pathological staining

Eosinophilic (pink) intranuclear and intracytoplasmic inclusions (Left) Immunohistochemical staining of canine distemper virus in the brain of a dog (Right)





PREVENTION AND CONTROL

CMI is important in the protective immune response

Adequate diagnosis, quarantine, sanitation, and vaccination

Recombinant (Canarypox virus vectored vaccine containing H and F proteins) and modified live virus (MLV) vaccines is considered high

Vaccinate puppies at 6 to 8 weeks, 9 to 11 weeks, and 12 to 14 weeks of age; booster 1 year later and additional boosters every 3 years thereafter

Antibiotic treatment

Killed vaccine to immunize wild animals

ORTHOMYXOVIRIDAE

six to eight segments of ssRNA

Influenza viruses are the most important members of the family, which are included in three genera (*Influenzavirus A*, *B*, and *C*)

Influenza viruses that are pathogenic to domestic animals are included in the genus *Influenzavirus A*, whereas viruses in the two other genera (B and C) circulate continuously in humans

Influenza A viruses infrequently are transmitted from their animal hosts to humans, but human epidemics and pandemics caused by influenza A viruses typically have no animal involvement beyond the initial incursion

Changes in agricultural practices can interrupt the evolutionary progression of some newly emergent influenza virus variants

Water fowl → Swine → Domestic Fowl → Human

Pandemic strain of influenza A virus in Mexico in 2009 was isolated upon intensive surveillance for the emergence of novel influenza viruses

This newly emergent virus had its origin in triple reassortant swine viruses (H1N1, H3N2, H1N2) that had been circulating in pigs in N. America since the late 1990s

The novel feature of this new virus was an additional reassortment that replaced two gene segments (*NA and M)* of the N. American swine virus with the respective segments of the Eurasian swine virus

PROPERTIES OF ORTHOMYXOVIRUSES

Classification

The family comprises the genera *Influenza virus A, B, C, Thogotovirus,* and *Isavirus*

Influenza is the Italian form of Latin, from *influentia*, "influence," so used because epidemics were believed to be caused by astrological or other occult influences

- The thogotoviruses are tick-borne viruses that infect livestock and humans in Africa, Europe, and Asia

- *Isavirus* is infectious salmon anemia virus, a highly fatal disease of marine-farmed Atlantic salmon

- **Influenza A: common pathogens of horses, swine, humans, and domestic poultry throughout much of the world, but they also are the cause of sporadic or geographically limited infections and disease in mink, seals, whales, and dogs**

- **Influenza B: are pathogens of humans**

- **Influenza C: infect humans and swine and reassortants have been detected**

The emergence of variant viruses depends not only on *genetic* drift—that is, point mutations (nucleotide substitutions, insertions, deletions), but also on *genetic* shift—that is, genomic segment reassortment

In the current classification system, influenza A viruses are categorized into 17 hemagglutinin (H) and 10 neuraminidase (N) types

In naming virus strains, influenza virus spp or type (A, B, or C), host (swine, horses, chicken, turkey, mallard, etc.), geographic origin, strain number, year of isolation and hemagglutinin and neuraminidase subtypes are included

The host of origin is not specified in viruses isolated from humans

Examples of virus strain names include:

- A/equine/Miami/1/1963 (H3N8), the prototypic equine influenza virus 2
- A/swine/Iowa/15/1930 (H1N1), the prototypic strain of swine influenza virus
- A/Hong Kong/1/1968 (H3N2), the virus that caused the human pandemic of 1968

A numerical *clade system* has been adopted to better relate the evolutionary changes in these related H5N1 isolates over time;

- **a *clade* is a taxonomic group comprising a single common ancestor and all descendants of that ancestor**

For the Eurasian–African H5N1 virus, the reference isolate is A/Goose/Guangdong/1/1996 (H5N1)

VIRION PROPERTIES

Orthomyxovirus virions are pleomorphic, often spherical but sometimes filamentous, and 80–120 nm in their smallest dimension

They consist of a lipid envelope with large spikes surrounding

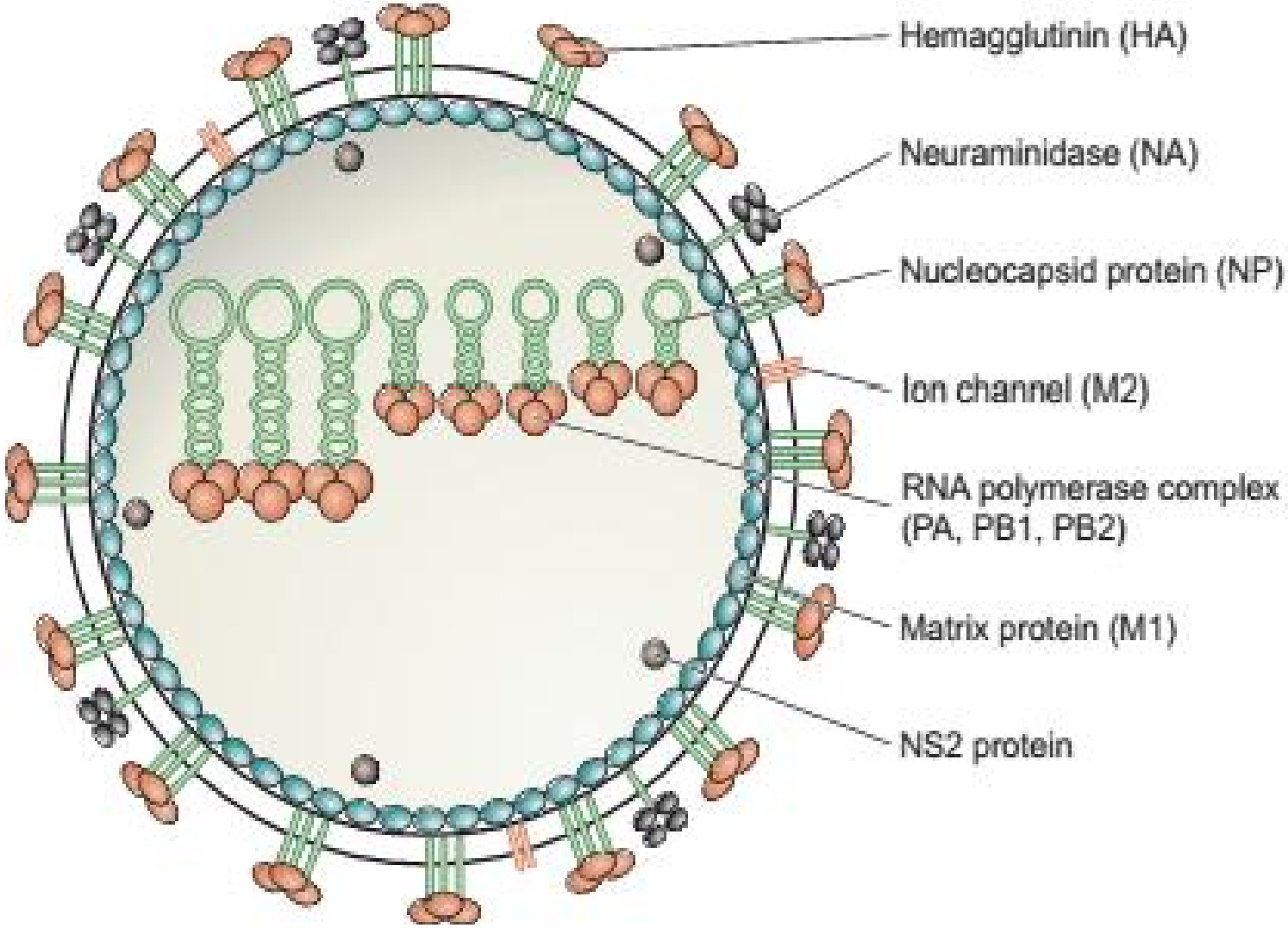
helical symmetry nucleocapsid segments of different sizes

For influenza A and B viruses, there are two kinds of glycoprotein spikes:

- homotrimers of the hemagglutinin protein and the neuraminidase protein

Influenza C viruses lack neuraminidase, and have only one type of glycoprotein spike that consists of multifunctional hemagglutinin- esterase molecules

SCHEMATIC DIAGRAM OF *INFLUENZA VIRUS* VIRION



Isavirus has hemagglutinin-esterase and an F protein

Regardless of the configurations, at least three functions are linked to the surface proteins: receptor binding, receptor cleavage, and membrane fusion

Virion envelopes are lined by the M1, on the inner surface of the lipid bilayer and are spanned by a small number of ion channels composed of tetramers of a second matrix protein, M2

Genomic segments consist of a molecule of viral RNA enclosed within a capsid composed of helically arranged nucleoprotein

- Three proteins that make up the viral RNA polymerase complex (PB1, PB2, and PA) are associated with the genomic RNA and nucleoprotein

The genome consists of 6-8 segments of linear negative-sense, ssRNA, and is 10–14.6 kb in overall size

The genome segments have non-translated regulatory sequences at both the 5' and 3' ends

The 13 nucleotides at the terminal 5' end and 12 at the 3' end are identical for each of the genomic segments and show partial inverted complementarity

- **This feature is essential for RNA synthesis**

They are sensitive to heat (56°C, 30 min), acid (pH 3), and lipid solvents, and are thus very labile under ordinary environmental conditions.

However, infectious influenza A virus has been recovered after 30 days in cold lake water.

VIRUS REPLICATION

Influenza virions attach to cells via the binding of their activated HA to sialic-acid-containing receptors on the PM

Different cells have d/t linkages of *N-acetyl neuraminic acid* (sialic acid) to a galactose residue, and the hemagglutinin recognizes these d/t linkages, which in turn determine the host range of the virus

The gut epithelium of ducks has a receptor with an α2,3 linkage (SA α 2,3Gal), whereas the predominant influenza virus receptor in the URT of humans is an α 2,6 linkage (SA α 2,6Gal)

There is also evidence that binding affinity for the SA α 2,6Gal glycan varies with the length of the oligosaccharide

Human-adapted H1 and H3 viruses show binding preference for long oligosaccharides present on epithelial cells in the URT, and mutations that affect this binding alter transmissibility

A single amino acid change in the hemagglutinin protein of the 1918 H1 Spanish flu virus at position 190 (E190D) changes binding preference from SA α 2,3Gal to SA α 2,6Gal

Influenza viruses enter cells via receptor-mediated endocytosis

The low pH of the endosome triggers a conformational change in the hemagglutinin protein

The hydrophobic domain of the HA2 trimer mediates fusion of the viral envelope with the endosomal membrane, releasing the RNA + nucleoprotein + polymerase proteins (RNP) into the cytoplasm

AVIAN INFLUENZA/FOWL PLAGUE

Many combinations of H and N antigens in influenza A virus are represented in isolates from avian species

Influenza A virus subtypes are distributed worldwide and are frequently recovered from clinically normal birds

Outbreaks of severe clinical disease, caused by H5 and H7 subtypes, occur periodically in chickens and turkeys.

Acute infection is referred to as fowl plague or highly pathogenic avian influenza (HPAI) and is categorized as a listed disease by the OIE

All H5 and H7 isolates are notifiable

Phylogenetic studies have shown close similarities between wild and domestic bird isolates and indicate that clades and lineages of isolates are associated with geographical and temporal factors.

