RHABDOVIRIDAE

Rhabdos (greek)-rod

Pathogens of mammals, birds, fish, plants

In the order ***Mononegavirales*** which also includes families *Bornaviridae*, *Filoviridae*, *Paramyxoviridae*

Classification

Group: **Group V ((-)ssRNA)**

Order: *Mononegavirales*

Family: ***Rhabdoviridae***

Genera

Cytorhabdovirus	
Lyssavirus	**Rabies**
Ephemerovirus	**Bovine ephimeral fever**
Nucleorhabdovirus	
Novirhabdovirus	Hematopoetic necrosis virus of fish
Vesiculovirus	**Vesicular Stomatitis Virus**
Dichorhavirus	Viruses of plants

- The family ***Rhabdoviridae*** includes **4 subfamilies**, 56 genera and 246 species of viruses.
- **Subfamily *Alpharhabdovirinae*** includes 33genera for viruses infecting only vertebrates, only invertebrates or vertebrate hosts and arthropod vectors. Important genera *includes*
  Genus *Ephemerovirus*, Genus *Lyssavirus*, Genus *Vesiculovirus*.
- **Subfamily *Betarhabdovirinae***
- The subfamily includes 9 genera for viruses infecting plant hosts and arthropod vectors.
- Eg. Genus *Cytorhabdovirus*, *Dichorhavirus*
- **Subfamily *Gammarhabdovirinae***
- The subfamily includes 2 genera infecting finfish
- **Subfamily *Deltarhabdovirinae- 11 genera***

Morphology

- **Bullet-shaped** or cone shaped or bacilliform virions are 70nm in diameter and 170nm in length.
- Enveloped with large peplomers and within this is a **helically coiled cylindrical nucleocapsid**.
- **Negative, single-stranded RNA** 11 to 15 kbp in size.
- Prototype for (-) RNA viruses
- **Vesicular stomatitis Indiana virus – type species**
- Replication in the cytoplasm.

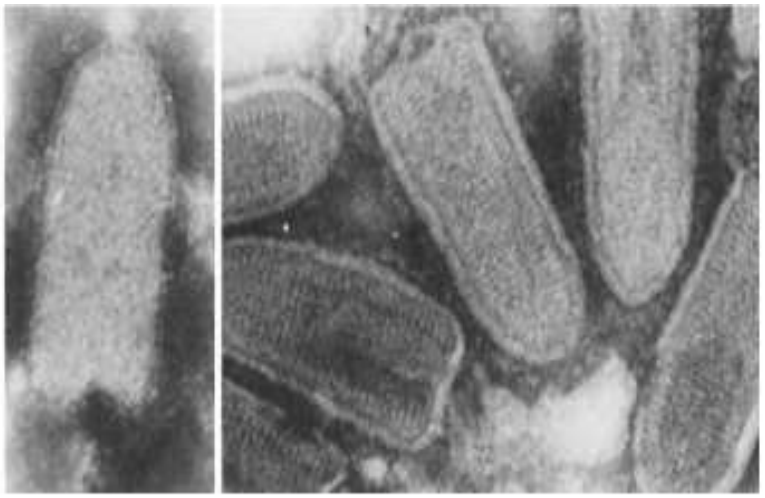
- They possess a three-layered structure: an outer lipid bilayer, a middle matrix protein layer, and an inner nucleocapsid composed of RNA and associated proteins.
- Peplomers consist of trimers of the viral envelope glycoprotein (G)
- A honeycomb pattern of peplomers is observed on the surface of some viruses

