Rhabdoviridae

VMC 321: Systemic Veterinary Virology

Dr. Manoj Kumar

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Department of Veterinary Microbiology

Bihar Animal Sciences University, Patna.

Introduction

- The members of the family *Rhabdoviridae* (from the Greek word *rhabdos,* meaning "rod")
- include pathogens for a variety of mammals, fish, birds, and plants.
- The family contains *Vesiculovirus* (vesicular stomatitis viruses [VSVs]); *Lyssavirus* (rabies and rabies like viruses), an unnamed genus constituting the plant rhabdovirus group; and other ungrouped rhabdoviruses of mammals, birds, fish, and arthropods.

RHABDOVIRIDAE

- Order: *Mononegavirales*
- Family: *Rhabdoviridae*
- Genus:
    - ✓ *Ephemerovirus*
    - ✓ *Lyssavirus*
    - ✓ *Vesiculovirus*

CLASSIFICATION AND CHARACTERISTIC MEMBERS

GENERA	PROPERTIES	MEMBERS
Lyssa virus	Infect vertebrates Some insects	Rabies virus Makola virus Lagos Bat virus
Vesiculovirus	VSV infection of animals & humans, mostly infects insects	Vesicular Stomatitis Virus Cocal virus Alogoas virus
Ephemerovirus	Infects vertebrates	Bovine ephemeral fever virus

Lyssa virus

Genus includes

- **Classical rabies virus** (genotype 1)

Six so-called rabies-related viruses,

- Lagos bat virus (genotype 2),
- Mokola virus (genotype 3),
- Duvenhage virus (genotype 4),
- European bat
- Lyssaviruses 1 and 2 (genotypes 5 and 6),
- Australian bat (genotype 7)

NONSEGMENTED NEGATIVE STRAND RNA VIRUSES
ORDER: *MONONEGAVIRALES*

- The family *RHABDOVIRIDAE*
- 45-100 X 100-430 nm ; bacilliform or bullet-shaped particles
- Membrane spikes composed of only G protein
- Helical nucleocapsids unwind to 20 X 700 nm
- 1 segment, 10-14kb genome
- 5-10 genes encode 5-10 proteins
- Virions contain an RNA polymerase activity transcribes and replicates genome RNA
- Replication occurs in the cytoplasm of host cell which acts as a virus "factory" creating **cytoplasmic inclusion bodies.**

Morphology

A. SIZE: 70-180 nm IN DIAMETER

B. ENVELOPE: YES

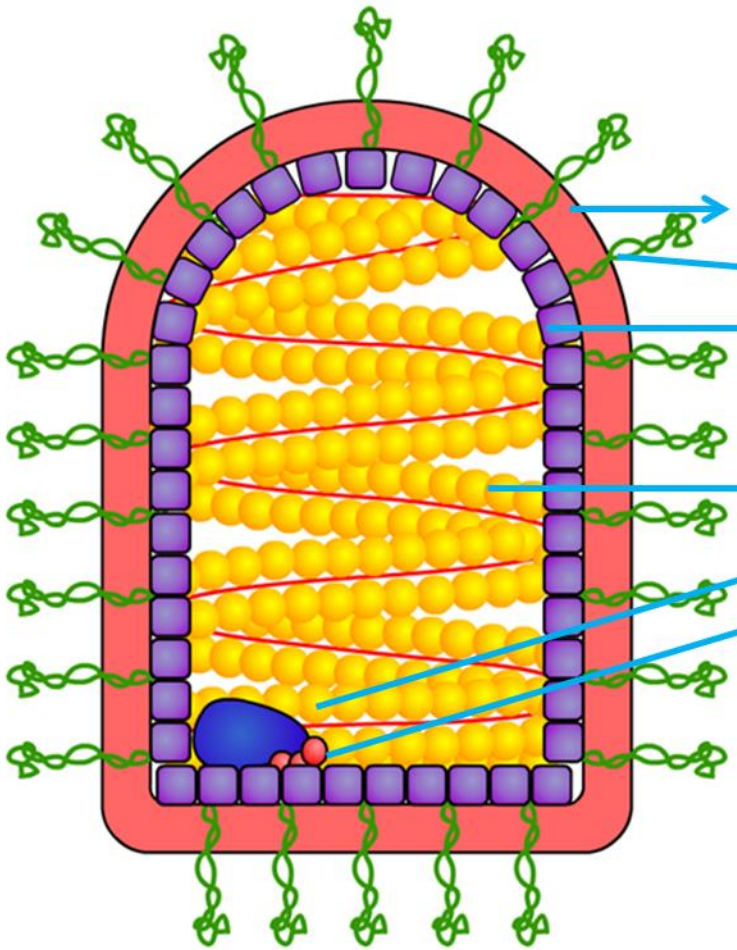
1. GLYCOPROTEINS: EXTERNAL (G) GLYCOPROTEIN 65kDa PROTEIN SPIKE
2. OTHER PROTEINS: NONE
3. MATRIX 1. PROTEINS: INTERNAL (M) MATRIX PROTEIN 26kDa LINE ENVELOPE

C. NUCLEOCAPSID

- NUCLEIC ACID: HELICAL NUCLEOCAPSID
  a. TYPE: RNA BALTIMORE TYPE: V
  b. STRANDED: SS
  c. POLARITY: (-)
- CAPSID
  - a. SYMMETRY: Elongated, rod “bullet” shape
  - b. COMPOSITION:
    - (1) proteins: nucleocapsid (n) protein