Infection is maintained in wild bird populations. Waterfowl are considered to be responsible for introducing the virus to domestic birds. Although ducks, particularly juveniles, regularly become infected with influenza A virus, they rarely show signs of illnes

Following replication in the intestinal tract, virus is shed in faeces. Secondary spread can result from the movement of personnel and contaminated equipment between poultry farms, and live-bird markets may contribute to the spread of infection in some countries

PATHOGENESIS

Spread of influenza virus in tissues is dependent on the type of proteases present in a given tissue and the structure of the viral HA molecule

The production of infectious virions requires post-translational cleavage of the viral precursor hemagglutinin (HA0) molecule into a disulphide-linked HA1–HA2 dimer to attain its full biological potential

In the majority of influenza A virus subtypes, HA is cleaved by particular host proteases such as trypsin-like enzymes found only in the epithelial cells of the respiratory and digestive tracts

The presence at the cleavage site of multiple basic amino acids, arginine or lysine, renders hemagglutinins of virulent subtypes susceptible to cleavage by intracellular host proteases present in many tissues, thereby facilitating the development of generalized infection

HPAI isolates arise by mutation from low virulence isolates probably after the transfer of virus from the natural wild bird host to poultry

Such change, which is unpredictable, may occur soon after introduction or following several months of circulation of low pathogenic avian influenza (LPAI) virus in poultry

CLINICAL SIGNS

The incubation period, which is variable, is up to 3 days in naturally-infected individual birds and 1 4 days in the entire flock.

The incubation period is influenced by virus and route of infection.

Clinically, the disease may be inapparent, mild or, in some instances, severe with high mortality.

Factors such as overcrowding, poor ventilation and concurrent infections may predispose to the development of severe disease.

Highly virulent subtypes cause explosive outbreaks of disease with high mortality. Clinical signs are more apparent in birds which survive for a few days.

Respiratory distress, diarrhoea, oedema in the cranial region, cyanosis, sinusitis and lacrimation are features of the clinical presentation.

Infection of laying birds results in a dramatic drop in egg production

DIAGNOSIS

The severe form of the disease may be difficult to distinguish from velogenic, viscerotropic Newcastle disease or from fowl cholera. Mild forms of the disease resemble other respiratory conditions in birds
Suitable specimens for laboratory examination include tracheal and cloacal swabs, faeces and pooled samples of organs

Tissue suspensions are inoculated into 9 to 11day-old embryonated eggs

Allantoic fluid, harvested after 4 to 7 days of incubation, is tested for hemagglutinating activity

Immunodiffusion using a suspension of chorioallantoic membrane from eggs inoculated with material from an outbreak and positive antiserum to the nucleocapsid or matrix antigens common to all influenza A viruses
HI or immunodiffusion tests to confirm

Definitive subtyping is performed by HI and NI in reference laboratories using mono-specific antisera prepared against the 16 hemagglutinins and nine neuraminidase determinants
To assess pathogenicity, ten chickens 4 to 8 weeks of age should be inoculated intravenously. Isolates that cause more than 75% mortality within 8 days or have an IVPI of greater than 1.2 are considered highly pathogenic.

Genomic sequencing

RT-PCR techniques

Serological testing for antibodies to influenza virus

- AGID, HAI and cELISA

RETROVIRIDAE

Retroviruses infect a wide variety of animals, including humans

causative agents of

- **cancer**
- **immunosuppressive or immunemediated diseases**
- **exist as stable components of the host genome**

Retroviruses were so named in the mid-1970s after the discovery reverse transcriptase but diseases described earlier in mid 1800s

- **Equine infectious anemia, bovine leukosis, and Jaagsiekte (pulmonary adenomatosis) of sheep**

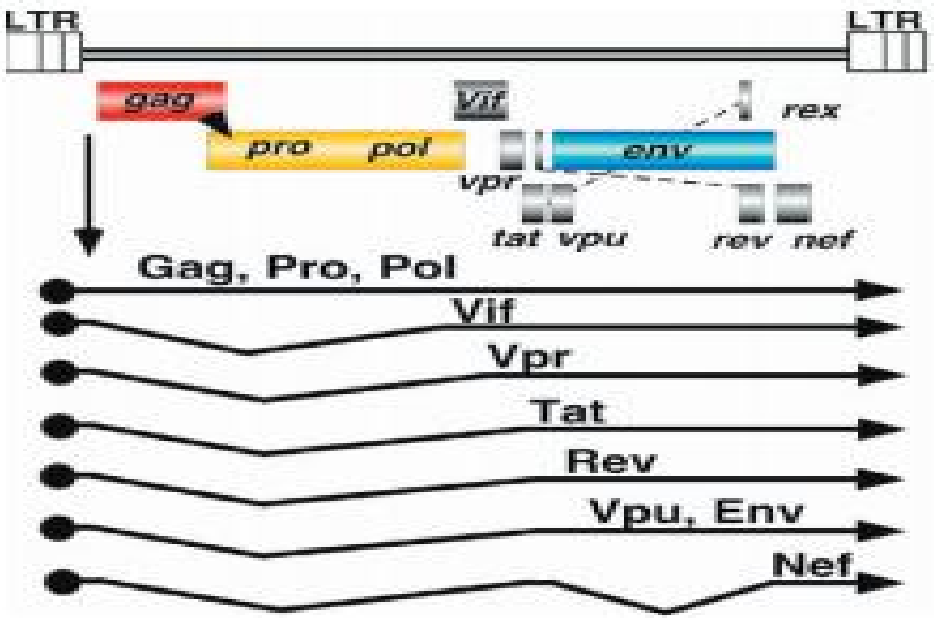
Rous (1911) - produced sarcomas by injecting filtrates in chickens

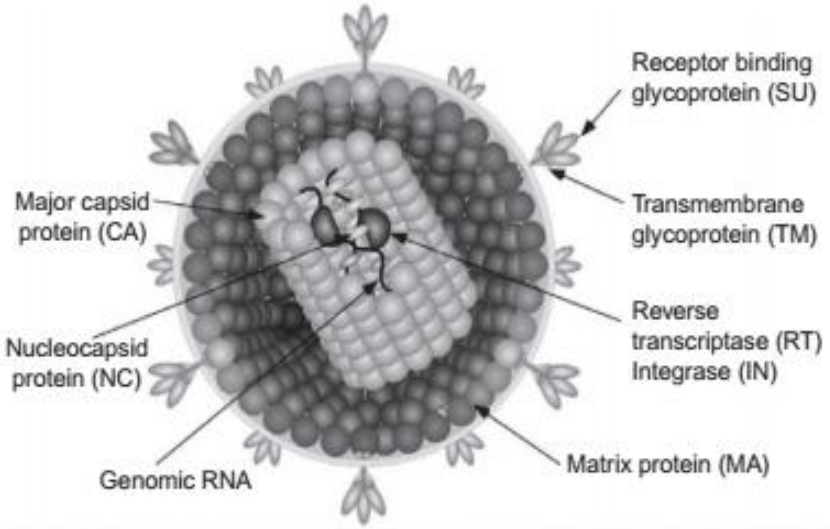
The two related viruses are prototypic of the etiologic agents of similar infectious malignant tumors now recognized in many other animal species, including cattle, cats, mice, and primates

The family *Retroviridae* is classified currently into two subfamilies (*Orthoretrovirinae and Spumaretrovirinae)* and seven genera

The term *retro* (reverse, backward) reflects the property of retroviruses to use their RNA genome to produce DNA intermediates using Reverse transcriptase, an RNA-dependent DNA polymerase

Since the 1980s, retroviruses have been demonstrated to cause a number of important human diseases, including lymphomas, leukemias and AIDS





PROPERTIES OF RETROVIRUSES

all replicate utilizing Reverse transcriptase (RT), a virus-encoded enzyme that synthesizes a DNA copy from the RNA viral genome

All contain RT and a diploid genome with two copies of positive-sense ssRNA

Virions are enveloped and typically form by budding from cell membrane

Retroviruses integrate into host cell genomes using another unique virus-encoded enzyme, integrase

This property allows them to acquire, but also alter, host genetic sequences

CLASSIFICATION

The family *Retroviridae* is subdivided into two subfamilies (*Orthoretrovirinae, Spumaretrovirinae)* and seven genera (*Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Deltaretrovirus, Epsilonretrovirus, Lentivirus,* and *Spumavirus)*

many are associated with some form of chronic disease characterized by immunopathology or cancer

The spumaretroviruses are an obvious exception as, to date, they have not been associated with disease

Initial classification of retroviruses was based on:

- **their respective host species of origin**
- **virion morphology and morphogenesis as determined by EM**
- **biological properties including their route of transmission (exogenous versus endogenous), host cell restrictions, and ability to resist cross-neutralization by specific antiserum**

Morphologically, four different types of particles were recognized, designated A, B, C, and D

- **Type C retrovirus morphogenesis involves the formation at the CM of distinctive crescent-shaped nascent nucleocapsids (hence the name *type C retrovirus)***

The antigenic relationships: envelope gp epitopes are retrovirus type-specific, whereas others are strain-specific

Core protein epitopes specified by the *gag gene* are often common to the retroviruses of particular animal species—that is, they are group-specific Ags and referred to as "Gag"

Some conserved epitopes (those of reverse transcriptases) are shared between retroviruses (interspecies antigens)

VIRION PROPERTIES

enveloped, 80–100 nm in diameter, have a unique three-layered structure

Inner most is the genome–nucleoprotein complex, which includes about 30 molecules of RT, and has helical symmetry

icosahedral capsid about 60 nm in diameter which in turn is surrounded by an envelope, from which surface envelope gp spikes (peplomers) project

The genomes of retroviruses are diploid>>>

- Two RNA copies packaged in virions as an inverted dimer of two molecules of linear positive-sense, ssRNA

Each RNA genome copy (7-11 kb) and has a 3'-polyadenylated tail and a 5' cap

Virions are inactivated relatively easily by lipid solvents or detergents and by heating

Retroviruses require RT for their replication

RT serves as an RdDp, a DdDp, and an RNase, with each distinctive function being carried out by a d/t part of the protein molecule

Replication of retroviruses: dependent on host-cell RNA polymerases for transcription of the integrated DNA copy of the viral genome

The genome of replication competent (exogenous/non-defective) retroviruses contains three major genes

The *gag gene* encodes the virion core proteins [capsid (CA); nucleocapsid (NC); and matrix (MA)]

the *pol gene* encodes RT and integrase (IN)

the *env gene* encodes the virion envelope proteins [surface (SU) and transmembrane (TM)]

Genome termini have several distinctive components, each of which is functionally important

- The R (repeat) and U (5' and 3' unique regions) are critical for reverse transcription, integration, and viral transcription after integration

Retroviruses are often classified as having acute or chronic transforming biologic characteristics, based on the presence or absence of key transforming genes in the viral genome

Viruses that are capable of acute cellular transformation have also been referred to as rapidly or strongly transforming

- These retroviruses typically contain viral oncogenes that, under direct control of a viral promoter, increase the probability of expression of v-*onc*

VIRUS REPLICATION

Cell Binding and Penetration

virion envelope gps bind to cellular receptors

The specific cellular receptors responsible for virus attachment are unique to each retrovirus genus, so many retroviruses are spp restricted in their host range

- **Moloney murine leukemia virus binds receptors that are present only on mouse cells**

After attachment, the viral envelope and the CM fuse, allowing the virion core to enter the cytoplasm

- **less commonly, entry involves receptor-mediated endocytosis**

Cells infected with a particular retrovirus are often resistant to super-infection by another closely related retrovirus

Even within a particular species, inbred animals or lines exhibit susceptibility to retrovirus infections based on receptor expression

- **Strains of avian leukosis virus have distinct interference patterns that reflect their individual effects on receptor expression**

Oncogenesis

Cellular "oncogenes" (c-*onc* or *proto-oncogenes)* are responsible for normal cell growth and differentiation

During retrovirus replication, *v-onc* can be created from *c-onc* through processes such as read-through transcription

***v-onc:* loss of cellular control of v-*onc* activity**

***v-onc:* cell growth control (dys-regulation) by influencing or acting as growth factors, receptors, intracellular signal transducers, or intranuclear factors such as transcription factors**

Oncogene capture is a unique feature of retrovirus replication and is considered an "illegitimate" recombination event

Current models favor a non-homologous recombination event during reverse transcription

occurs by two potential mechanisms each occurring when retroviruses integrate within or adjacent to a proto-oncogene; specifically, packaging of deleted and wild-type genomes can be followed by recombination, resulting in additional sequences "captured" by the virus

Alternatively, packaging of read-through transcripts can occur as RNA polymerases transcribe the provirus, and subsequent recombination results in the incorporation of additional sequences into the new viral genome

Many of these events may block replication, but those that lead to a transformation event may ultimately be expressed as cancer in the infected host

REOVIRIDAE

This family is one of the most complex in all of virology, currently comprising 12 recognized and three proposed genera of viruses with genomes composed of several (10–12) segments of dsRNA.