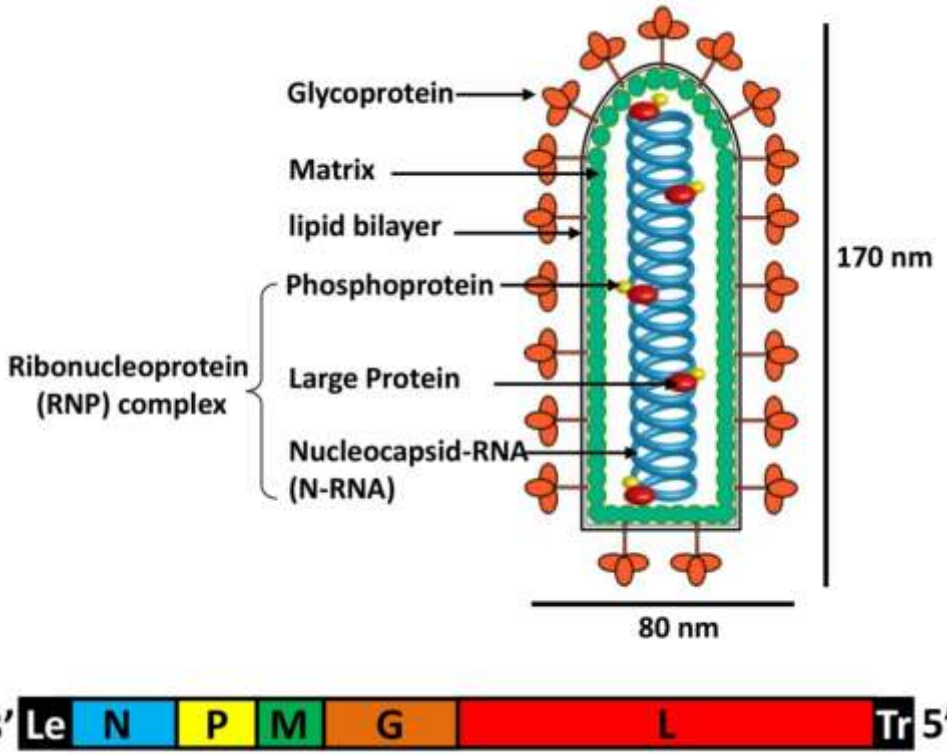
Genome

- The RNA has a 3′-terminal free hydroxyl group and a 5′-triphosphate and is not polyadenylated
- The ends have inverted complementary sequences encoding transcription and replication initiation signals.
- The nucleocapsid consists of a ribonucleoprotein (RNP) complex comprising the genomic RNA and tightly bound nucleoprotein (N) together with an RNA-dependent RNA polymerase (L) and polymerase-associated phosphoprotein (P).
- The individual genes are flanked by conserved transcription stop and start signals separated by short untranscribed intergenic sequences

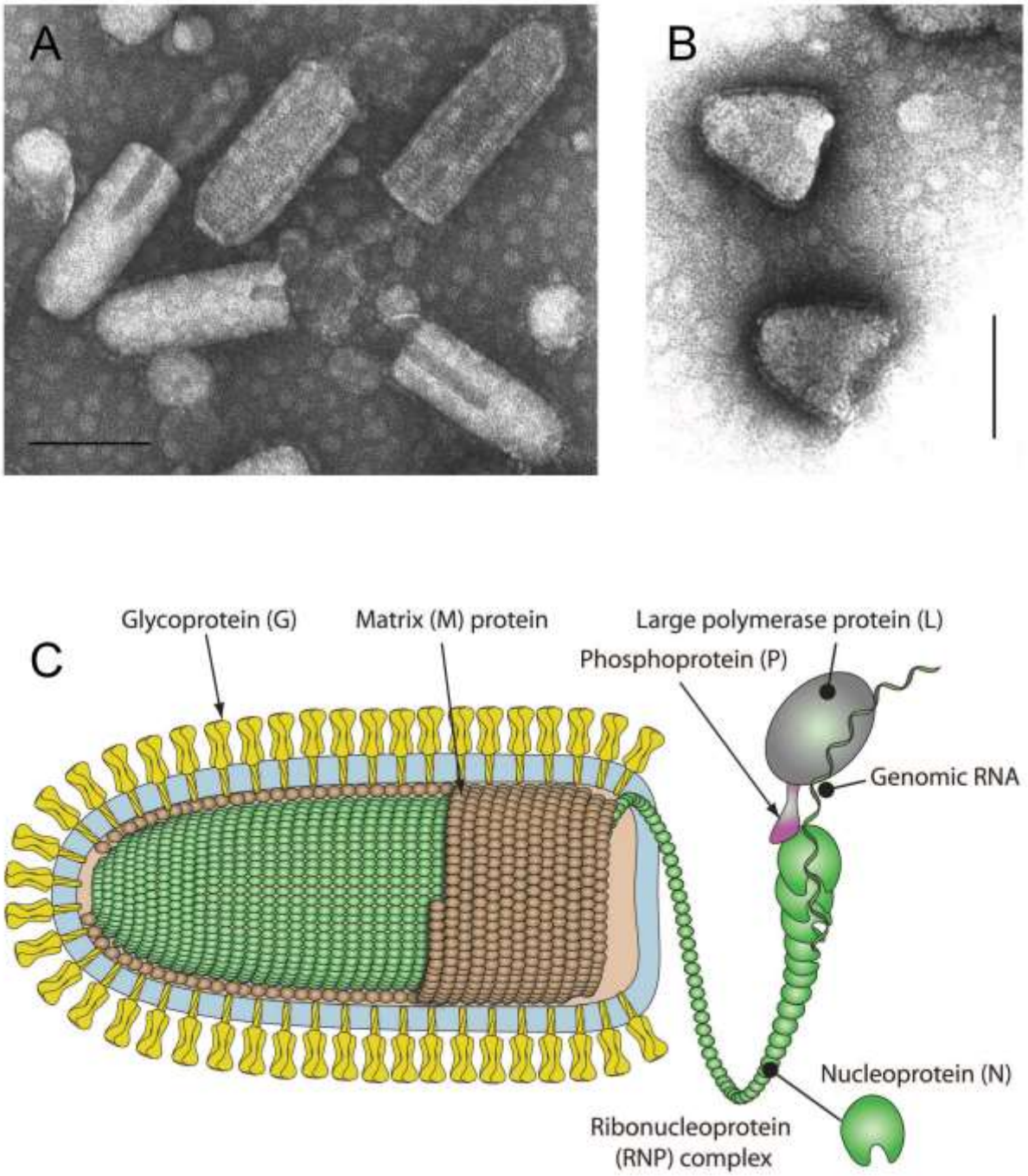
The genome encodes **five genes** in the order **3'-N-P-M-G-L-5‘.**