RHABDOVIRIDAE

Virus Structure



MEMBRANE

(G) Glycoprotein Spike

(M) Matrix Protein

NUCLEOCAPSID

(N) Nucleocapsid Protein

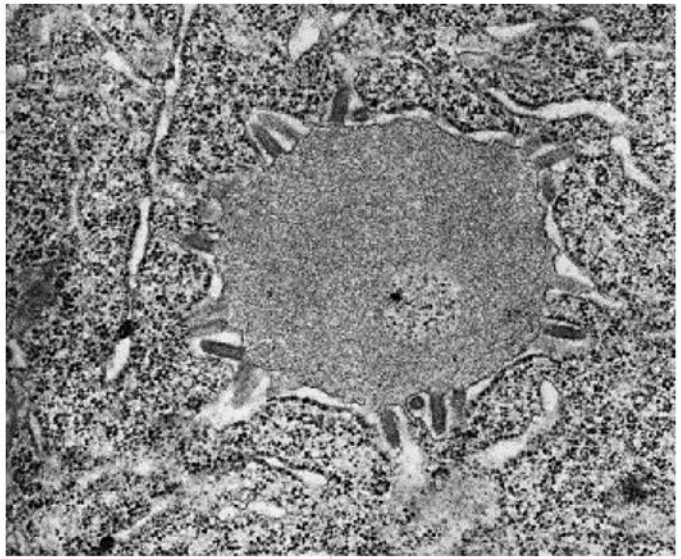
(P) Phosphoprotein

(L) Protein

RNAdRNAp associated with the core structure

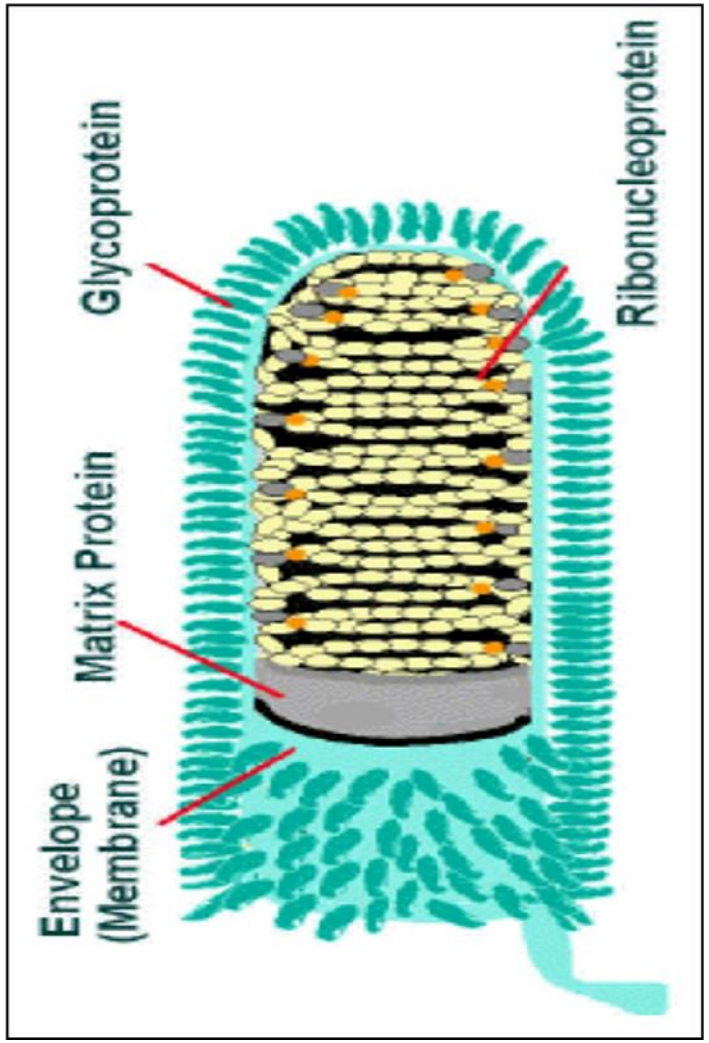