Viruses in the d/t genera can be distinguished on the basis of several different features,

Including their capsid structure, number and size of genome segments, host range and associated diseases, serological properties, and, increasingly, by the nucleotide sequence of their genomes.

The root term "reo" is an acronym for "respiratory enteric orphan,"

- **The first members were identified in the respiratory and the enteric tracts of animals and humans as "orphans"—that is, not associated with any disease.**

These viruses are now members of the genus *Orthoreovirus*.

The later inclusion in the family of the genera *Orbivirus, Coltivirus, Rotavirus, Seadornavirus,* and *Aquareovirus* added important pathogens of humans and a variety of animal spp, including aquatic animals.

The distribution of the member viruses of the genus *Orthoreovirus* is ubiquitous,

- **Viruses from cattle, sheep, swine, humans, non-human primates, bats, and birds;**
- **however, most of these infections are not associated with any significant clinical disease.**

Only reovirus infections of poultry, and perhaps primates, are of major pathogenic significance.

Viruses of the genus *Orbivirus* are transmitted to animals primarily by arthropod vectors, which, depending on the individual virus, can be certain spp of *Culicoides* midges, mosquitoes, black flies, sandflies, or ticks.

The global and seasonal distribution of individual viruses coincides with that of their specific biological vector and appropriate climatic conditions.

Bluetongue and African horse sickness viruses economically are the most important members of this genus, although several others are potentially important, either regionally or globally.

There is concern that AHS or other pathogenic orbiviruses might follow.

PROPERTIES OF REOVIRUSES

Classification

All animal viruses with multi-segmented dsRNA genomes are included in the family *Reoviridae*

Reassortment of genome segments among d/t strains of these viruses is common within the same virus species, as is a high rate of RNA mutations in individual genes.

The resulting genetic shift and drift leads to a remarkable diversity of viruses, which is reflected by the numerous serotypes and myriads of strains of individual viruses within each genera.

The genus *Orbivirus* is divided into at least 21 virus subgroups, which represent distinct virus spp.

Several of these include viruses that cause disease in domestic animals

- **Separate virus species encompass 25 (very likely 26) serotypes of bluetongue virus;**
- **9 serotypes of AHSV; 10 serotypes of epizootic hemorrhagic disease virus, including Ibaraki virus; 7 serotypes of equine encephalosis virus;**
- **1 serotype of Peruvian horse sickness virus; 13 serotypes of Palyam virus, including Chuzan virus; certain other viruses affecting different animal spp.**

VIRION PROPERTIES

Orthoreoviruses and rotaviruses are resistant to lipid solvents and are stable over a wide range of pH, but orbiviruses and coltiviruses have a narrower zone of pH stability (pH 6–8) and lose some, but not all, infectivity on exposure to lipid solvents.

Proteolytic enzymes increase the infectivity of orthoreoviruses and rotaviruses

- **chymotrypsin in the SI cleaves an outer capsid VP4 protein of rotavirus, thereby enhancing infectivity.**
- **Proteolytic cleavage of the outer capsid protein VP2 of bluetongue virus also increases its infectivity for cells of its insect vector (*Culicoides* species) but not for mammalian cells.**

Orbiviruses and rotaviruses are remarkably stable.

Bluetongue viruses are relatively stable in the presence of protein, and have been re-isolated from blood stored for years at room temp.

Viral infectivity is inactivated by phenols, formalin, 95% ethanol, and β-propriolactone.

Reovirus particles are non-enveloped, spherical, and have a diameter of ~85 nm.

Virions consist of a multilayered capsid, each with icosahedral symmetry.

The precise virion morphology varies with the genus.

The genome consists of linear dsRNA divided into 10 (genus *Orthoreovirus and Orbivirus)*, 11 (genus *Rotavirus* and *Aquareovirus*), or 12 (genus *Coltivirus, Seadornavirus,* and some currently designated members of genus *Aquareovirus)* segments.

The overall genome size is approximately 23 kbp (*Orthoreovirus)*, 19 kbp (*Orbivirus*), 16–21 kbp (*Rotavirus*), 29 kbp (*Coltivirus*), 21 kbp (*Seadornavirus*), or 24 kbp (*Aquareovirus*).

The positive strands of each double-stranded segment have 5'-terminal caps (type 1 structure).

The 3' termini of both strands lack 3'-poly(A) tails.

Each RNA segment is present in equimolar proportion in virions.

Genus Orbivirus

The outer capsid consists of a diffuse layer formed by two proteins, VP2 and VP5.

This outer capsid is dissociated readily from the core particle, which has a surface composed of 260 VP7 trimers arranged in ring-like structures for which the genus is named.

Both VP2 and VP5 are attached to VP7, which in turn is associated with a subcore shell composed of 120 copies of VP3 surrounding the transcriptase complex (VP1, VP4, and VP6), and the genomic RNA segments.

Surface projections are observed only on virions that have been stabilized (frozen for cryoelectron microscopy).

- **Otherwise, the surface of virions appears smooth and unstructured.**

The 10 genome segments of the orbiviruses are all monocistronic, except the smallest gene segment (10), which includes a single ORF, but with two functional initiation codons near the 5' end of the positive-sense strand.

As with the orthoreoviruses, the gene segments form distinct size patterns during electrophoresis that can be used to identify the different orbivirus species.

Variation can be detected b/n the RNAs of closely related viruses with this method, which can be used to distinguish strains within a single viral serotype.

The 10 genome segments encode seven structural proteins (VP1– 7) and four non-structural proteins (NS1–NS3, NS3A).

VIRUS REPLICATION

The σ1 protein of orthoreoviruses mediates attachment to target cells.

Virions or infectious subviral particles enter susceptible cells by receptor-mediated endocytosis.

Junctional adhesion molecule A is a serotype-independent receptor for orthoreovirus, and sialylated glycoproteins can serve as a co-receptor for some strains.

Variations in the σ1 protein, a filamentous trimer that projects as a spike from the virion, determine the cell and tissue tropism of each virus.

Once internalized, virions are degraded to core particles, within which virion-associated RNA polymerase and capping enzymes repetitively transcribe 5'-capped mRNAs that are extruded into the cytoplasm through channels at core particle vertices.

RNA polymerase (transcriptase) utilizes the negative strands of each of the dsRNA segments as templates; only certain genes are transcribed initially, four mRNAs appearing before the other six.

The proportion of the various mRNAs found in infected cells varies, and the efficiency of the translation of each also varies (over a 100- fold range).

How this regulation is mediated is not known.

After early mRNA synthesis, genomic RNA replication takes place within nascent progeny subviral particles in the cytoplasm of infected cells.

The mechanism of genomic RNA replication is complex and not fully understood.

Newly synthesized, dsRNA in turn serves as a template for the transcription of more mRNAs, which this time are uncapped.

These mRNAs are translated preferentially to yield a large pool of viral structural proteins that self assemble to form virions.

- **The mechanism that ensures that one copy of each dsRNA segment is encapsidated into nascent virions is not known.**

Shortly after virus entry, host-cell protein synthesis decreases abruptly;

One proposed mechanism is that the cap-dependent host-cell mRNAs are less efficient in driving protein translation than uncapped viral mRNAs.

Structures, termed viroplasms/ factories, form in localized areas of the cytoplasm—these intracytoplasmic inclusion bodies can be dramatic in size and the number of associated virions.

- **Inclusion bodies have a granular and moderately electron-dense appearance in thin-section EM.**

Progeny virions tend to remain cell associated, but are eventually released by cell lysis.

Replication of orbiviruses and rotaviruses is generally similar to that of orthoreoviruses.

Rotavirus infectivity requires triple-layered virus particles containing the outer capsid proteins, VP4 and VP7, for attachment.

Proteolytic cleavage of VP4 (i.e., by chymotrypsin in the SI), is imp't for virus entry into the cells and increased infectivity.

Some rotavirus strains bind to sialic acid residues on the cell surface,

- **There are suggestions that rotavirus can enter cells either by receptor-dependent endocytosis or by direct penetration.**

Regardless, the process of cell entry removes the outer shell of the virion to generate the transcriptionally active double-layered particle that mediates transcription.

Rotavirus progenitor particles acquire a temporary lipid envelope as they bud into cisternae of the ER of infected cells, which then breaks down, leaving VP7 as the major outer capsid protein.

Bluetongue virus—and presumably other orbiviruses— enter cells through clathrin-dependent, receptor-mediated endocytosis.

Outer capsid (VP2 and VP5) and core (VP7) proteins have all been implicated in cell attachment and penetration.

Indeed, bluetongue virus core particles (that lack the outer capsid proteins, VP2 and VP5) have infectivity comparable to that of fully intact virus particles for cells of the insect (*Culicoides)* vector, whereas core particles are less infectious for mammalian cells.

Cell membrane glycoproteins can function as receptors, but these interactions are otherwise poorly characterized.

Virus entry results in removal of the outer capsid, which activates the core-particle-associated transcriptase and capping enzyme.

Transcription occurs within core particles associated with viral inclusion bodies.

Distinctive tubules composed of the viral NS1 protein are characteristically present in the cytoplasm of infected cells, although their precise function remains uncertain.

Before release of newly formed virus particles by lysis, particles can bud through the CM in a process mediated by the NS3 protein.

Virus / Hosts / Serotypes	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Orthoreovirus			
Reoviruses in mammals Serotypes 1–4	Asymptomatic infection experimental disease	Fecal–oral route; systemic infection	For mouse colonies good sanitation, regular testing, and preventive quarantine
Reoviruses in poultry Reoviruses in many bird species, multiple serotypes	Tenosynovitis/arthritis Respiratory disease, enteritis, with weight loss, stunted growth; often subclinical	Fecal–oral route; systemic infection Feces, serum, multiple target tissues	Good sanitation, regular testing, and preventive quarantine Attenuated and inactivated virus vaccines available
Orbivirus			
Bluetongue virus Sheep, cattle, goats, deer Serotypes 1–25	Bluetongue Fever, hyperemia, cyanosis, edema, oral, cavity erosions, nasal discharge, lameness	Vector transmission: *Culicoides* spp. Blood—virus detection; serum—antibody testing; spleen, lung, lymph nodes	Attenuated and inactivated virus vaccines available Prevent contact with *Culicoides* spp.
African horse sickness virus Horses; donkeys, mules, zebras (subclinical) Serotypes 1–9	Respiratory or cardiovascular failure Fever, edema	Vector transmission: *Culicoides* spp. Blood—virus detection; serum—antibody testing, spleen, lung, lymph nodes	Attenuated and inactivated virus vaccines available Prevent contact with *Culicoides* spp.