- **L** -the RNA-dependent RNA polymerase that functions in transcription and RNA replication;
- **G** -the glycoprotein that forms trimers that make up the peplomers
- **M** (or M2 for rabies virus)- Matrix protein that facilitates virion budding by binding to the nucleocapsid and to the cytoplasmic domain of the glycoprotein.
- **P-**(also called NS or M1), a component of the viral polymerase;
- **N** -the nucleoprotein, the major component of the viral nucleocapsid
- Three proteins (N, P, and L), in association with viral RNA, constitute the nucleocapsid and comprise the transcription complex





rabies virus (*left*) and vesicular stomatitis virus (*right*).


A
B
C
Glycoprotein (G)
Matrix (M) protein
Large polymerase protein (L)
Phosphoprotein (P)
Genomic RNA
Nucleoprotein (N)
Ribonucleoprotein
(RNP) complex

Transcription

- There is only a single promoter site, located at the 3' end of the viral genome; the polymerase (transcriptase) attaches to the genomic RNA template at this site and, as it moves along the viral RNA, it encounters stop/start signals at the boundaries of each of the viral genes.
- The stop signal present at the end of each gene comprises a stretch of U on which the viral polymerase acquires a stuttering behaviour.

- This is called **stop-start or stuttering transcription-** it also accounts for the addition of poly(A) tails on the 3' ends of each mRNA

Viral replication

1, Rhabdoviruses bind to the cell surface and are

(*2*) endocytosed. The envelope fuses with the endosome vesicle membrane to deliver the nucleocapsid to the cytoplasm.