Negative ssRNA

terminal inverted sequences allow for panhandle formation

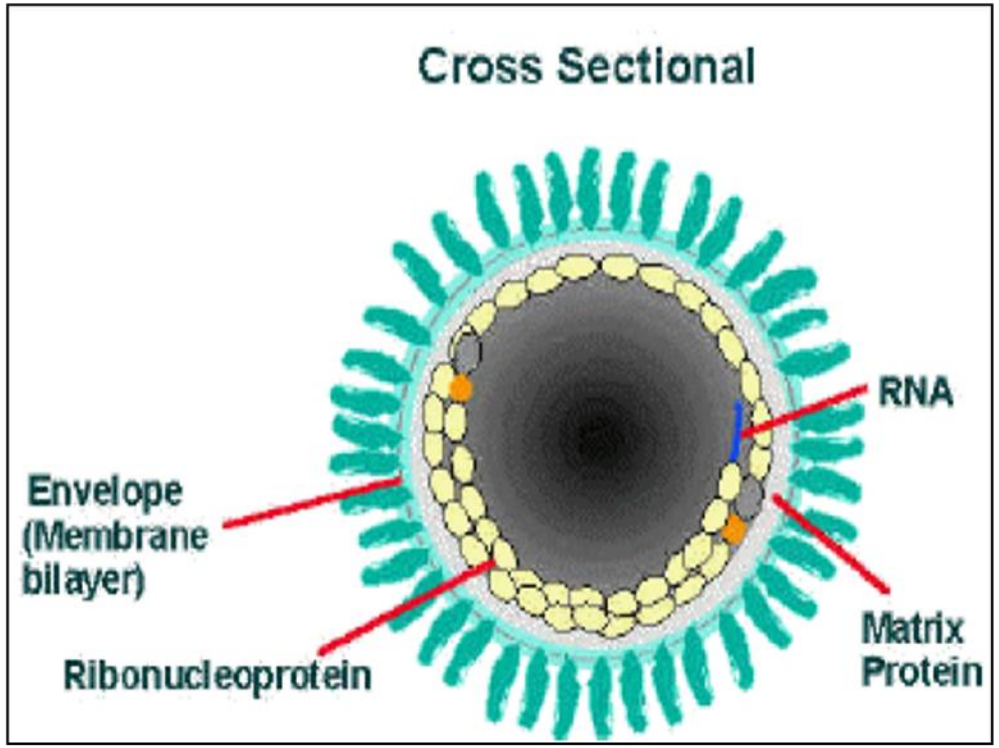


Structure of Rabies virus

LS of Rabies virus

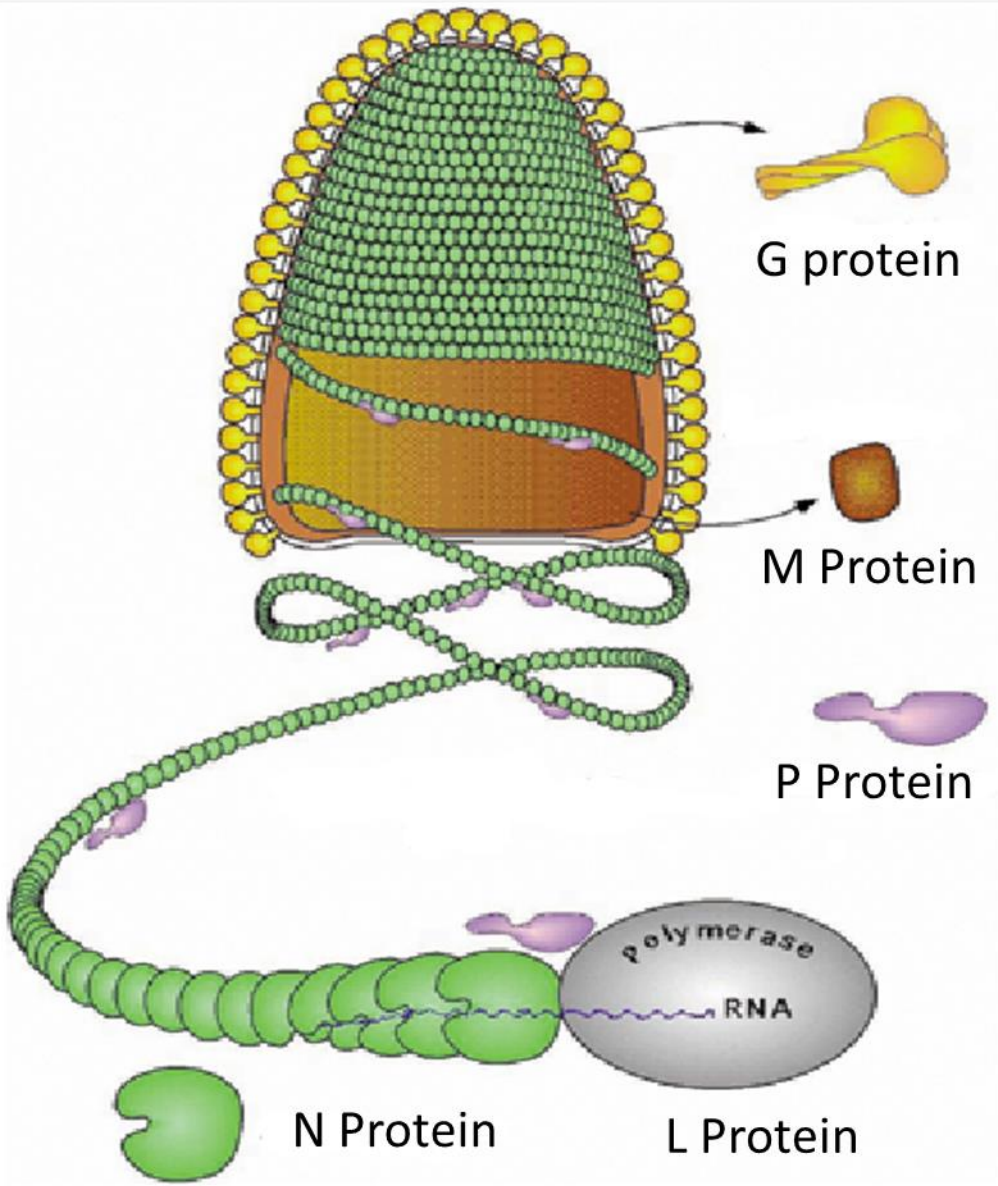


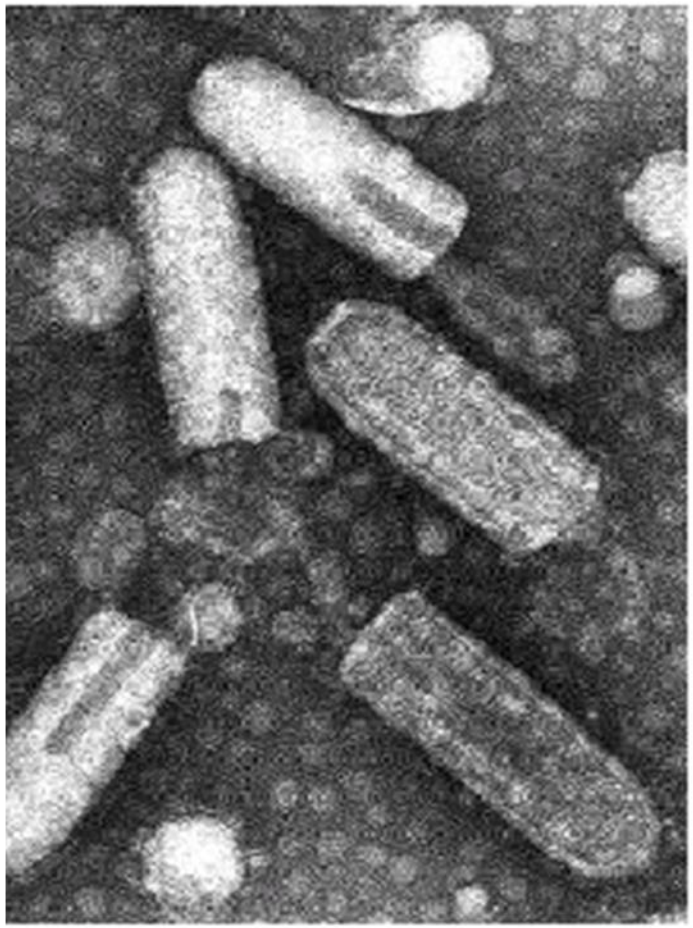
CS of rabies virus