Virus / Hosts / Serotypes	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Equine encephalosis viruses Horses; donkeys, mules, zebras (subclinical) Serotypes 1–7	Subclinical often Fever, African horse sickness-like disease	Vector transmission: *Culicoides* spp. Blood—virus detection; serum—antibody testing, spleen, lung, lymph nodes	No vaccines Prevent contact with *Culicoides* spp.
Epizootic hemorrhagic disease of deer viruses Deer, cattle, sheep Serotypes 1–10 Ibaraki virus (EHDV setorype 2)	Hemorrhagic disease Fever, hyperemia, cyanosis, edema	Vector transmission: *Culicoides* spp. Blood—virus detection; serum—antibody testing, spleen, lung, lymph nodes	No vaccines Prevent contact with *Culicoides* spp.
Palyam virus Cattle, serotypes 1–13	Reproductive, central nervous system disease; Abortion, congenital abnormalities; hydranencephaly	Vector transmission: *Culicoides* spp.	No vaccines
Peruvian horse sickness Horses in South America and Australia	Neurological disease	Vector transmission: likely mosquitoes	No vaccines

Virus / Hosts / Serotypes	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Rotavirus			
Rotaviruses Virtually all species	Gastroenteritis/diarrhea	Fecal–oral route Feces	Dam innoculation with attenuated or inactivate virus Oral attenuated vaccine for neonates
Coltivirus			
Colorado tick fever virus Small animals, humans—zoonosis	Tick fever/Saddle-back fever Retro-orbital pain, myalgia, leukopenia	Trans-stadial vector transmission: wood tick (*Dermacentor andersonie*) Blood and serum	No vaccines or treatments Prevent contact with ticks
Eyach virus Small animals, humans—zoonosis	Antibodies found in patients with meningoencephalitis and polyneuritis	European *Ixodidae* ticks Blood and serum	No vaccines or treatments Prevent contact with ticks
Aquareoviruses			
Fish, shellfish	Uncertain	Uncertain	No vaccines or treatments

TOGAVIRIDAE

Viruses possess a lipid envelope (or cloak: "toga") surrounding an icosahedral capsid.

There are two genera within the family: specifically, the genera *Alphavirus* and *Rubivirus*.

The single member of the rubivirus genus is the exclusively human pathogen, rubella virus.

Alphaviruses are distributed worldwide, and several are important pathogens of humans and/or animals.

With the notable exception of the salmonid alphaviruses, alphaviruses are maintained in an enzootic infection cycle that includes insect vectors and animal reservoir hosts.

Individual alphaviruses typically exist in geographically limited habitats defined by transmission cycles that involve specific mosquito and vertebrate hosts that contribute to virus persistence, geographic distribution, overwintering, and amplification.

With the exception of chikungunya and perhaps Ross River viruses, domestic animals and humans are "dead-end" hosts that are not involved in primary enzootic transmission cycles in nature.

But they can serve as critical amplifying hosts that contribute to geographic extension and disease outbreaks.

- **A mosquito–horse–mosquito transmission cycle is responsible for explosive spread during epizootics of Venezuelan equine encephalitis.**

The host range for many alphaviruses is extensive, and may be restricted only by the feeding preferences of their insect hosts.

Important alphavirus pathogens of vertebrate animals include eastern, western, and Venezuelan equine encephalitis and related viruses (the so-called equine alphavirus encephalitides), and Getah virus.

Although the pathogenic significance to animals of other alphaviruses is largely undefined, particularly in wildlife spp, several alphaviruses in addition to those just listed are imp't zoonotic pathogens:

- **Sindbis virus and a group of viruses related to Semliki Forest virus, including chikungunya, o'nyong-nyong, Ross River, Barmah Forest, and Mayaro viruses.**

With regard to disease in mammals, alphaviruses can be classified into three groups:

- **those that cause neurologic disease (encephalitis);**
- **those that cause a febrile illness with polyarthritis;**
- **those that cause no apparent disease.**

In contrast to other alphaviruses, salmonid alphaviruses appear to infect only fish, without any requirement for an arthropod vector.

These three equine encephalitis viruses initially were referred to as arboviruses because of their transmission by arthropods.

Their further initial designation as "group A arboviruses" ultimately led to their current designation as alphaviruses.

VIRION PROPERTIES

Alphavirus virions are spherical, uniform in appearance, enveloped, and 70 nm in diameter.

Virions consist of a lipid envelope with fine spikes surrounding an icosahedral nucleocapsid that is 40 nm in diameter.

The spikes are composed of heterodimers of the E1 and E2 gps that are organized in a (T = 4) icosahedral lattice consisting of 80 trimers.

The genome is a single molecule of linear, positivesense, ssRNA, 11–12 kb in size.

The RNA has a 5'-methylated nucleotide cap and its 3' end is polyadenylated.

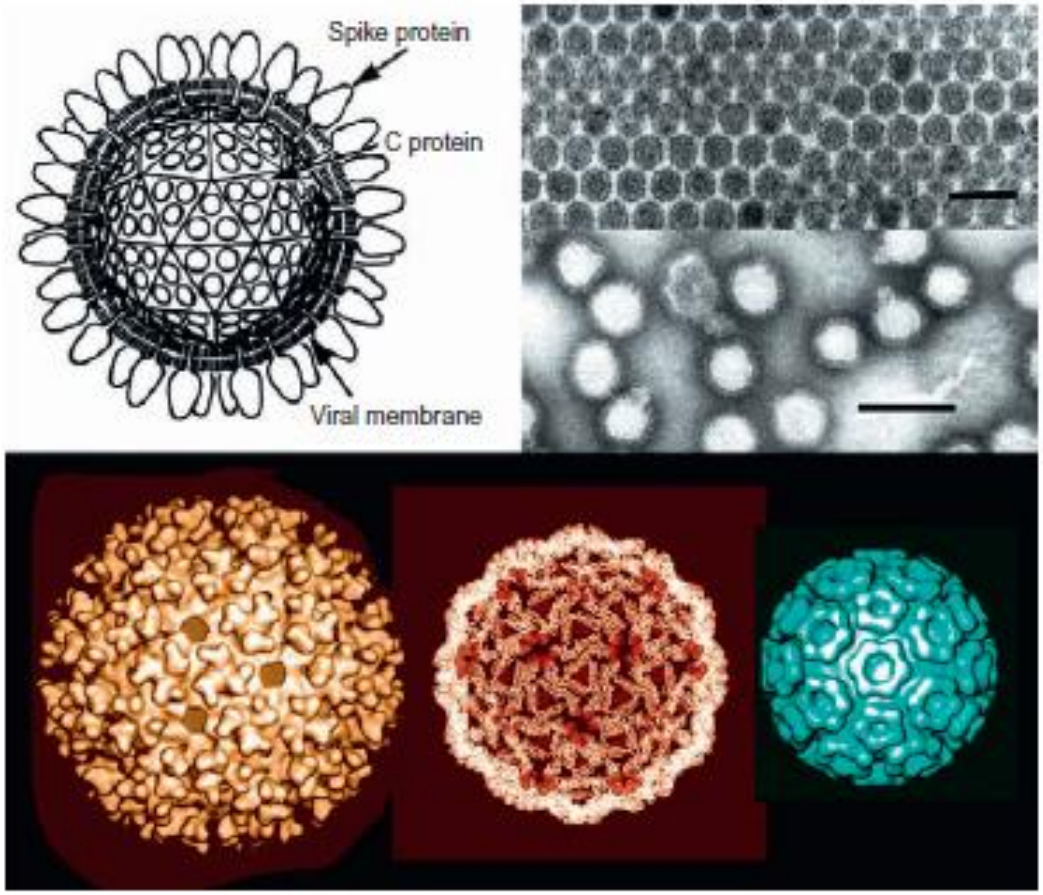
The 5’ two-thirds of the genome encodes NS proteins;

The 3’ one-third is not translated from genomic RNA, but is expressed from a subgenomic mRNA molecule that is transcribed from a full-length, negativesense intermediate.

The subgenomic mRNA encodes five proteins, including a nucleocapsid protein (C, *Mr 30–33 kDa)* and two envelope gps (E1 and E2, *Mr 45–58 kDa).*

Some alphaviruses have a third envelope protein, E3 (*Mr* 10 kDa) which is a cleavage product of a precursor protein, PE2.

Alphaviruses are relatively unstable in the environment, and are inactivated easily by common disinfectants and high temperature.



(Top panel–left). Diagrammatic representation of a Sindbis virus particle. The spike proteins on the surface represent the external portions of the E1+E2 heterodimers that associate to form trimers. C, capsid protein. (Upper right), Thin section of pelleted particles of Semliki Forest virus. (Lower right), Negative contrast electron micrograph of particles of Semliki Forest virus. (Bottom panel), Structure of Sindbis virus (SINV). (Left), Surface shaded view as determined by cryo-electronmicroscopy and image reconstruction. (Center), Surface view of SINV showing the organization of the E1 glycoprotein on the surface of the particle. (Right), Image represents the nucleocapsid core showing the pentameric and hexameric capsomeres (T = 4 icosahedron).

VIRUS REPLICATION

Alphaviruses can replicate to very high titers and cause severe cytopathic changes in many kinds of vertebrate cells.

- **Vero (African green monkey kidney), BHK-21 (baby hamster kidney), and primary chick and duck embryo cells.**

They also grow in, but do not cause cytopathic changes in, mosquito cells, such as C6/36, which are derived from *Aedes albopictus*.

In mammalian and avian cells, infection causes a complete shutdown of host-cell protein and nucleic acid synthesis.

In mosquito cells there is no shutdown, and cell division can continue, with the cells becoming persistently infected and continuously shedding virus.

Viral attachment to the host cell first involves interaction b/n the viral E2 gp and receptors on the cell surface.

The broad host range of alphaviruses suggests that either E2 contains several receptor binding sites or the cell receptor is ubiquitous.

- Various lectins, integrins, and laminin have been identified as putative cellular receptors of individual alphaviruses.

Once virions bind to the cell, the virus–receptor complex is endocytosed into coated vesicles using the clathrin-dependent pathway.

The acidification of the vesicles causes a rearrangement of the E1–E2 dimer with the formation of an E1 trimer inducing fusion with the vesicle membrane with release of the nucleocapsid into the cytoplasm.

Upon entry into the cytoplasm, the virion RNA has two main functions.

The 5’ end of the genomic RNA serves as mRNA that, in some alphaviruses, is first translated to produce two polyproteins,

- the larger of which is produced by a read-through of a weak stop codon.

These NS proteins in their uncleaved and cleaved forms direct the synthesis of the template negative-sense RNA genome from the input virion RNA and then genomic-size plus-strand RNA, along with a subgenomic RNA.

The full-length plus-strand RNA is encapsidated into new virions, whereas the subgenomic RNA acts as message for the synthesis of the structural viral proteins.

