

Order: Mononegavirales

Family: Rhabdoviridae

Rhabdovirus (rhabdos = rod) / (bullet - shaped).

Rhabdoviridae encompasses more than 175 viruses of vertebrates, invertebrates, and plants.

General properties:

► Morphological characters:

- **Shape: Bullet or conical.**
- **Size: 75 x 180 nm.**
- **Nucleocapsid symmetry: Helical.**
- **The virus is enveloped.**



➡ Virus structure:

• Genome structure and organization:

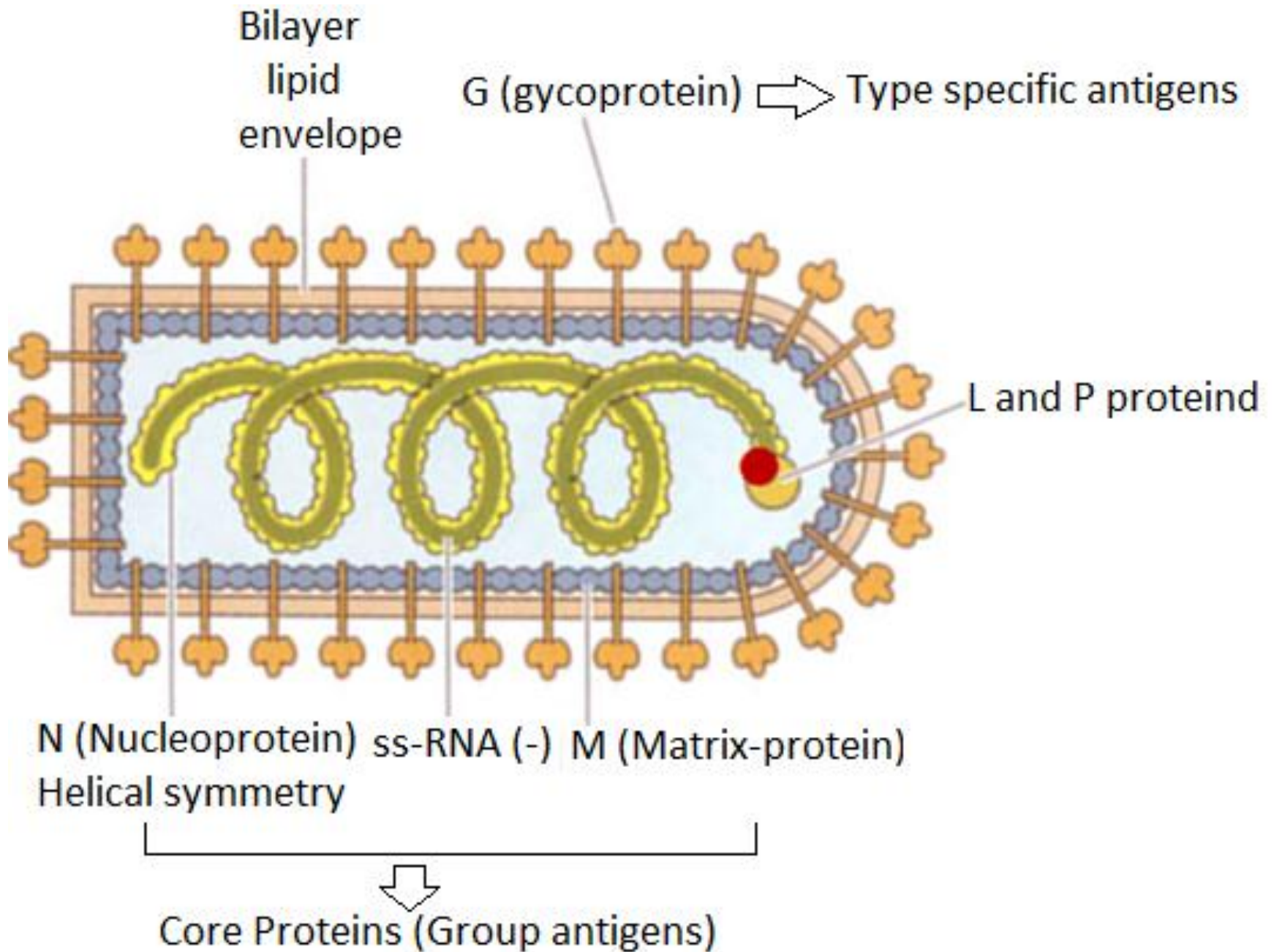
It contains a single strand, linear RNA (-) sense (11-15 Kb).