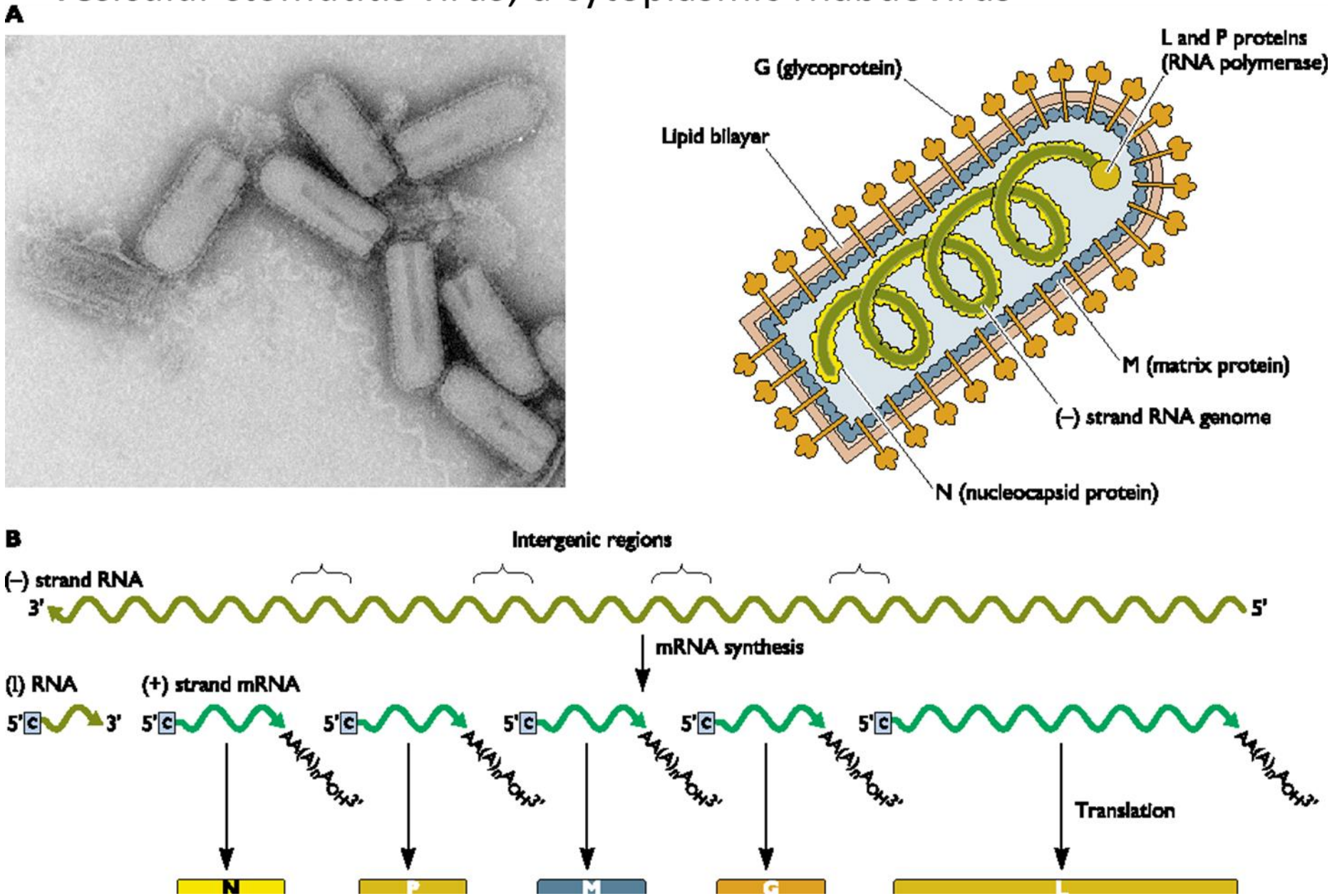
Rhabdovirus structure

All 5 proteins encoded by most animal rhabdovirus genomes are part

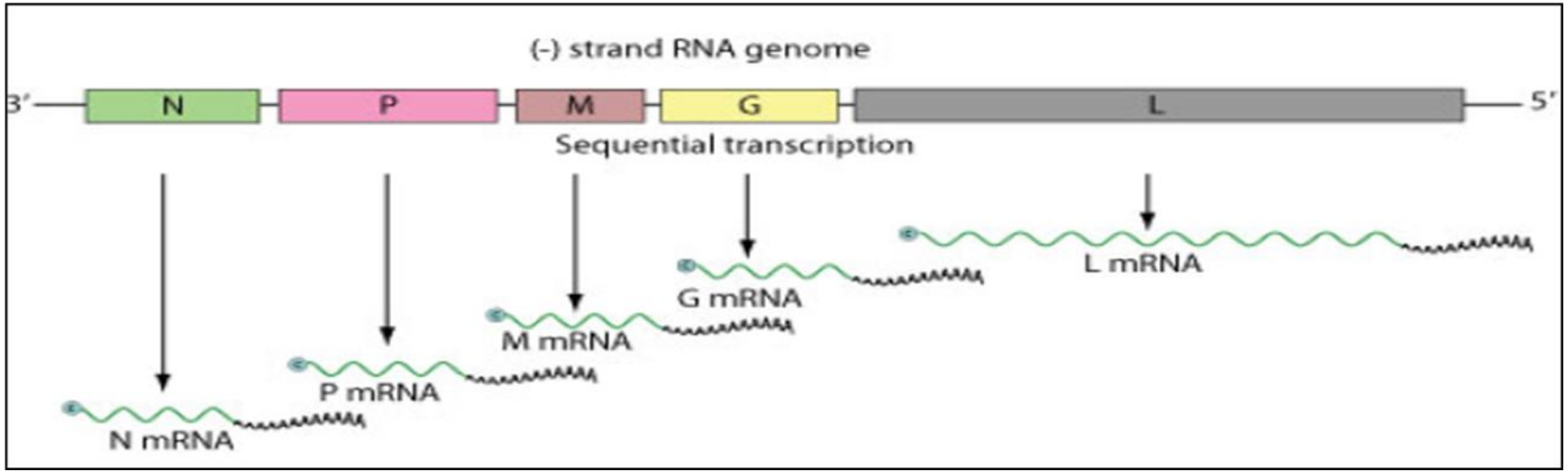




Particle structure, genome organization, and expression of *Vesicular stomatitis virus*, a cytoplasmic rhabdovirus