The structural proteins are expressed from the subgenomic RNA as a polyprotein that is then cleaved to form the individual proteins.

In mammalian cells, nucleocapsids are assembled in the cytoplasm and move to the PM, where they align under patches containing viral gp spikes.

Finally, virions are formed by budding of nucleocapsids through patches of PM that are studded with spike gp.

The budding process in insect cells may be localized to internal cellular membranes.

New World and Old World apparently utilize d/t mechanisms to interfere with the host interferon response, which is key to survival in infected animals.

For Sindbis virus, the multifunctional NS protein, nsP2, inhibits host-cell transcription, whereas in Venezuelan and eastern equine encephalitis virus the nucleocapsid protein, C, inhibits RNA transcription.

Host-cell macromolecular synthesis, including innate immune responses, is compromised by both mechanisms, thus enhancing the yield of infectious virus.

Virus	Arthropod Vector	Domestic Animal Host	Disease	Geographic Distribution
Eastern equine encephalitis virus	Mosquitoes	Horses (humans)	Encephalitis	Americas
Western equine encephalitis virus	Mosquitoes	Horses (humans)	Encephalitis	Americas
Highlands J virus	Mosquitoes	Horses	Encephalitis	Americas
Venezuelan equine encephalitis virus	Mosquitoes	Horses (humans)	Febrile disease, encephalitis	Americas
Getah virus	Mosquitoes	Horses	Febrile disease	Southeast Asia

BUNYAVIRIDAE

It is the largest virus family, with more than 350 member viruses included in five genera:

- ***Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus,* and *Tospovirus*.**

The family name is derived from the place in Uganda where the prototype bunyavirus was isolated.

The common features of the bunyaviruses pertain both to the nature of the virions and to their biological properties.

Viruses in three genera (*Orthobunyavirus, Nairovirus,* and *Phlebovirus)* are maintained in arthropod–vertebrate–arthropod cycles (arboviruses), which have specificity in regard to both arthropod vectors and vertebrate reservoir hosts.

This specificity is the basis for the usually narrow geographic and ecologic niches occupied by each virus.

Similarly, viruses in the genus *Tospovirus* can be transmitted b/n plants by thrips, and replicate in both thrips and plants.

Viruses in the genus *Hantavirus* are an exception, in that they are maintained in vertebrate–vertebrate cycles without arthropod vectors;

Nevertheless, the hantaviruses also exhibit great specificity in vertebrate reservoir hosts, and therefore also have distinct geographic and ecologic niches.

Arthropod-borne bunyaviruses are transmitted by specific mosquitoes, ticks, midges, or biting flies, whereas the individual hantaviruses are disseminated by specific rodents.

Bunyaviruses cause transient infection in their vertebrate hosts, whether mammal or bird.

Life-long persistent infection in their arthropod vectors,

Hantaviruses cause persistent infection in their rodent reservoir hosts.

Most bunyaviruses never infect domestic animals or humans, but those that do can cause important diseases that vary from congenital fetal malformation to systemic "hemorrhagic fever" disease syndromes.

PROPERTIES OF BUNYAVIRUSES

Classification

The very large number and diversity of the bunyaviruses offer a considerable taxonomic challenge, and current nomenclature is confusing.

Genomic features are used to define genera, particularly the organization of each RNA genome segment and the sequences of conserved nucleotides at the termini of each segment.

Classical serological methods are used to classify these viruses further.

In general, antigenic determinants on the nucleocapsid protein are relatively conserved, and so serve to define broad groupings among the viruses,

Shared epitopes on the envelope gps, which are the targets in neutralization and hemagglutination-inhibition assays, define narrow groupings (serogroups).

Unique epitopes on envelope gps, also determined by neutralization assays, define individual virus species.

With few exceptions, viruses within a given genus are related antigenically to each other, but not to viruses in other genera.

The lack of adequate biochemical characterization of many named bunyaviruses confuses their precise classification.

Genetic reassortment occurs when cultured cells or mosquitoes are coinfected with closely related bunyaviruses.

Within its particular ecologic niche, each bunyavirus evolves by genetic drift and selection;

- **Isolates of La Crosse virus from d/t regions in the US differ considerably, as a result of cumulative point mutations and nucleotide deletions and duplications.**

The evolution of La Crosse virus has also involved genome segment reassortment, and reassortant viruses have been isolated from mosquitoes in the field.

The bunyaviruses are assigned to five genera, four of which include viruses that infect animals and a fifth (the genus *Tospovirus*) that contains only plant viruses.

A very substantial number of bunyaviruses have not yet been assigned to a genus or serogroup.

The genus *Orthobunyavirus* contains a large number of viruses that share common genetic features and are serologically unrelated to viruses in other genera of the *Bunyaviridae*.

Most of these viruses are mosquito-borne, but some are transmitted by sandflies or *Culicoides* spp.

The genus includes a number of pathogens of domestic animals and humans, including Akabane and La Crosse viruses and their relatives.

The genus *Phlebovirus* contains over 50 viruses, all of which are transmitted by sandflies or mosquitoes.

The genus contains important pathogens, including Rift Valley fever virus and the sandfly fever viruses.

The genus *Nairovirus* contains a large number of viruses, most of which are tick-borne, including the pathogens Nairobi sheep disease and Crimean-Congo hemorrhagic fever viruses.

The genus *Hantavirus* also includes a substantial number of viruses, many of them relatively recently discovered.

All are transmitted by persistently infected reservoir rodent hosts via urine, feces, and saliva; the same transmission pattern has occurred among rats in lab colonies.

In humans, several of these viruses from Asia cause hemorrhagic fever with renal syndrome, whereas those from Europe are typically associated with a d/t and less severe disease syndrome designated “neuropathica epidemica.”

Some of the hantaviruses from the Americas cause a severe acute respiratory distress syndrome referred to as “hantavirus pulmonary syndrome.”

VIRION PROPERTIES

Morphological properties vary among viruses in the various genera, but bunyavirus virions are spherical, ~80–120 nm in diameter,

are composed of a lipid envelope with gp spikes, inside which are three circular ribonucleoprotein (RNP).

These RNP complexes are stabilized by a panhandle structure generated by non-covalent bonds b/n inverted palindromic sequences on the 3' and 5' ends of each RNA genome segment.

The terminal sequences are identical for all three RNA segments within each virus spp, and are critical for recognition by the viral polymerase for virus genome replication and initiation of virus mRNA transcription.

The genome of bunyaviruses is 11–19 kb and consists of three segments of negative-sense (or ambisense), ssRNA, designated large (L), medium (M), and small (S).

The RNA segments differ in size among the genera:

the L RNA segment ranges in size from 6.3 to 12 kb, the M RNA segment from 3.5 to 6 kb, and the S RNA segment from 1 to 2.2 kb.

The L RNA encodes a single large protein (L), the RdRp (transcriptase).

The M RNA encodes a polyprotein that is processed to form two gps (Gn and Gc) and, in some cases, a NS protein (NSm).

The S RNA encodes the nucleocapsid (N) protein and, for members of the *Orthobunyavirus* and *Phlebovirus* genera, a NS protein.

The N and NSs proteins of viruses in the genus *Phlebovirus* are each translated from a separate subgenomic mRNA.

The N protein is encoded in the 3' half of the S RNA, and its mRNA is transcribed using genomic RNA as template.

However, the NSs protein, occupying the 5' half of the same S RNA molecule, is encoded in the reverse complementary sense, with the NSs mRNA being transcribed only after the synthesis of full-length viral genome RNA intermediates;

- **thus the S segment RNA exhibits an ambisense coding strategy.**

All bunyaviruses have at least four virion proteins,

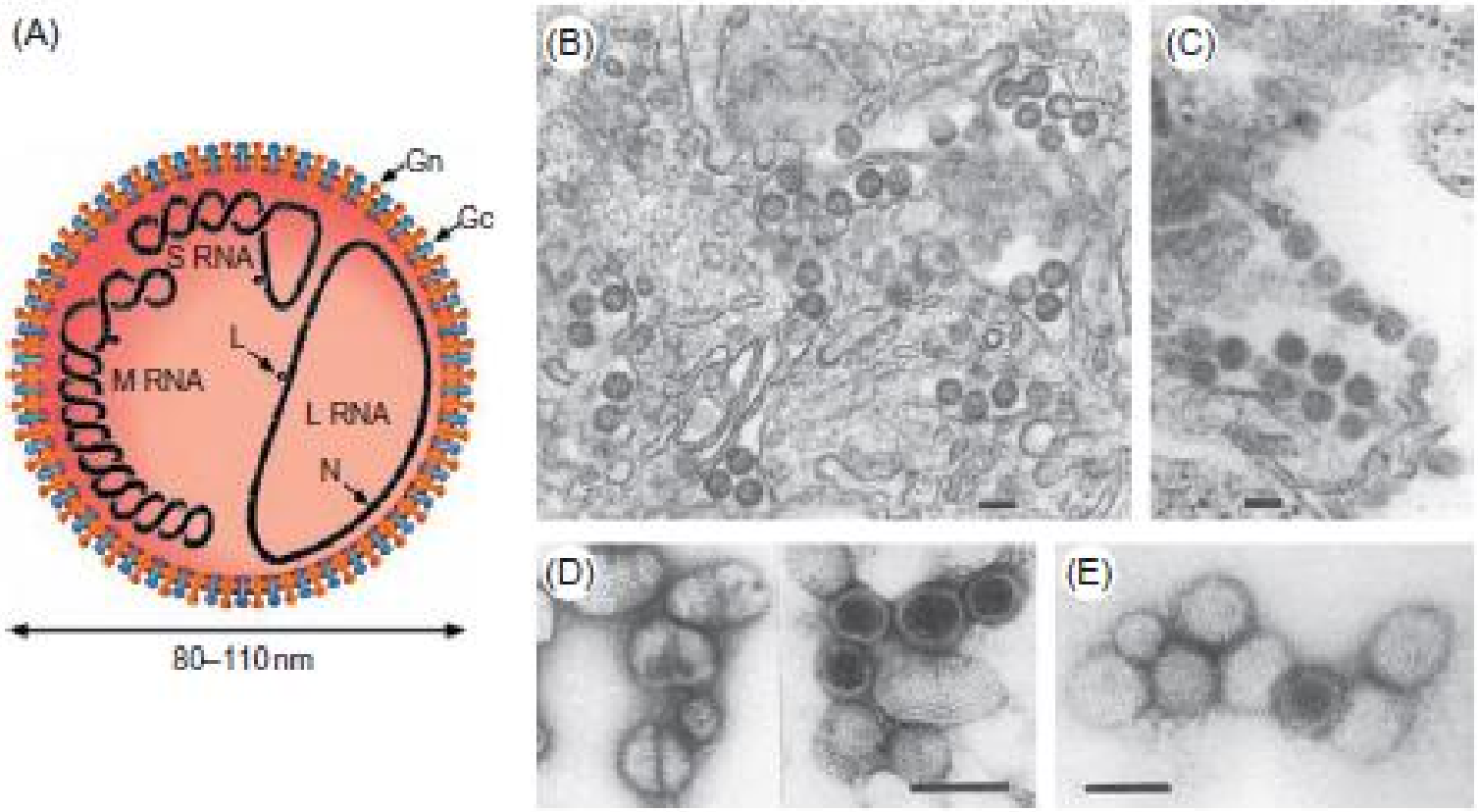
- **two external gps(Gn, Gc), the L protein (transcriptase), and the N protein (nucleoprotein).**

Virions also contain lipids, with their composition reflecting the composition of host CM (principally derived from the Golgi membrane, but also cell-surface membrane) and carbohydrates as side chains on the gps.