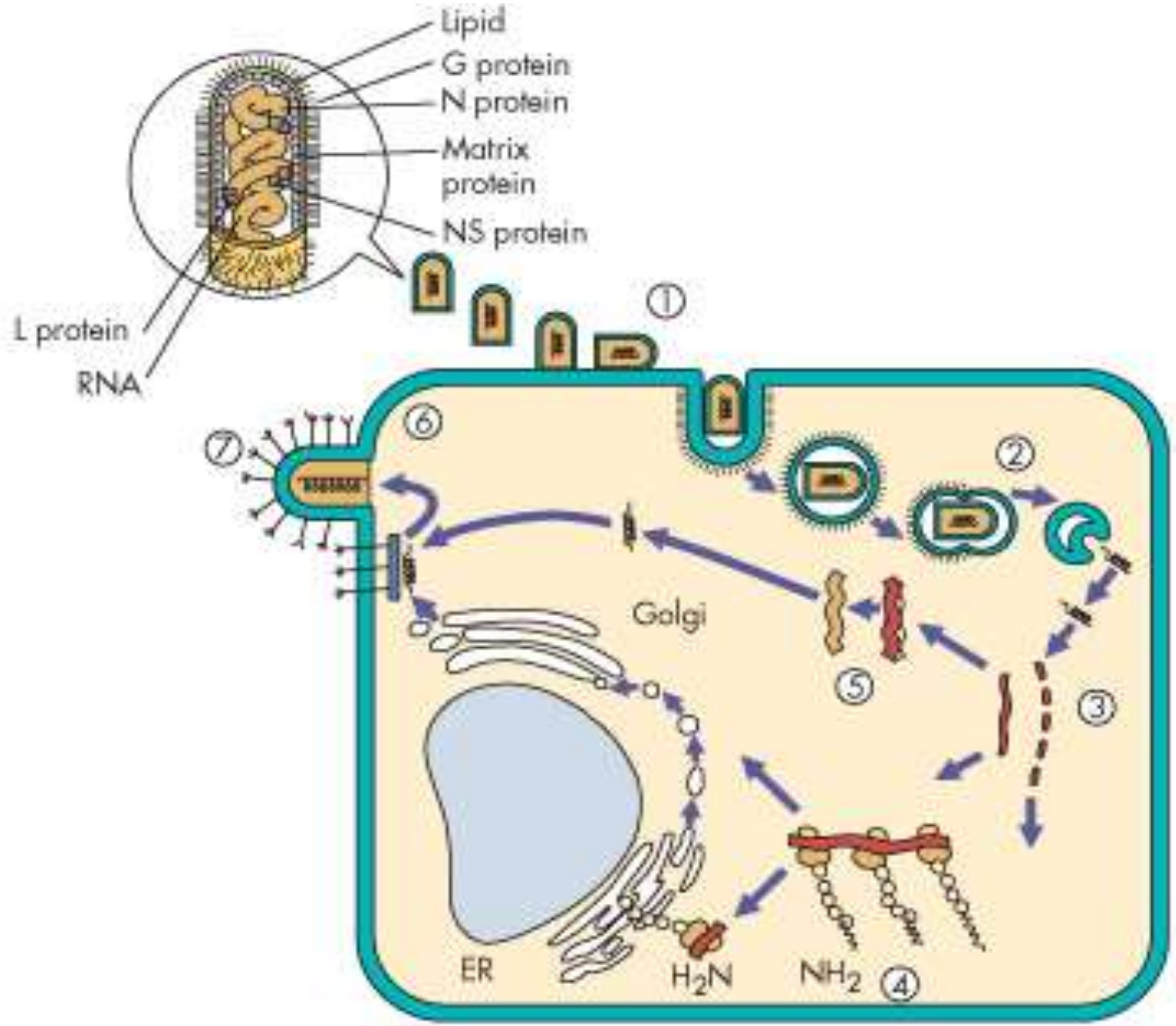
The virion must carry a polymerase, which (*3*) produces five individual messenger RNAs (mRNAs) and a full-length (+) RNA template.

4, Proteins are translated from the mRNAs, including one glycoprotein (G), which is co-translationally glycosylated in the endoplasmic reticulum (ER), processed in the Golgi apparatus, and delivered to the cell membrane.

5, The genome is replicated from the (+) RNA template, and N, L, and NS proteins associate with the genome to form the nucleocapsid.

6, The matrix protein associates with the G protein-modified membrane, which is followed by assembly of the nucleocapsid.

7, The virus buds from the cell in a bullet-shaped virion.



Rabies

- Rabies otherwise ‘rabere’ in Latin means ‘to be mad.’
- **Syn.** Hydrophobia
- Rabies is a **zoonotic**, fatal and progressive neurological infection caused by rabies virus of the genus ***Lyssavirus*.** Most rabies cases are caused by genotype 1 / serotype 1 strains**.**
- Identified by **Louis Pasteur** in 1880's
- It affects **all warm-blooded animals** and the disease is prevalent throughout the world and endemic in many countries **except in Islands like Australia and Antarctica.**
- The natural hosts are terrestrial carnivores and bats. Most mammals can be experimentally infected.
- **Disease has long asymptomatic period**