GENETIC (PHYSICAL) MAP



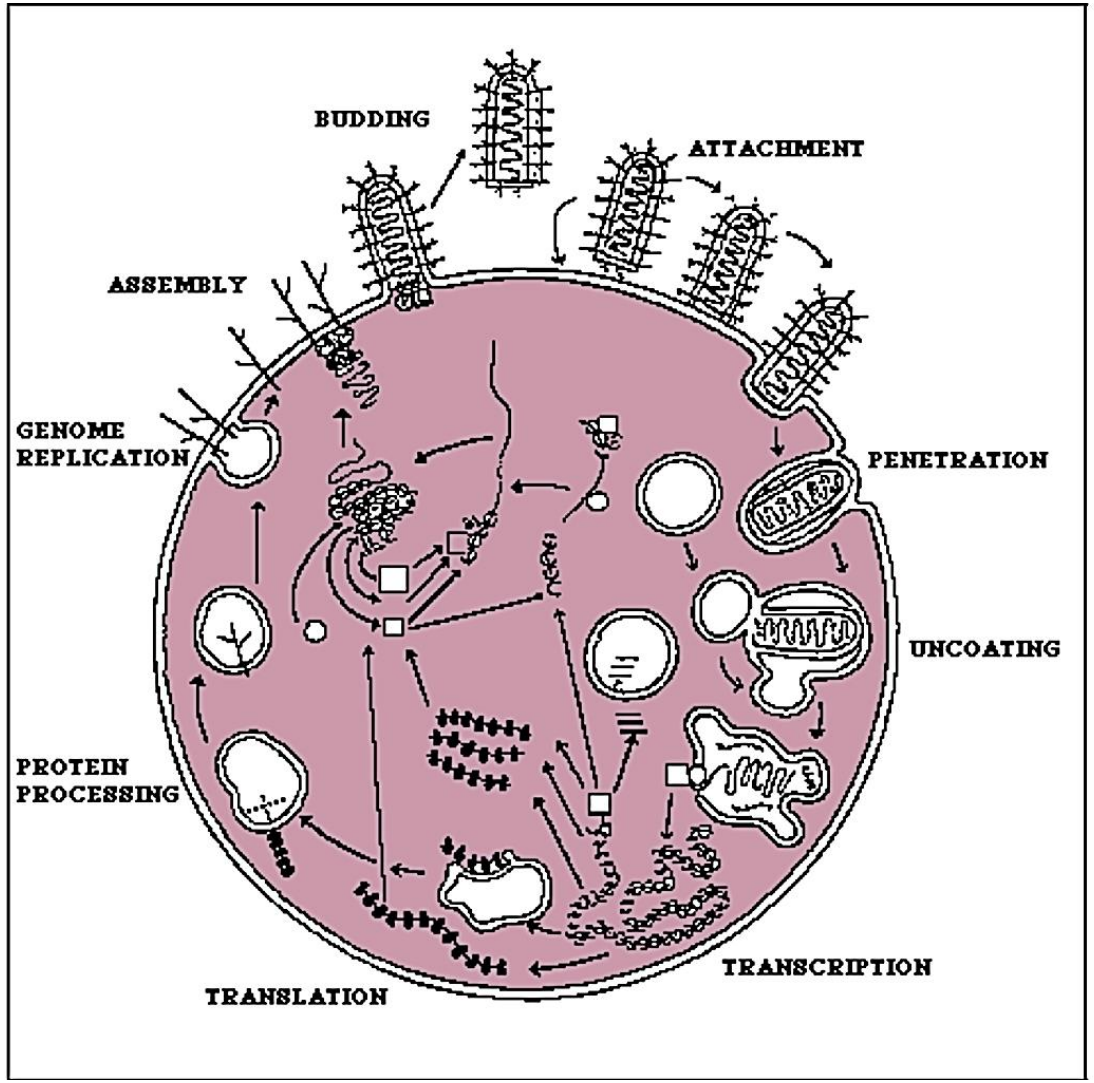
Replication

- G protein attaches to the host cell and is internalized by endocytosis.
- viral envelope fuses with the membrane of the endosome
- uncoating releases the nucleocapsid to be released into the cytoplasm; replication ensues.
- The RNA-dependent RNA polymerase associated with the nucleocapsid transcribes the viral genomic RNA, producing five individual messenger RNAs (mRNAs).
- mRNAs are translated into the five viral proteins.
- viral genomic RNA is also transcribed into a full-length positive sense RNA template that is used to generate new genomes.
- G protein is synthesized by membrane-bound ribosomes, processed by the Golgi apparatus, and delivered to the cell surface in membrane vesicles.
- M protein associates with the G protein-modified membranes.

Cont…

- Assembly of the virion occurs in two phases:
  (i) assembly of the nucleocapsid in the cytoplasm and
  (ii) envelopment and release at the cell plasma membrane.
- The genome associates with the N protein and then with the polymerase proteins L and NS to form the nucleocapsid.
- Association of the nucleocapsid with the M protein at the plasma membrane induces coiling into its condensed form.
- The virus then buds through the plasma membrane and is released when the entire nucleocapsid is enveloped.
- Cell death and lysis occur after infection with most rhabdoviruses, with the important exception of rabies virus, which produces little discernible cell damage.

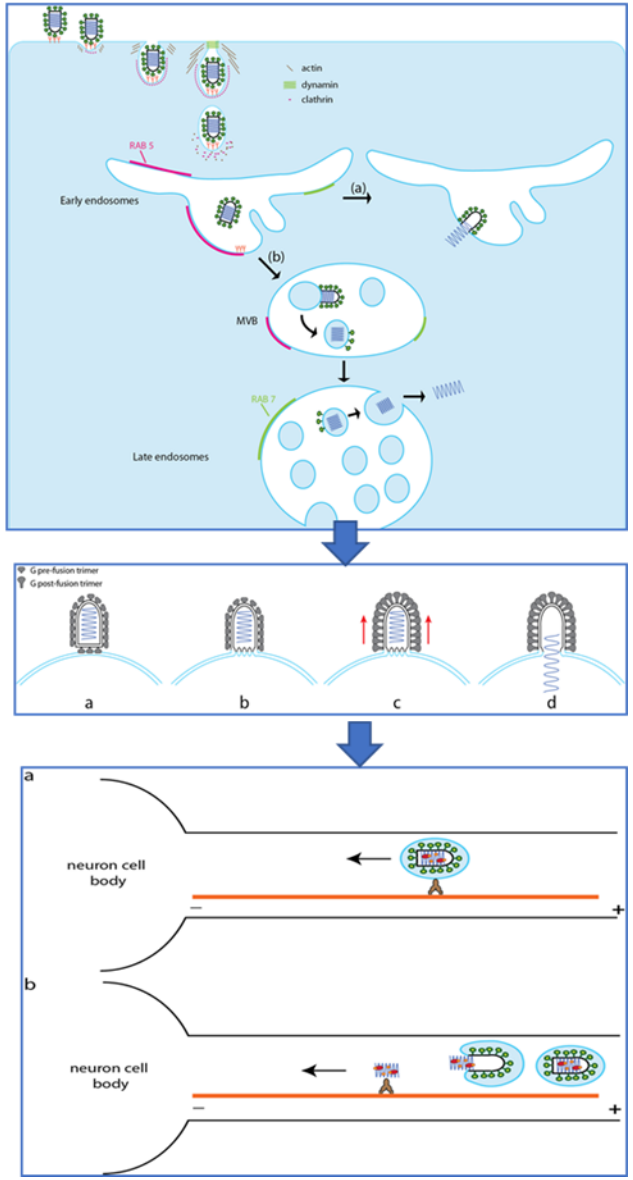
Replication: Schematic representation



RABIES IN ANIMALS

Pathogenesis

- Virus enters cell by endocytic pathway
- Multiplies in muscle and connective tissue.
- Enters peripheral nerves to CNS
- First symptoms of malaise, sore throat, fever.
- Increased sweat, hydrophobia, difficulty in swallowing, muscle spasms, convulsions
- Levels of virus in the blood is not very high.
- Invariably fatal.
- Death by respiratory paralysis.
- Virus can be diagnosed in brain tissue by staining for Negri bodies and by fluorescent antibody tests.



Pathogenesis

Step 1

- Rabies virus gains entry into a new host by introduction of virus-containing saliva into a bite wound
- Entry may also be gained by saliva contamination of the mucous membranes of the mouth, eyes and nasal passages
- Local viral proliferation in non-neural tissue

Step 2

- Viral attachment to nerve cell receptors and entry into peripheral nerve endings
- Virus is transported along afferent axons, eventually reaching the central nervous system along cranial nerves and along motor and sensory pathways as well as the spinal cord (centripetal transport)

Pathogenesis

Step 3

- Proliferation in nervous tissue

Step 4

- Widespread distribution of the virus throughout the brain and spinal cord

Step 5

- Centrifugal transport along efferent cranial nerves

Step 6

- Virus concentrated → nervous tissue, salivary glands, saliva & cerebrospinal fluid (CSF)

Step 7

- Salivary glands become infected

Step 8

- Virus concentrated → nervous tissue, salivary glands, saliva & cerebrospinal fluid (CSF)

Step 9

- Salivary glands become infected

Step 10

- Virus particles are shed in the saliva

Step 11

- Infection of the brain commonly leads to behavioural changes (FURIOUS FORM)