The Gn gp (formerly known as G2) is responsible for receptor binding of California serogroups bunyaviruses.

The NSs protein of RVFV interferes with the innate host-cell antiviral response via inhibition of the cell signaling molecules (protein kinase and the transcription factor, TFIIH), leading to global suppression of the type I interferon response.

The viruses are quite sensitive to heat and acid conditions, and are inactivated readily by detergents, lipid solvents, and common disinfectants.



Diagrammatic representation of a bunyavirus virion in cross section. Family Bunyaviridae. (A) ***Gc, Gn, glycoproteins produced by*** processing of M RNA polyprotein; L, transcriptase encoded by L RNA; L, M, and S RNA, large, medium, and small RNA segments; N, nucleoprotein encoded by S RNA. (B) Hepatocyte of a rat infected with Rift Valley fever virus, showing virions budding in Golgi vesicles. (C) Thin section of mouse brain infected with California encephalitis virus, showing extracellular virions. (D) Negatively stained Hantaan virus virions, showing the pattern of spike placement in squares that is characteristic of all hantaviruses. (E) Negatively stained Rift Valley fever virus virions, showing the delicate spike fringe. Bars represent 100 nm

VIRUS REPLICATION

Most bunyaviruses replicate well in many kinds of cells, including Vero (African green monkey) cells, BHK-21 (baby hamster kidney) cells, and, except for hantaviruses, C6/36 mosquito (*Aedes albopictus*) cells.

Except for hantaviruses and some nairoviruses, these viruses are cytolytic for mammalian cells, but are non-cytolytic for invertebrate cells.

Most of the viruses also replicate to high titer in suckling mouse brain.

Viral entry into its host cell is by receptor-mediated endocytosis;

All subsequent steps take place in the cytoplasm.

Cell receptors are not described for many bunyaviruses, but those that contribute to binding of the hantaviruses include αβ integrins and other cell receptor proteins such as gC1qR/ p32, which is expressed on endothelial cells, dendritic cells, lymphocytes, and platelets.

Because the genome of the negative-sense ssRNA viruses cannot be translated directly.

The first step after penetration of the host cell and uncoating is the activation of the virion RNA polymerase and its transcription of viral mRNAs from each of the three virion RNAs.

The exception, as noted earlier, is that in the genus *Phlebovirus* the 5' half of the S RNA is not transcribed directly; instead, the mRNA for the NSs protein is transcribed after synthesis of full-length complementary RNA.

The RNA polymerase also has endonuclease activity, cleaving 5'-methylated caps from host mRNAs and adding these to viral mRNAs to prime transcription (cap snatching).

After primary viral mRNA transcription and translation, replication of the virion RNA occurs and a second round of transcription begins, amplifying, in particular, the genes that encode structural proteins necessary for virion synthesis.

Virions mature by budding through intracytoplasmic vesicles associated with the Golgi complex and are released by the transport of vesicles through the cytoplasm and release by exocytosis from the apical and/or basolateral PM.

Genus	Virus	Geographic Distribution	Arthropod Vector	Target Host Species or Amplifier Host	Disease in Animals	Disease in Humans
Phlebovirus	Rift Valley fever virus	Africa	Mosquitoes	Sheep, cattle, buffalo, humans	Systemic disease, hepatitis, abortion	Flu-like illness, hepatitis, hemorrhagic fever, retinitis
Nairovirus	Nairobi sheep disease virus	Eastern Africa	Ticks	Sheep, goats	Hemorrhagic enteritis	Mild febrile illness
	Crimean-Congo hemorrhagic fever virus	Africa, Asia, Europe	Ticks	Sheep, cattle, goats, humans	Mild if any	Hemorrhagic fever, hepatitis

Genus	Virus	Geographic Distribution	Arthropod Vector	Target Host Species or Amplifier Host	Disease in Animals	Disease in Humans
Bunyavirus	Akabane virus	Australia, Japan, Israel, Africa	Mosquitoes, Culicoides	Cattle, sheep	Arthrogryposis, hydranencephaly	None
	Cache Valley virus	United States	Mosquitoes	Cattle, sheep	Arthrogryposis, hydranencephaly rarely	Very rarely congenital infection
	La Crosse and other California encephalitis group viruses	North America	Mosquitoes	Small mammals, humans	None	Encephalitis
Hantavirus	Hantaan virus	China, Russia, Korea	None	*Apodemus agrarius* (striped field mouse)	None documented	Hemorrhagic fever with renal syndrome
	Puumala virus	Scandinavia, Europe, Russia	None	*Clethrionomys glareolus* (bank vole)	None documented	Hemorrhagic fever with renal syndrome
	Seoul virus	Worldwide	None	*Rattus norvegicus* (Norway rat)	None documented	Hemorrhagic fever with renal syndrome
	Sin Nombre virus and other New World hantaviruses	The Americas	None	*Peromyscus maniculatus* (deer mouse) and other reservoir rodent species	None documented	Hantavirus pulmonary syndrome

FLAVIVIRIDAE

The family *Flaviviridae* comprises three genera (*Flavivirus, Pestivirus* and *Hepacivirus*), the members of which, although similar in genomic organization and physicochemical properties, are genetically distinct and biologically quite different.

The genus *Flavivirus* contains at least 70 viruses; several of which are of vet importance,

- **Japanese encephalitis, West Nile, louping ill, and Wesselsbron viruses.**

Some 30 members of this genus are arthropod-borne human pathogens, the causative agents of diseases varying from fevers with rash to life-threatening hemorrhagic fevers to encephalitis to hepatic necrosis.

Members such as the four dengue viruses, West Nile virus, Japanese encephalitis virus, and several tick-borne encephalitis viruses rank among the most imp't human viral pathogens.

The genus *Pestivirus* contains important vet pathogens,

- **BVDV, border disease virus of sheep, and CSFV.**

The genus *Hepacivirus* contains only the human pathogens, hepatitis C and the inappropriately named hepatitis G viruses.

Yellow fever virus, the prototype of the genus *Flavivirus*, was discovered in the course of investigating epidemic yellow fever.

Yellow fever was one of the great scourges of humankind during the 18th and 19th centuries, with epidemics repeatedly affecting coastal cities in the Americas, Europe, and West Africa, transmitted by the mosquito, *Aedes aegypti* .

Following the discovery of the virus and its vector, mosquito eradication programs quickly eliminated the disease from cities in the western hemisphere.

Hemispheric eradication was envisioned, but in 1932 the enzootic/zoonotic jungle cycle involving monkeys and jungle-canopy mosquitoes was discovered—a cycle that precludes eradication.

The global significance and impact of other members of the genus are increasing, especially dengue, Japanese encephalitis and West Nile viruses.

Members of the genus *Pestivirus* occur worldwide as economically important veterinary pathogens.

CSF has been conjectured that the virus might have emerged at that time by species jumping—that is, by a host-range mutation of another pestivirus.

Early in the 20th century, as intensive swine production expanded, CSF became a most imp't disease in developed countries.

Subsequent eradication programs were so successful that today it is reintroductions of the virus, rather than enzootic infection, that attract attention.

BVD was first described in New York in 1946 as an apparently new disease of cattle.

Mucosal disease was described in 1953, and is another clinical entity caused by the same virus, but with markedly d/t severity and herd incidence pattern.

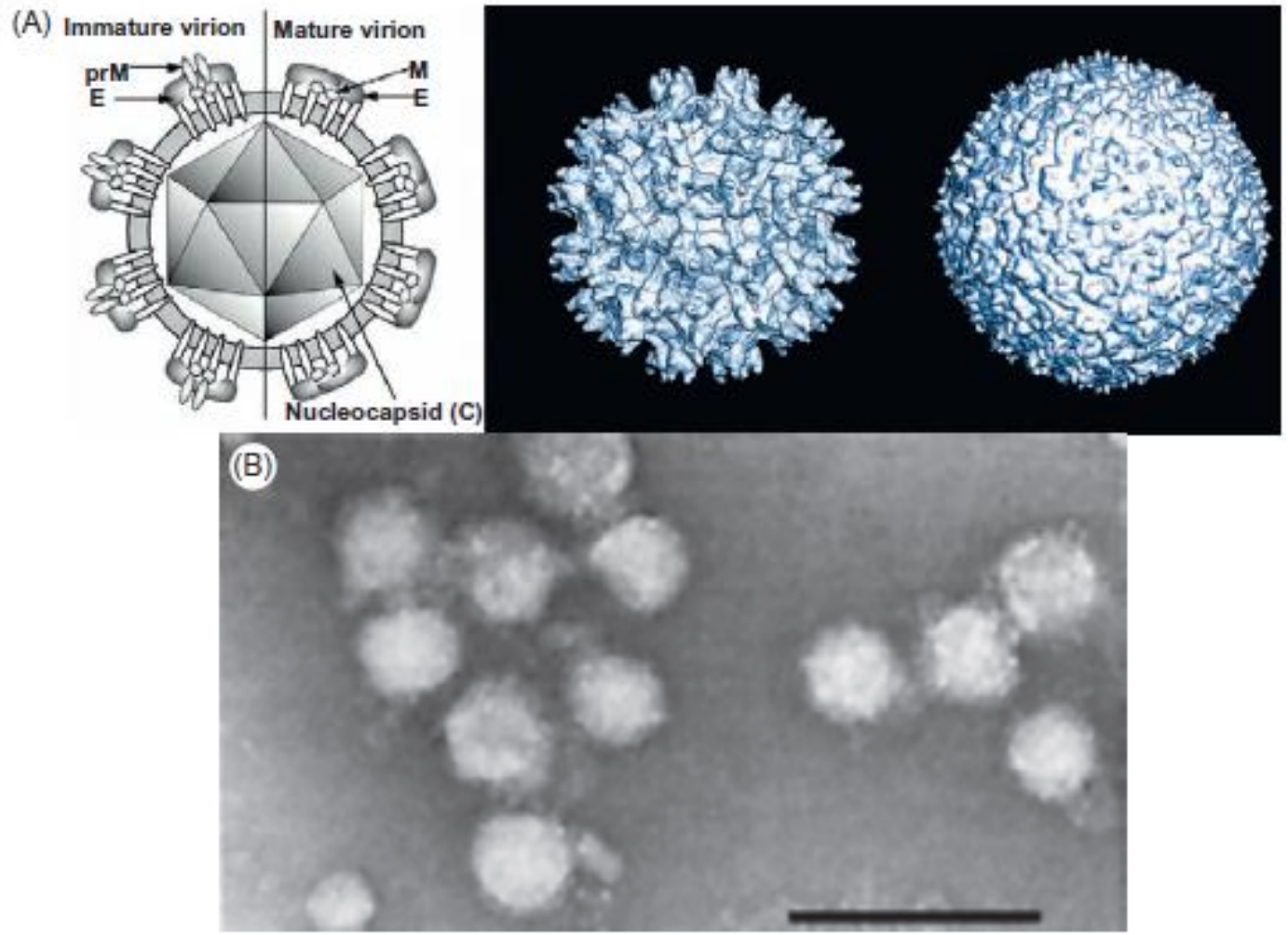
Border disease originally was described in 1959 in sheep in the border region b/n Wales and England, and the infection still is common in intensive sheep production areas worldwide.