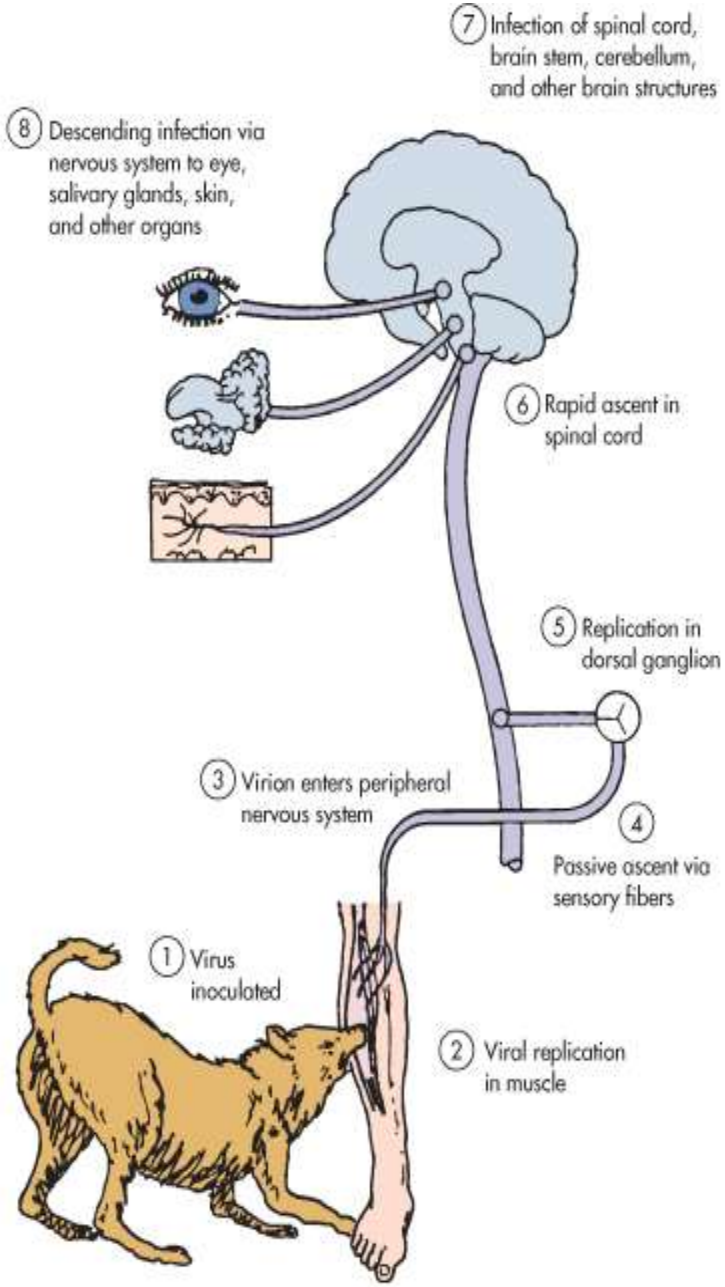
Transmission

- Spread to humans and animals is almost always **by bites** of a rabid animal via introduction of virus-laden saliva into tissues.
- Vampire bats, insectivorous, and fruit bats also transmit the virus by bites.
- There are rare instances of human infection by inhalation, e.g., in bat-infected caves and laboratories and by infected tissues used as transplants.
- Saliva is infectious at, or before, the time clinical signs occur.
- Domestic dogs, cats, and ferrets may shed virus up to 10 days before onset of clinical signs.
- Viral shedding in wildlife has been reported for several weeks before onset of signs.

Pathogenesis

- Virus is not very cytolytic and seems to remain cell-associated
- Virus replicates in the smooth muscle at the site of the bite with minimal or no symptoms
- The length of the incubation phase is determined by the infectious dose and the proximity of the infection site to the CNS and brain
- Moves to nerves and binds to acetylcholine receptors at the neuromuscular junctions.
- Moves centripetally to the spinal chord and then moves to brain.

- An ascending neuronal dysfunction occurs.
- In brain virus multiplies extensively leading to behavioral changes.
- In reservoir host virus moves from the brain centrifugally to adrenals, pancreas, salivary glands.
- For the movement of the virus amino acid at position 333 of the rabies virus glycoprotein (G) is critical. If here arginine or lysine is there, the virus is virulent.



Pathogenesis

- After weeks to months, the virus infects the peripheral nerves and travels up the CNS to the brain ***(prodrome phase)***
- Infection of the brain causes classic symptoms, coma, and death ***(neurologic phase)***
- During the neurologic phase, the virus spread to the glands, skin, and other body parts, including the salivary glands, from where it is transmitted.
- Replication in the neocortex leads to **dumb form** of the disease, there is paralysis and finally death.
- Antibody response occurs at the late stages
- Antibody can block the progression of the virus
- The long incubation period allows active immunization as a post-exposure treatment.