Step 12

- Induce the host to bite other animals

Step 13

- Transmitting the virus

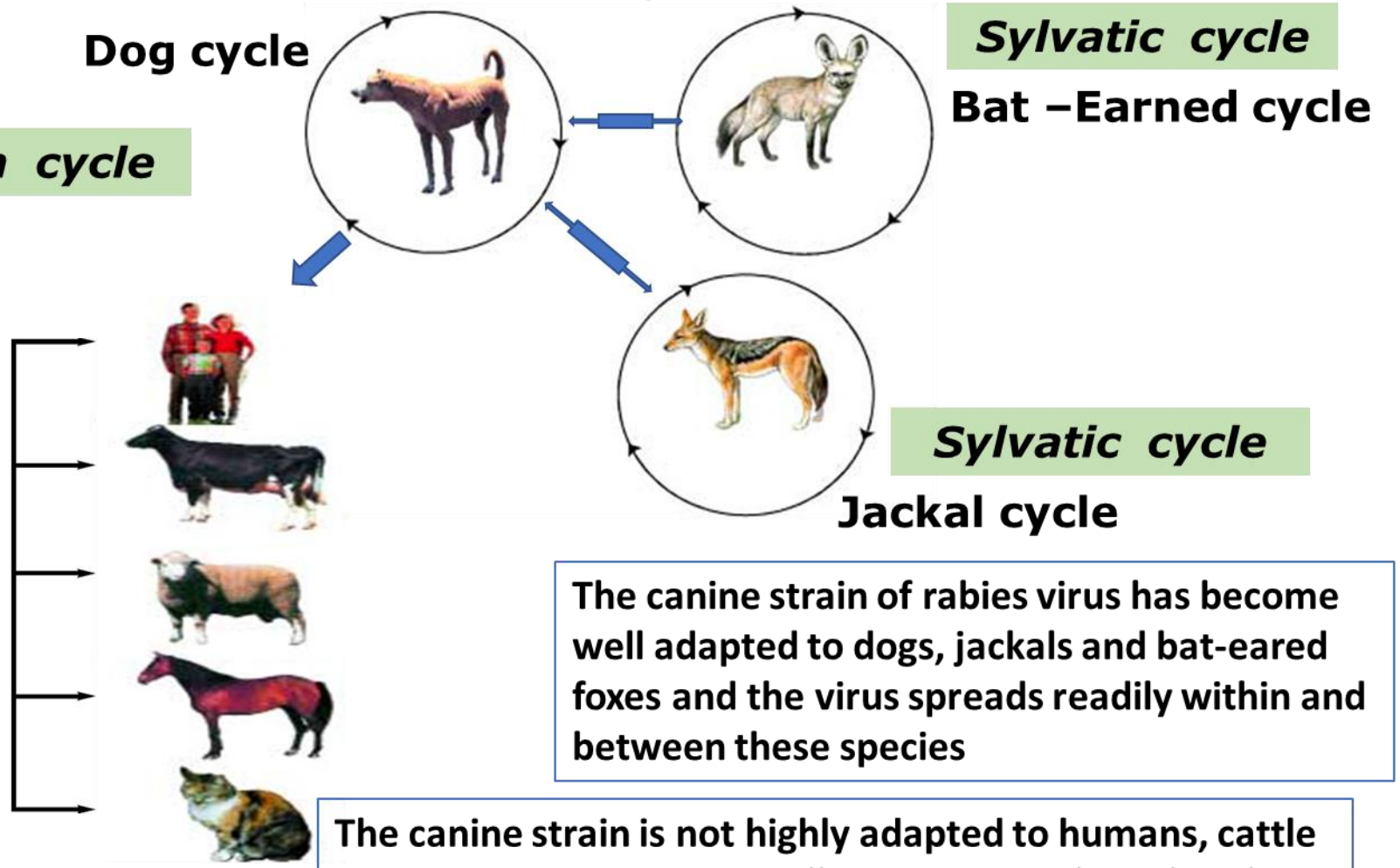
Step 14

- Widespread central nervous system infection

Step 15

- Respiratory paralysis
- Inevitable death

Canid rabies virus strain cycles



The canine strain of rabies virus has become well adapted to dogs, jackals and bat-eared foxes and the virus spreads readily within and between these species

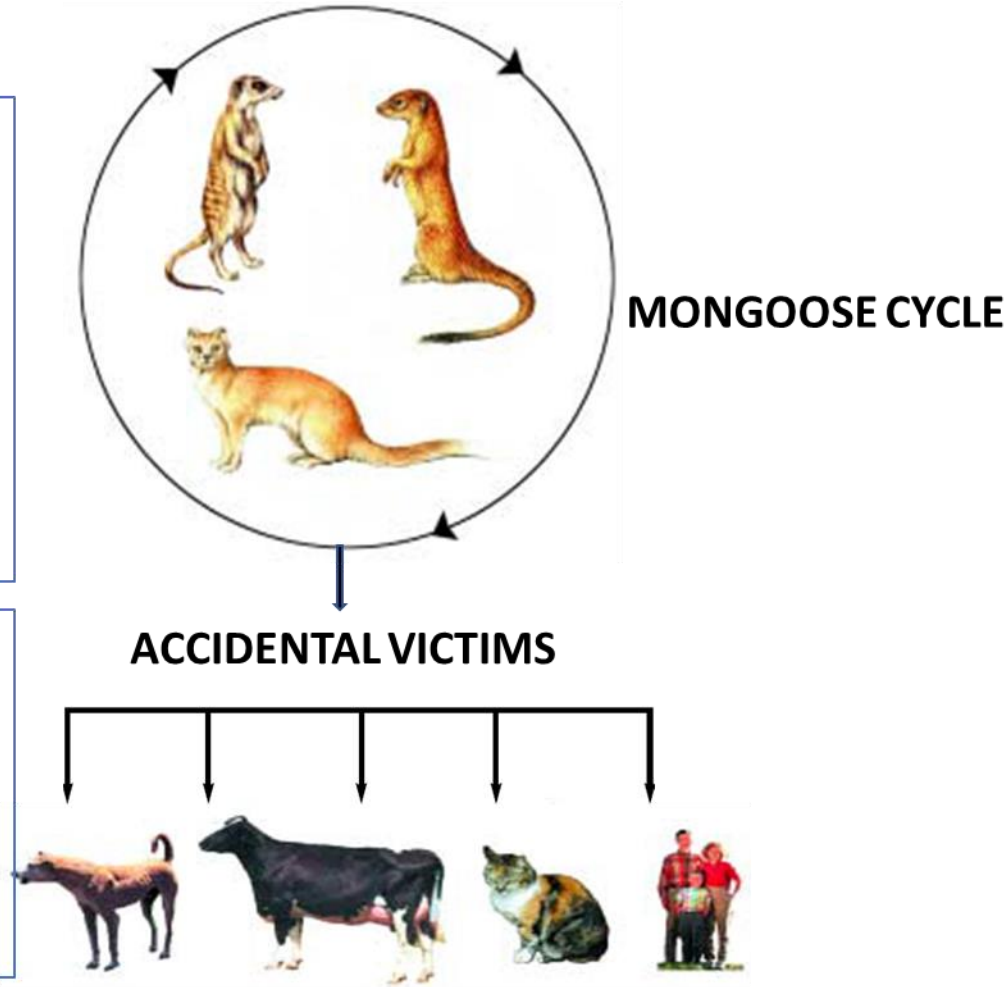
The canine strain is not highly adapted to humans, cattle and cats and does not usually spread readily within these species. Cats are, however, potentially very dangerous and are adaptable of transmitting the disease