Vertical transmission of border disease virus is characteristic of infection of pregnant ewes, and the resultant fetal abnormalities have given rise to colloquial names such as "hairy shaker disease" and "fuzzy lamb syndrome."

Hepatitis C virus was discovered in 1989 by a *tour de force* of modern molecular biology.

Although the virus still has not been successfully grown in either cell culture or laboratory animals other than the chimpanzee, it has been completely sequenced and its diagnosis made routine using reagents produced with recombinant DNA technology.

This success now serves as a model for the detection, characterization, and diagnosis of other uncultivable viruses.



Family *Flaviviridae, genus Flavivirus.* *(A) (Left), Schematic of immature and mature virion. (Center and right), Three-dimensional* cryo-electron microscopic reconstructions of immature and mature particles of an isolate of Dengue virus. C, nucleocapsid protein; E, major spike protein; M, transmembrane protein; prM, precursor glycoprotein. (B) Central European tick-borne encephalitis virus. Negative-stain electron microscopy. Bar represents 100 nm.

PROPERTIES OF FLAVIVIRUSES

Classification

The genus *Flavivirus* includes the vet pathogens, Japanese encephalitis, West Nile, Wesselsbron, and louping ill viruses, in addition to many important human pathogens, including the dengue viruses.

Members of the genus are subdivided on the basis of their mode of transmission into four groups:

- **tick-borne viruses;**
- **mosquitoborne viruses;**
- **viruses with no known arthropod vector;**
- **viruses with no known animal host.**

The mosquito- and tick-borne flaviviruses are maintained in nature in arthropod–vertebrate–arthropod cycles, whereas the non-arthropod-borne viruses are transmitted directly among bats or rodents.

The genus *Pestivirus* includes BVDV, border disease virus, and CSF.

Genomic sequence analysis indicates that these three viruses are related very closely.

Segregation of individual viruses can be difficult, and is based on sequence analysis, including that of the 5'-UTR of the genome, serological analysis with type-specific antisera, and host spp of origin.

Experimentally, the viruses have an overlapping host spectrum: swine fever virus can be transmitted to cattle; bovine viral diarrhea virus can infect swine, sheep, goats, New World camelids, and a variety of other wild and domestic ungulates, including deer, antelope, and buffalo; border disease virus infection rarely has been documented in cattle.

A putative pestivirus of giraffe has been described, but is otherwise uncharacterized, and a highly pathogenic and genetically novel pestivirus in pigs (Bungowannah virus) recently was described in Australia.

VIRION PROPERTIES

Virions are spherical, 50 nm (flaviviruses) or 40–60 nm (pestiviruses) in diameter, and consist of a tightly adherent lipid envelope that may display indistinct glycoprotein spikes, surrounding a spherical nucleocapsid with icosahedral symmetry.

The genome consists of a single molecule of linear, positive-sense, ssRNA of approximately 11, 12.3, and 9.6 kb for flavi-, pesti-, and hepaciviruses, respectively.

Flaviviruses contain a 5'-terminal cap structure, whereas pestiviruses and hepaciviruses do not.

The viral genome contains one long open reading frame encoding 10 or more proteins, which are created by co- and post-translational processing and cleavage of a single, large polyprotein.

Virions contain three (genus *Flavivirus*) or four (genus *Pestivirus*) structural proteins that are encoded in the 5' end of the genome; the non-structural proteins are encoded in the 3' end.

Structural proteins of flaviviruses include: C, the nucleocapsid protein; prM, a precursor glycoprotein that is cleaved during virus maturation to yield M, the transmembrane protein; E, the major spike glycoprotein, which also is the major target for neutralizing antibodies.

Pestiviruses have four structural proteins, including C, the nucleocapsid protein, and the Erns, E1, and E2 envelope glycoproteins.

There are seven or eight non-structural proteins, including NS5, the RNA-dependent RNA polymerase, and NS3, which has several functions, including helicase and protease activities, in addition to contributing to the RNA polymerase complex.

NS2B and NS3 largely are responsible for cleavage of the polyprotein, and host-cell proteases are responsible for the remainder of this processing.

Pestiviruses encode a unique non-structural protein, Npro, that autocatalytically releases itself from the polyprotein; this protein is not essential for virus replication in cell culture, but modulates interferon responses in infected cells.

The viruses are inactivated easily by heat and by common disinfectants, and the lipid envelope is susceptible to organic solvents.

However, the stability of swine fever virus in meat products and offal for weeks or even months has contributed importantly to its spread and reintroduction into previously virus-free areas.

VIRUS REPLICATION

Members of the genus *Flavivirus* replicate well and cause cytopathic changes in many cell cultures:

- **Vero (African green monkey kidney), BHK-21 (baby hamster kidney), and mosquito (C6/36) and primary chick and duck embryo fibroblasts are commonly used for isolation and propagation of these viruses.**

Many flaviviruses infect and kill newborn and, in some cases, adult mice; indeed, most of the flaviviruses were first isolated in newborn mice.

Members of the genus *Pestivirus* generally replicate well in primary and continuous cell cultures derived from the principal host species—bovine viral diarrhea virus in bovine embryonic fibroblast or kidney cells.

Border disease virus is best isolated in ovine cells, and classical swine fever virus in porcine lymphoid or kidney cells.

Pestiviruses isolated from naturally infected animals are predominantly non-cytopathic in cell culture.

Cellular attachment of all members of the family *Flaviridae*, regardless of genus, appears to be mediated by ligands on the E glycoprotein(s), although cellular receptors have not been unambiguously identified.

The viruses enter cells via receptor-mediated endocytosis and replication takes place in the cytoplasm.

Flaviviruses only partially shut down protein and RNA synthesis of mammalian host cells.

Infection commonly is accompanied by a characteristic proliferation of perinuclear membranes.

Replication involves the synthesis of complementary negative-sense RNA, which then serves as a template for positive-sense (genome-sense) RNA synthesis.

The only viral mRNA is the genomic RNA—translation yields a single polyprotein that is cleaved and processed to form the various viral structural and non-structural proteins.

For mosquito-transmitted flaviviruses, virion assembly occurs on membranes of the endoplasmic reticulum and plasma membrane in mosquito cells, but preformed capsids and budding are not seen.

Instead, fully formed virions appear within the cisternae of the endoplasmic reticulum and are released via exocytosis or cell lysis.

CORONAVIRIDAE

The family *Coronaviridae* comprises at least two genera.

Genus *Coronavirus,* contains a substantial number of pathogens of mammals and birds that individually cause a remarkable variety of diseases,

- **Pneumonia, reproductive disease, enteritis, polyserositis, sialodacryoadenitis, hepatitis, encephalomyelitis, nephritis, and various other disorders.**

Coronavirus and coronavirus-like infections have been described in swine, cattle, horses, cats, dogs, rats, birds, bats, rabbits, ferrets, mink, and various wildlife species.

Many coronavirus infections are subclinical or asymptomatic.

In humans, coronaviruses are included in the spectrum of viruses that cause the common cold and, recently, SARS, which is a zoonosis.

Berne virus neutralizing Abs have been detected in sera of sheep, goats, rabbits, and mice, and torovirus-like particles have also been observed by EM in feces of swine, cats, turkeys, and humans.

A nidovirus from fish—white bream virus, which is most closely related to the toroviruses—recently was proposed as the prototype member of a new genus, *Bafinivirus*.

PROPERTIES OF CORONAVIRUSES

Classification

Despite profound differences in virion structure and genome size, coronaviruses, toroviruses, arteriviruses, and roniviruses exhibit remarkable similarities in their genome organization and replication strategy.

In infected cells, these viruses all utilize a distinctive "nested set" transcription strategy in which the expression of genes encoding structural viral proteins is mediated via a nested set of 3' co-terminal subgenomic mRNAs.

This unique strategy has been recognized by the establishment of the order *Nidovirales (nidus, nest)*, encompassing the family *Coronaviridae*, with two genera (*Coronavirus* and *Torovirus*), the family *Arteriviridae*, with one genus (*Arterivirus*), and the family *Roniviridae* containing invertebrate nidoviruses.

Sequence analysis of the gene encoding portions of the viral RdRp (transcriptase) suggests that the member viruses of the order *Nidovirales* probably evolved from a common ancestor.

Extensive genome rearrangements through heterologous RNA recombination have resulted in the variations seen—that is, viruses with similar replication and transcription strategies but disparate structural features.

The genus *Coronavirus* can be subdivided into at least three cluster groups on the basis of genetic and serologic properties, with subgroups in two of these.

- **Group 1a includes transmissible gastroenteritis virus of swine, porcine respiratory coronavirus, canine coronavirus, feline enteric coronavirus (feline infectious peritonitis virus), ferret and mink coronaviruses, and spotted hyena coronavirus.**
- **Group 1b includes certain human coronaviruses, porcine epidemic diarrhea virus, and bat coronavirus.**

Group 2a includes mouse hepatitis virus, bovine coronavirus, sialodacryoadenitis virus of rats, porcine hemagglutinating encephalomyelitis virus, canine respiratory coronavirus, and other human coronaviruses.

Group 2b includes human SARS coronavirus and civet cat, raccoon dog, and horseshoe bat coronaviruses.

Group 3 includes avian infectious bronchitis virus, turkey coronavirus, and several potential but still largely uncharacterized new species from ducks, geese, and pigeons.

Further taxonomic subdivision of these viruses is likely in the future.

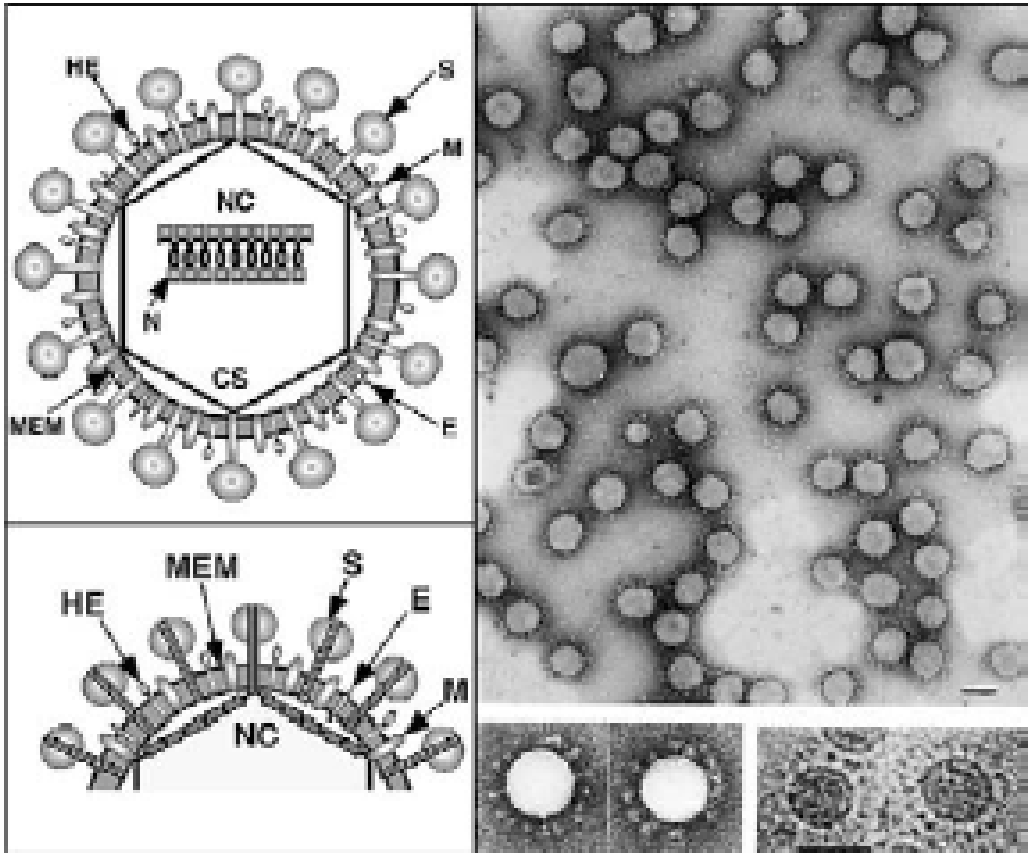
Viruses in the genus *Torovirus* are all apparently closely related and genetically distinct from coronaviruses; however, many toroviruses have yet to be fully characterized.