Clinical features

- Incubation period from 14 to 90 days but as long as 7 years has been reported.
- **The prodromal phase**: Involves change in behavior and lasts 2 - 3 days. Anxiety, irritability and unease are characteristic. Some are more alert, restless and sensitive to light and noise.
- **The excitive or furious phase**: Signs include restlessness, depraved appetite, hiding, wandering, aggressive biting, excessive salivation, dysphagia, muscle tremors, incoordination and staggering.
- **The paralytic or dumb phase**: This develops in several days with seizures, paralysis, coma and death in 3 - 4 days.
- In horses and cattle the paralytic phase appears to be predominant

Rabies virus/Epidemiology

- **At risk:**
  - Veterinarians and animal handlers
  - Person bitten by a rabid animal
  - Inhabitants of countries with no pet vaccination program

  Modes of control
  - Vaccination
  - For pets
  - For at-risk personnel
  - “Vaccination program have been implemented to control rabies in forest mammals”

Diagnosis

- **Clinical specimens**: Brain.
- The **fluorescent antibody procedure (FAT)** is widely used and is the preferred method for rabies diagnosis.
- The FA test is used occasionally on formalin fixed brain tissue (when fresh tissue is unavailable) to confirm a rabies diagnosis based on the microscopic finding of **Negri bodies**.
- Rabies virus can be propagated in cell cultures and in suckling mice inoculated intracerebrally.
- RT-PCR

Rabies virus/Treatment & Prophylaxis

- Clinical rabies is almost always fatal unless treated
- Only hope:
  - Post exposure prophylaxis
  - For anyone exposed by bite or by contamination of an open wound or mucous membrane to the saliva or brain tissue of an animal suspected to be infected with the virus

Rabies virus/Treatment & Prophylaxis

- First protective measure
  - Local treatment
    - Washing with soap and water.
    - Rabies antiserum Purified human antirabies immunoglobulin at a dose rate of 20 IU/Kg body weight.
- Then
  - Vaccination

Rabies virus/Treatment & Prophylaxis

- Vaccine (inactivated virus)
  - Human diploid cell vaccine (HDCV), purified Vero cell rabies vaccine (PVRV), purified chick-embryo cell vaccine (PCECV) and purified duck embryo vaccine (PDEV).
  - **Post exposure** On**, 0-3-7-14-28 days**. The person should also receive another shot called rabies immune globulin (RIG).
    - In humans at high risk three injections at 0-7 and 21 days are given as **pre-exposure** vaccine.
    - Pets should be vaccinated at 12 to 16 weeks with a booster 9 months later.
    - Then annual vaccination is done.