Mongoose rabies virus strain cycles

Some mongoose strains of rabies virus may be well adapted to other
mongoose species,

This virus type spreads readily within and between these species

The mongoose strain is not highly adapted to dog, bat-eared fox and
black-backed jackal species and does not usually spread readily between these animals



Transmission

- Humans, cattle and other domestic animals main victims of canid rabies in dog rabies-endemic regions
- Spreading infection = migration
- Rabies virus is usually spread between animals in the saliva, during a bite from an infected animal
- Humans and domestic animals → sheep, cattle
- dogs → dead-end hosts

Species affected



FAMILIES

- *Canidae* (dogs, jackals, coyotes, wolves, foxes and raccoon dogs),
- *Mustelidae* (e.g., skunks), Viverridae (e.g., mongooses)
- *Procyonidae* (raccoons)



ORDER

- Chiroptera (bats).




Hosts

- All warm blooded animals at variable degrees of susceptibility
- Foxes, coyotes, jackals, wolves and certain rodents are among the most susceptible animal groups
- Skunks, bats, raccoons, rabbits, cattle, some members of the felidae family and viverridea civet, mongoose etc. High susceptibility

Table 42–2. Animal Susceptibility to Rabies.[1]			
Very High	**High**	**Moderate**	**Low**
Foxes	Hamsters	Dogs	Opossums
Coyotes	Skunks	Sheep	
Jackals	Raccoons	Goats	
Wolves	Cats	Horses	
Cotton rats	Bats	Nonhuman primates	
	Rabbits		
	Cattle		

[1]Modified from Baer GM, Bellini WJ, Fishbein DB: Rhabdoviruses. In: *Fields Virology*. Fields BN et al (editors). Raven Press, 1990.

- NATURAL HOST
    - Vertebrates

TROPISM

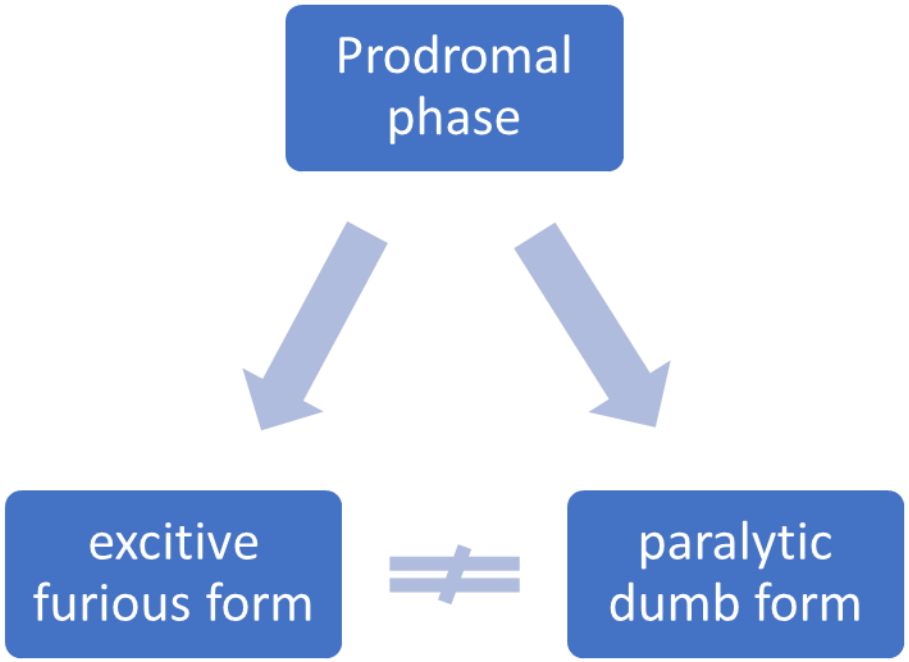
- Rabies virus replicates in neurons

Pathogenicity

Rabies affects the central nervous system

Associated with behavioural changes

Manifest in many different ways



Progression of Rabies Disease

Disease Phase	Symptoms	Time (days)	Viral Status	Immunologic Status
Incubation phase	Asymptomatic	60-365 after bite	Low titer, virus in muscle	-
Prodrome phase	Fever, nausea, vomiting, loss of appetite, headache, lethargy, pain at site of bite	2-10	Low titer, virus in CNS and brain	-
Neurologic phase	Hydrophobia, pharyngeal spasms, hyperactivity, anxiety, depression	2-7	High titer, virus in brain and other sites	Detectable antibody in serum and CNS
	CNS symptoms: loss of coordination, paralysis, confusion, delirium			
Coma	Coma: cardiac arrest, hypotension, hypoventilation, secondary infections	0-14	High titer, virus in brain and other sites	-
Death	-	-	-	-

Clinical phases

- *Prodromal phase:*

Involves change in behavior and lasts 2 - 3 days. Anxiety, irritability and unease.

Some are more alert, restless and sensitive to light and noise.

- FURIOUS

- *Excitive or furious phase:*

- *Include restlessness, depraved appetite, hiding, wandering, aggressive biting,* excessive salivation, dysphagia, muscle tremors, incoordination and staggering.

- *Paralytic or dumb phase:*

- *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.

Symptoms : Dogs

- Change in temperament, attacking and biting anything (often injuring mouth and breaking teeth),
- Exaggerated responses to sound and light
- Restlessness, nervousness, snapping at imaginary flying insects
- Disorientation, wandering aimlessly
- A fixed stare,
- Drooling saliva
- Hoarse howling, choking sounds, "bone in throat" syndrome
- A febrile reaction,
- Uncoordinated actions and progressive paralysis,
- Dilated pupils,
- Irritability,
- Photophobia,
- Infliction of self-injury,
- Convulsions,
- Muscle spasms.