VIRION PROPERTIES

Coronaviridae **are enveloped, 80–220 nm in size, pleomorphic although often spherical (coronaviruses), or 120–140 nm in size and disc, kidney shaped**

They have large (20 nm long) club-shaped spikes enclosing what appears to be an icosahedral internal core structure within which is a helical nucleocapsid.

Some coronaviruses also have a second fringe of shorter (5 nm long) spikes (hemagglutinin).



Structure of coronavirus virions. (Top left) Schematic diagram of virus structure; (Bottom left) Diagram of virion surface. (Top right) Electron micrograph of virus particles of transmissible gastroenteritis virus (TGEV) stained with uranyl acetate (top right) or sodium phosphotungstate (insert bottom left) showing the surfae of the virus particles. The spikes (peplomers) are better defined using sodium phosphotungstate. (insert bottom right) Cryo-electron microscopic visualization of unstained TGEV in vitreous ice. The particles contain an internal structure inside the viral envelope and well extended peplomers. MEM, lipid membrane; S, spike protein; M, large membrane protein, E, small envelope protein; HE, hemagglutinin-esterase; N, nucleocapsid protein; CS, core-shell; NC, nucleocapsid. The bars represents 100 nm.

The genome of the family *Coronaviridae* consists of a single molecule of linear positive-sense, SSRNA, 27.6–31 kb in size for coronaviruses and 25–30 kb for toroviruses, the largest known non-segmented RNA viral genomes.

The genomic RNA is 5' capped and 3' polyadenylated, and is infectious.

The major virion proteins of the genus *Coronavirus and Torovirus* include a nucleocapsid protein (N, 50–60 kDa, 19 kDa) and several envelope/spike proteins:

- **the major spike glycoprotein (S, 180–220 kDa);**
- **a triple-spanning transmembrane protein (M, 23–35 kDa);**
- **a minor transmembrane protein (E, 9–12 kDa), which together with the M protein is essential for coronavirus virion assembly.**

Toroviruses lack a homolog of the coronavirus E protein, which may explain the structural differences b/n the coronaviruses and toroviruses.

The secondary, smaller spikes, seen in some group 2 coronaviruses and in toroviruses, consist of a dimer of a class I membrane protein (65 kDa), a hemagglutinin esterase (HE) that shares 30% sequence identity with the N-terminal subunit of the HE fusion protein of influenza C virus.

- Sequence comparisons indicate that the *HE* genes of coronaviruses, toroviruses, and orthomyxoviruses were acquired by independent, non-homologous recombination events (probably from the host cell).

Although there is no sequence similarity b/n the torovirus proteins and their counterparts in coronaviruses, they are similar in structure and function, and are related phylogenetically.

Virus neutralizing Abs generated are directed at the surface gps, with the majority being conformational epitopes located at the N-terminal portion of the S protein.

Cellular immune responses are principally directed toward the S and N proteins.

Besides the canonical structural proteins, coronaviruses are unique among nidoviruses b/c their genomes encode (within differing regions) variable numbers of accessory proteins (4/5 in most; 8 in the SARS).

They are dispensable for *in-vitro* virus replication, but which increase virus fitness *in vivo*.

The accessory proteins encoded by the SARS virus ORF 3b and 6, are antagonists of innate immune responses,

- specifically interfering with the development of type I interferon responses;
- the specific roles of other accessory proteins are still largely unknown.

The accessory proteins have homologous versions within coronavirus groups, but lack similarity with proteins in different groups.

In group 2 coronaviruses, the HE protein is considered an accessory protein.

- mouse hepatitis virus HE-deletion mutants replicate like wild-type virus *in vitro,* but in mice they have an attenuated phenotype.

VIRUS REPLICATION

The host spectrum of individual coronaviruses appears to be largely determined by the S protein, portions of which mediate receptor binding and virus cell fusion that occur at either the PM or within endosomes of susceptible cells.

Individual coronaviruses utilize a variety of cellular proteins as receptors.

Aminopeptidase N serves as a receptor for several group 1 coronaviruses.

SARS utilize angiotensin converting enzyme 2.

Mouse hepatitis virus utilizes carcino-embryonic antigen-related cell adhesion molecule 1 (CEACAM-1), and other group 2 coronaviruses utilize *N-acetyl-9-O-acetyl neuraminic acid*.

The functional receptor for group 3 coronaviruses such as infectious bronchitis virus is undefined, although heparan sulfate and sialic acid residues may serve as non-specific attachment factors.

The strategy of expression of the coronavirus genome is complex.

First, the viral RNA serves as mRNA for synthesis of the RdRp.

The two large ORF encoding the units of the polymerase are translated—the larger via ribosomal frameshifting—as a single polyprotein that is then cleaved.

These proteins then assemble to form the active RNA polymerase.

This enzyme is then used to transcribe full-length complementary (negative-sense) RNA, from which in turn are transcribed, and also a 3' co-terminal nested set of subgenomic mRNAs.

The nested set comprises up to 10 overlapping mRNAs that extend for d/t lengths from common 3' ends and share a common 5' leader sequence.

They are generated by a leader-primed mechanism of discontinuous transcription:

- **the polymerase first transcribes the noncoding leader sequence from the 3' end of the complementary (negative-sense) RNA.**

The capped leader RNA then dissociates from the template and reassociates with a complementary sequence at the start of any one of the genes, to continue copying the template right through to its 5' end.

Only the unique sequence that is not shared with the next smallest mRNA in the nested set is translated;

- **this strategy yields the various viral proteins in regulated amounts.**

Intergenic sequences serve as promoters and attenuators of transcription.

Torovirus transcription and replication apparently are similar to those of coronaviruses, except that there are no common 5' leader sequences on the mRNAs.

A puzzling finding is that subgenomic negative-sense RNAs complementary to the nested set of mRNAs are also present in torovirus-infected cells.

The fact that these subgenomic RNAs contain 5'- and 3'-terminal sequences that are identical to those of genomic RNA implies that they may function as replicons.

The synthesis, processing, oligomerization, and transport of the several envelope glycoproteins of coronaviruses display some unusual features.

E.g., the envelope protein M, which in some coronaviruses contains *O-linked* rather than *N-linked glycans,* is directed exclusively to cisternae of the ER and other pre-Golgi membranes.

As a result, virions bud only there and not from the PM.

Virions are then transported in vesicles to the PM and are released by exocytosis.

After their release, many of the mature enveloped virions remain adherent to the outside of the cell.

In addition to the accumulation of point mutations as a result of polymerase errors during transcription, genetic recombination occurs at high frequency b/n the genomes of different but related coronaviruses.

This may be an important mechanism for the generation of the genetic diversity seen with these viruses in nature, and provides a constant potential source of new viruses with novel phenotypic properties, including host spp tropism and virulence.

Coronavirus or Torovirus	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Group 1a			
Feline enteric coronavirus (formerly feline infectious peritonitis virus)	Peritonitis, pneumonia, meningoencephalitis, panophthalmitis, wasting syndrome Anorexia, chronic fever, malaise, weight loss, abdominal enlargement, CNS signs	Direct contact; fecal–oral route from maternal shedding Feces, blood, body fluids	Attenuated (TS) vaccine Interruption of transmission cycle, quarantine, high-level hygiene
Canine coronavirus	Mild gastroenteritis Mild diarrhea	Ingestion by fecal–oral route Acute feces; small intestinal sections or smears	Inactivated vaccine
Transmissible gastroenteritis virus of swine	Gastroenteritis Watery diarrhea, vomiting, dehydration	Fecal–oral route Acute feces; small intestinal sections or smears	Oral attenuated vaccine to pregnant sows Good sanitation
Porcine respiratory coronavirus	Interstitial pneumonia Mild respiratory disease or subclinical	Aerosols Nasal swabs; trachea, lung sections	No vaccine available

Coronavirus or Torovirus	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Group 1b			
Porcine epidemic diarrhea virus	Gastroenteritis Watery diarrhea, vomiting, dehydration	Fecal–oral route Acute feces; small intestinal sections or smears	Oral attenuated virus vaccine (Asia) to pregnant sows
Group 2a			
Porcine hemagglutinating encephalomyelitis virus	Vomiting, wasting disease, encephalomyelitis Anorexia, hyperesthesia, muscle tremors, emaciation	Aerosols, oronasal secretions Nasal swabs, tonsil, lung, brain	Good husbandry, maintain immune sows No vaccine available
Mouse hepatitis virus	Enteritis, hepatitis, nephritis, demyelinating encephalomyelitis Various	Introduction of virus into a naïve colony: aerosols and direct contact, Target tissues, secretions	Depopulation Preventive quarantine
Sialodacryoadenitis virus of rats	Inflammation and necrosis of salivary and nasolacrimal glands Lacrimation, anorexia, weight loss, chromodacryorrhea	Direct contact, fomites, and aerosols Nasopharyngeal aspirates, respiratory tissues	Depopulation and repopulation, preventive quarantine
Bovine coronavirus	Gastroenteritis, winter dysentery, shipping fever Profuse or bloody diarrhea, dehydration, decreased milk, respiratory disease	Fecal–oral route, aerosols, respiratory droplets Feces, large intestinal sections or smears, nasal swabs, lung sections	Maternal immunization: inactivated or attenuated vaccines; no vaccine for winter dysentery

Coronavirus or Torovirus	Disease / Symptoms	Transmission / Diagnostic Specimen	Prevention / Control
Group 2b			
SARS coronavirus (humans)	Severe acute respiratory syndrome (10% patients) Fever, myalgia, diarrhea, dyspnea	Aerosol droplets, ?fecal–oral route Nasopharyngeal aspirates, stools, serum	Quarantine, stringent isolation of patients
SARS coronavirus (civet cats, bats)	Subclinical?	Fecal–oral route Feces	Testing and depopulation of animals in live markets
Group 3			
Avian infectious bronchitis virus	Tracheobronchitis, nephritis Rales, decreased egg production	Aerosols and ingestion of food contaminated with feces Tracheal swabs and tissue, cloacal swabs, cecal tonsils, kidney	Multivalent attenuated and inactivated vaccines available Good sanitation and testing
Turkey coronavirus, Bluecomb virus	Enteritis Diarrhea, depression, cyanotic skin	Fecal–oral route, aerosol Feces, intestinal sections or smears	Inactivated virus vaccine
Torovirus			
Breda virus (cattle)	Enteritis Diarrhea, dehydration	Fecal–oral route Feces, large intestinal sections or smears	No vaccine available