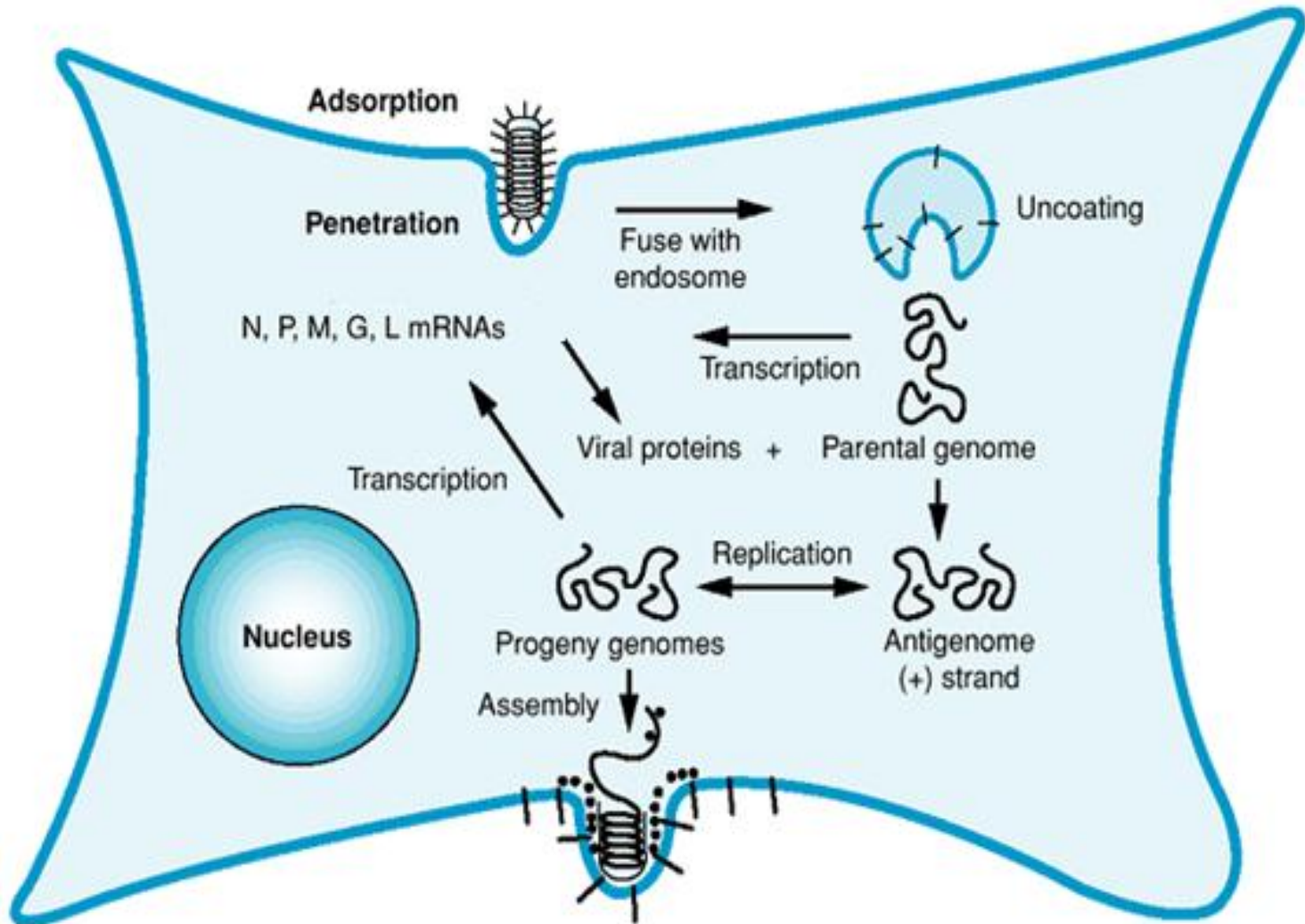
The virion contain five proteins coding genes:

- Nucleoprotein (N),
- Phosphoprotein (P),
- Matrix protein (M),
- Glycoprotein (G)
- Transcriptase (L),

Protein structure and antigenicity:



➡ Virus Multiplication cycle:



➡ **Hemagglutination:**

- BHK cell culture adapted Rabies virus, VSV, and BEFV possess a lipoprotein haemagglutinin which agglutinates erythrocytes of goose or chicken RBCs.