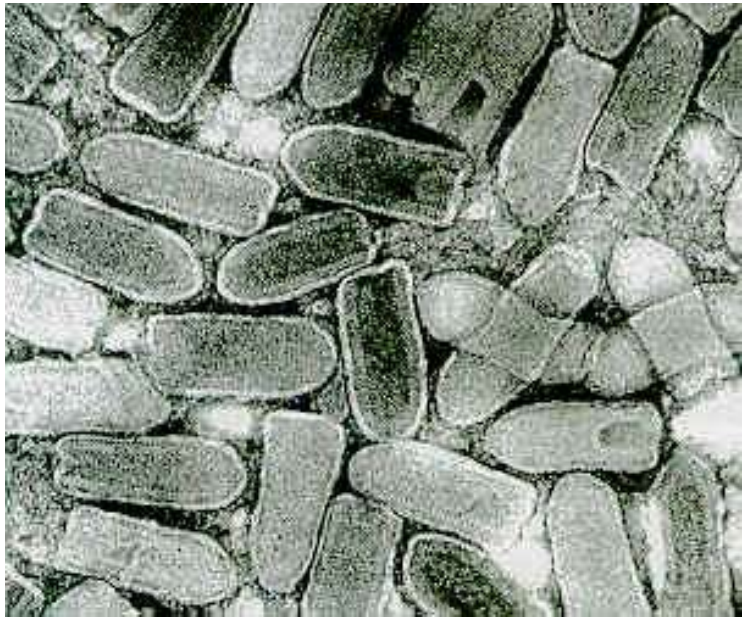
BOVINE EPHEMERAL FEVER

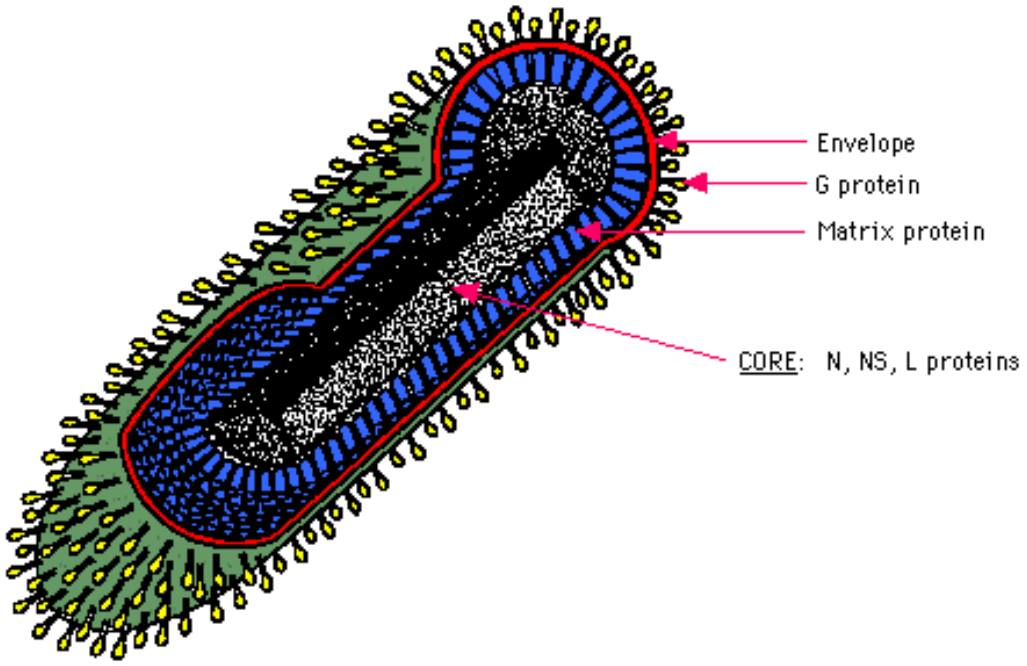
(Three-day sickness, Bovine Enzootic Fever, Three-day stiff sickness,

Dragon boat disease)

Definition

- A **non-contagious epizootic arthropod-borne viral disease**
- Bovine ephemeral fever virus (BEFV) an arthropod-borne rhabdovirus which is classified as the **type species** of the **genus *Ephemerovirus***.
- It causes an acute febrile illness of cattle and water buffalo with a sudden episode of fever accompanied by muscle involvement with arthritis, stiffness of the limbs, and lameness, followed by rapid recovery.
- It is of economic importance because it reduces milk production and fertility and causes abortion.





•Bullet-shaped morphology, although virions (~185 nm × ~75 nm) appear to be more tapered at one end

• BEFV genome is much larger and more complex

Transmission

- **Insect bite**
- not spread from cow to cow
- In enzootic areas, ephemeral fever is a seasonal disease that occurs in the summer and autumn, especially in the rainy season.
- Bovine ephemeral fever virus most probably is transmitted by arthropod vectors
- Potential vectors include **culicine and anopheline mosquitoes**, and possibly Culicoides midges; both enzootic and epizootic spread is limited by the distribution of appropriate vectors.

Clinical Signs

- Depressed
- High fever (105-107 F) with biphasic or triphasic fever
- Serous ocular and nasal discharge
- Anorexia
- Decreased milk production
- Weight loss
- Stiffness and lameness
- **More severe in high BW animals**

Clinical Signs

- **Severe cases**
  - Muscle stiffness
  - Drag feet when forced to walk
  - Lying down, with hide limbs outstretched- to relieve muscle cramp
  - Lie down for three days
  - cessation of rumination- constipation
  - abortion

Clinical Signs

- Morbidity may reach to 30%
- Usually, recovery is dramatic and complete in 3 days (range 2–5 days), with the exception of a return of milk production.
- Low mortality
- Causes of the death
  - Pneumonia from secondary infection
  - Muscle damaged and inflammation from long period lying down
  - Pregnancy toxemia (fatty liver syndrome)

Pathogenesis

- The pathogenesis of the disease is complex and probably reflects pathophysiologic and immunologic effects mediated by the release and activity of various inflammatory mediators (so-called "**cytokine storm**").

- 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.

- In all cases, there is an 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 anti-inflammatory 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

- Clinical signs
- Sero-conversion: paired serum
  - SN test
  - ELISA
- Gross lesion

Prevention and Control

- Vector control
- Vaccine: Modified-live virus and killed vaccines are available
- Infection results in solid, long-lasting immunity.

Vesicular Stomatitis

Vesicular Stomatitis Virus

- **Vesiculovirus**
  - Major serotypes are Indiana and New Jersey variants
    - **VSV-NJ and VSV-I**
- Affects horses, cattle, South American camelids swine, humans
  - Sheep and goats resistant
- Vesicular stomatitis closely resembles three vesicular diseases exotic to the U.S.: FMD, swine vesicular disease, and vesicular exanthema of swine.