Symptoms : Cattle

- Typical hoarse bellow,
- Aggressive particularly on provocation,
- Vicious attacks on inanimate objects,
- Butting other cattle,
- Attacking humans,
- Wind-sucking,
- "bone in throat" syndrome,
- Separate themselves from rest of herd,
- Anorexia,
- Knuckling of fetlocks especially hind limbs,
- Swaying gait,
- Tail and posterior limb paralysis,

- Jaw and tongue paralysis,
- Profuse salivation,
- Dragging hooves,
- Pseudo-oestrous, hypersexual behaviour,
- Decreased milk production,
- Dilated pupils,
- Fixed stare,
- Grinding teeth,
- Pica,
- Tenesmus with diarrhoea,
- Frequent urination
- Loss of condition, Emphysema

Knuckling fetlocks and hind-quarter paralysis in a bovine

Hind-quarter paralysis in a calf

Bitten animals exhibit; unusual behaviour

Continuous bellowing

Symptoms : Sheep/goats

Symptoms resemble those of cattle

Prominet symptoms than cattle:

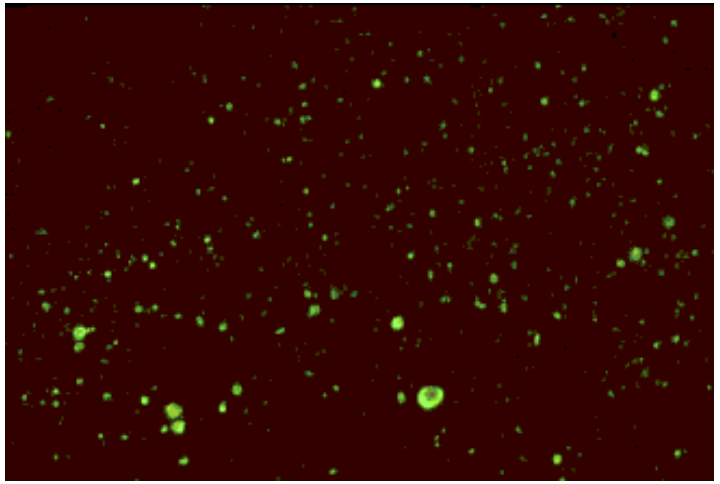
- Hypersexual behaviour
- Sexual excitement
- Incessant bleating
- Aggression
- Aimless running
- Pawing and paddling
- Grinding of teeth

Symptoms : Pigs

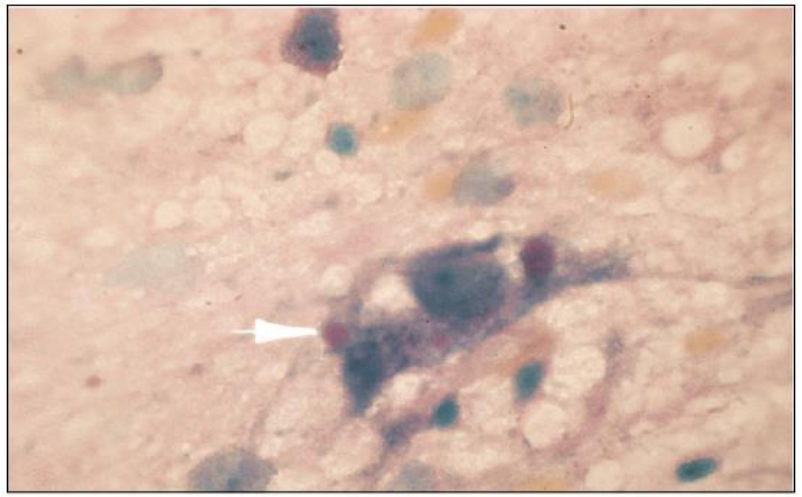
- Hiding in corners of pen,
- Hypersexual behaviour,
- Aggression,
- Biting,
- May kill offspring

Diagnosis

- **Identification of the agent-** Neuroblastoma cell lines, BHK-21
- **Serological tests -** virus neutralisation (VN) tests , Indirect ELISA , Fluorescent antibody test



Positive Fluorescent Antibody Test



Brain tissue showing Negri bodies.

Diagnosis

- Direct FAT demonstrate rabies antigen in touch impressions of brain tissue (medulla, cerebellum, hippocampus)
- Reverse transcription-polymerase chain reaction RT-PCR to test for the presence of viral RNA in the brain of the suspect animal
- FAT, RT-PCR is performed using a skin biopsy,, corneal impression, saliva
- Negri bodies in the hypothalamus, thalamus, pons, cerebral cortex and dorsal horns of the spinal cord; Not all virus positive brains show Negri bodies
- Virus isolation by intracerebral inoculation of weanling mice with fresh homogenized tissue.
- Control mice are inoculated with extracted tissue incubated with specific neutralizing antibody.
- Mice develop encephalitis within 14 days

Vaccine

- Live Attenuated Viruses
- Live attenuated viruses adapted to growth in chick embryos (eg, Flury strain) are used for animals but not for humans.
- Occasionally, such vaccines can cause death from rabies in injected cats or dogs.
- Rabies viruses grown in various animal cell cultures have also been used as vaccines for domestic animals.
- A recombinant viral vaccine consisting of vaccinia virus carrying the rabies surface glycoprotein gene has successfully immunized animals following oral administration.
- This vaccine may prove valuable in the immunization of both wildlife reservoir species and domestic animals.

Rabies Vaccine and Vaccination

Parenteral administration

- Target popultation: **Domestic animals**
- Recombinant, modified live and inactivated virus vaccines
- Primary vaccination > 3 months (e.g. for animal movement/trade),
- or according the manufacturer‘s prescription
- Annual boosters
- Monitor vaccination coverage in the population

Oral Vaccination

- Target population: **Stray or wild animals**
- Mainly administered as bats
- Modified live virus or recombinant vaccines (VRG and SAG2)

VACCINES: domesticated species

- Killed virus vaccines
- One year protocols
  - Puppies & kittens >3 months of age should be vaccinated with a 1 year vaccine; next year,
  - repeat annually

WOUND TREATMENT

- 1. Wash wound thoroughly under running water with soap, or saline or chlorhexidine, or cetrimide, for 5 minutes
- 2. Apply disinfectant, e.g. Betadine or aqueous iodine (Zepharin)
- 3. Do not suture or apply compressive bandages
- 4. Administer anti-tetanus treatment and antibiotics if necessary

ANTIRABIES TREATMENT

1. Unimmunised patient: infiltrate immunoglobulin (20 IU/kg)

- on day 0 into and around wound, with remainder into gluteus.
- Inject single dose vaccine into deltoid muscle on days0, 3, 7, 14 and 28

2. Previously immunised patient:

   inject single dose vaccine into deltoid muscle on days 0 and 3

3. Late presentation (more than 48 hours after exposure):

   inject double dose vaccination (one dose into each deltoid) on day 0, single dose on day 3, 7,14, 28

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