➡ **Effect of physico-chemical agents:**

- The virions are sensitive to ethyl ether and other lipid solvents.
- They persist in the soil for many days at 4-6°C.

➡ **Classification:**

Order Mononegavirales, Family Rhabdoviridae

- * **Genus Lyssavirus:** Rabies V. (human and animals).
- * **Genus Vesiculovirus:** Vesicular Stomatitis V. (cattle, Horse, Pig).
- * **Genus Ephemerovirus:** Bovine Ephemeral fever V. (3-day sickness),
in cattle and buffloes

GENUS LYSSAVIRUS

RABIES

An infectious notifiable disease of man and animals caused by rabies virus transmitted through the bite of rabid animal to other animals resulting in a rapidly fatal encephalomyelitis.

Hosts affected:

wide host range susceptibility:

- Man and all warm - blooded animals.
- Dogs, cats, cattle and wild carnivores as fox are mostly affected.
- Vampire bats also act as natural reservoir of the virus.



Hemagglutination:

- Rabies virus produce haemagglutinins when propagated on BHK-21 cells, maintained in media containing 0.4% bovine albumin and no serum.
- There is no neuraminidase enzyme.
- The optimum conditions for HA test:
 - low temperature 4°C and low pH (6.2).
 - one day - old chick or goose RBCs
 - serum (inhibitor) free medium.
- The rabies haemagglutinin - inhibitors is present in high titer in sera of many mammals and is difficult to remove even after adsorption by 25% kaolin at pH 9.

Physicochemical characters:

- The virus resist:
 - 7-10 days in autolyzed brain tissues.
 - Nervous tissues in 50% glycerol for months.
- Temperature:
 - Resist in 4°C for several weeks and at lower temperatures for many months and lyophilization.
 - Inactivated by repeated freezing and thawing.
 - Labile at room temperature after only a few days.
 - Inactivated by boiling for 2 min. and at 56°C for 15-30 minutes.
- Inactivated by:
 - UV, proteolytic enzymes, acid pH, bile salts, formalin and 20% ether.
 - Exposure for 15 minutes to 1% formol, 3% cresol or 0.1% mercuric chloride.
- Preservation: PBS at pH 7-9 containing 2% inactivated normal guinea -pig serum or 0.75% bovine albumin, -70°C .

***Antigenic properties:**

Surface glycoproteins (G) are the type specific Ag (induce production of neutralizing antibodies in vaccinated animals that cause protection of them against subsequent challenge with rabies virus.

All strains of rabies virus are antigenically homogeneous, detected by SNT.

Antigenic relationship between rabies virus and other lyssaviruses and rhabdoviruses was recognized using FAT and AGPT.

According to the host adaptation there are two types of Rabies virus strains:

*** Street virus:**

*** Fixed virus:**

Street rabies virus

Strains of rabies virus occur in animals under natural conditions, (eg. Flurry strain, Kelev strain)

They are characterized by:

- 1- Variation in virulence,
- 2- Long incubation period,
- 3- Production of intracytoplasmic inclusions (Negri Bodies) on histopathology.
- 4- Variation in being fixed by serial passage in experimental hosts (Some become fixed immediately, Others after 50-60 passages and Some not at all).

Fixed rabies virus

They derived from street rabies viruses by serial passage in experimental hosts (Lab. animal, Fertile egg and Cell culture) that cause changes in the biological properties of the fixed virus.

They are characterized by:

- 1- Increased virulence for experimental host and decreased virulence for other animal species (used as vaccine).
- 2- Shortening of incubation period.
- 3- Failure to produce typical Negri bodies.
- 4- Increased neurotropism

Examples:

Flurry avianized strain (136 serial passage I/C in one day old chick).

Flurry LEP (50 serial passage in ECE).

Flurry HEP (180 serial passage in ECE).

CVS strain (serial I/C passage in Mice).

ERA strain (Serial passage in BHK cells)

**Laboratory diagnosis:
The preferred samples:**



A-Viral samples include:

- Brain tissues (cerebral cortex, cerebellum and portions of the hippocampus) placed in 50% glycerol-saline to preserve the virus at -70°C for direct demonstration, isolation and identification.
- Saliva, salivary gland tissue can be used for mice inoculation.