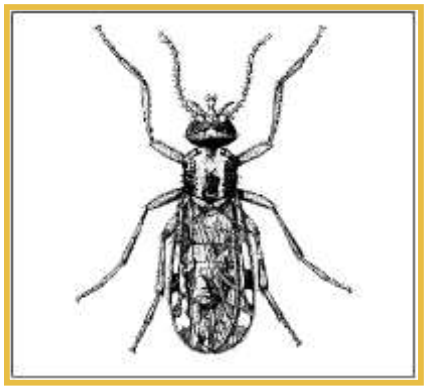
Morbidity/ Mortality

- Morbidity
  - Range: 5 to 90%
  - Most animals seroconvert
- Mortality
  - Higher in adults
  - Death rare in cattle and horses

Animal Transmission

- Vectors
  - Sandflies
  - Blackflies
  - Seasonal outbreaks
- Direct contact
  - Infected animals-saliva, exudate, epithelium of open vesicles
  - Contaminated objects

Sandfly



Blackfly

Human Transmission

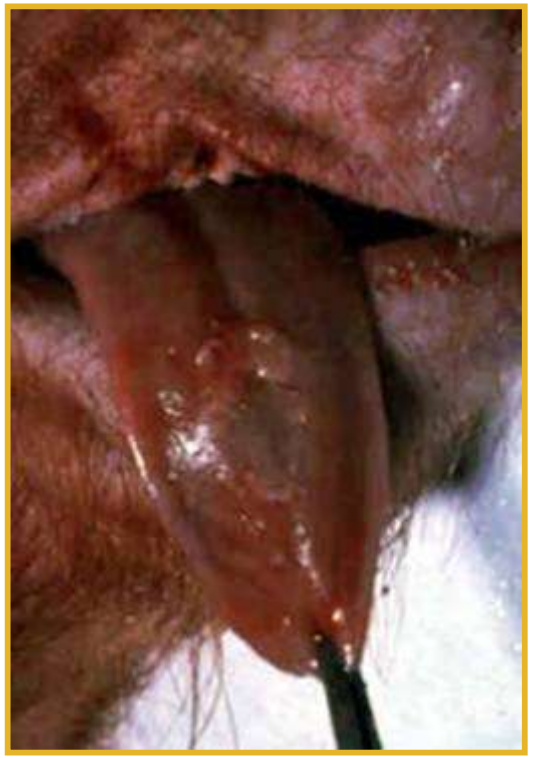
- Direct contact
  - Infected tissues, vesicular fluid, saliva
- Insect bites
  - Blackfly, sandfly
- Aerosol
  - Laboratory settings

Clinical Signs

- Incubation period
  - 3 to 5 days
- Fever and vesicles that resemble FMD
- **Horses severely affected**
  - Oral lesions
    - Drooling, chomping, mouth rubbing, lameness
  - Coronary band lesions

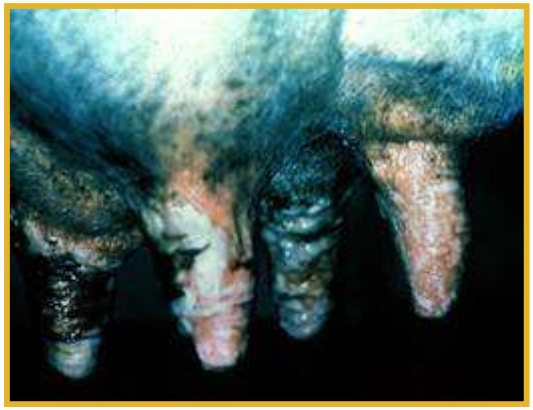
Clinical Signs

- Cattle, pigs
  - Vesicular lesions
    - Oral, mammary gland, coronary band, interdigital region
  - Vesicles usually isolated to one body area
  - Salivation, lameness
- Recover within 2 weeks



Post Mortem Lesions

- Gross lesions
  - Erosive, ulcerative lesions
  - Oral cavity, nostrils, teats, coronary band
- Histopathology
  - Degeneration of epithelial cells



Clinical Diagnosis

- Vesicular diseases are clinically indistinguishable!
- But, symptoms in horses are suggestive
  - Salivation and lameness
- VSV vs. FMD
  - VSV less contagious
  - VSV lesions generally found in one area of the body

Laboratory Diagnosis

- Virus isolation
- Viral antigen detection
  - Vesicular fluid or epithelium
  - ELISA, complement fixation, virus neutralization
- Antibody tests
  - Paired serum samples
  - ELISA, complement fixation, virus neutralization