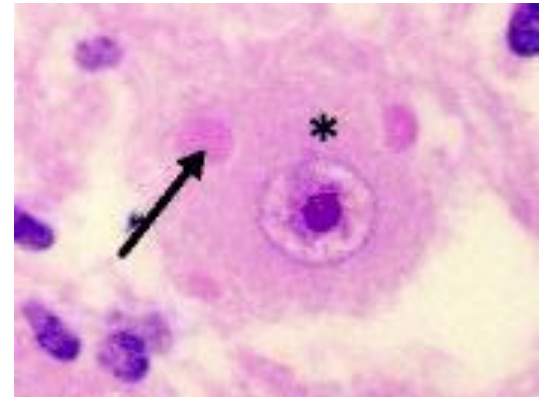
B- Serum samples for serological identification of antibodies.

Direct demonstration:

Impression smear from brain tissue on glass slide and subjected for:

1- Fixation by acetone then examined using FAT and conjugated serum show cytoplasmic fluorescence as a positive result.

2- Staining using sellers stain and examined microscopically for demonstration of Negri bodies (histopathology= intracytoplasmic inclusion bodies).



Virus Isolation:

1- Mice or hamsters (3-6 weeks) by I/C route: muscular tremors, in coordination of gait, paralysis then death after 6 days post-inoculation.

- Mouse brain examined microscopically for presence of Negri bodies especially in mice showing paralysis for 24 hours before death.

2- Tissue culture: as BHK21 and WI 38 cell lines: cell rounding then cell lysis 72 h after inoculation.

- The virus make intracytoplasmic Inclusion bodies (Negri bodies).

Virus identification:

A- Serological identification:

1- Virus Neutralization Test (VNT) : Detect the virus using specific antiserum.

2- Fluorescent Antibody Technique (FAT): It is rapid and highly specific test for detection of viral antigen in infected dog, inoculated mice and tissue culture (FAT detect viral antigen in epithelial cells of corneal smears and on impression smears from brain of inoculated mice several days before death).

B- Non Serological identification:

1- Reverse transcription – Polymerase Chain Reaction (RT-PCR):

Diagnose viral nucleic acid using primers specific for G gene.

2- Electron Microscope (EM) examination:

for detection of the virus through specific morphological characters.

Indirect Serological identification using Serum Neutralization Test

(SNT): for detection of neutralizing antibodies in sera.

Virus Control:

- A highly purified tissue culture adapted inactivated rabies virus vaccines used for human and animals.
- Rabies is a notifiable public health disease with strict control measures applied.

BOVINE EPHEMERAL FEVER (THREE- DAY SICKNESS)

An arthropod – borne virus disease of cattle and buffaloes characterized by sudden fever, stiffness, lameness and spontaneous recovery within few days.

***Hosts affected: Cattle and buffaloes.**

***General properties:**

- The virus possesses some of the characteristics of Rhabdoviruses.
- Citrated whole blood from affected cattle remains infective for 8 days when stored at 2-4°C.
- The virus can be stored for years at - 70°C in lyophilized state.
- Low pH (2.5) or high pH (12) destroys the infectivity of the virus within 10 minutes.
- The virus is inactivated at 56°C within 10 minutes, 37°C within 18 hours at 25°C in 120 hours.

Laboratory diagnosis:

- The preferred sample is the buffy coat from citrated blood samples is preferred for both detection as well as isolation of the virus.
- The virus also can be detected in cells from lungs, spleen and lymph nodes (but not for isolation).



- Virus isolation:

- **Lab. animal:** unweaned mice or hamsters (1-3 days age) by intracerebral inoculation, causing paralysis and death after 2-3 days.
- **Tissue culture:** BHK-21 and Vero cells, CPE develop after 48 hrs, characterized by cell rounding, granular cytoplasm then cell lysis.
- **Identification:**
 - Serological diagnosis of the virus using neutralization test (one serotype), FAT, ELISA and Immunoperoxidase technique.
 - Non serological diagnosis using RT-PCR and EM examination.

*SNT, ELISA and CFT for antibody detection are considered of diagnostic value only when clinical signs of disease were presented.

Vaccine: Cell culture adapted inactivated vaccine