Rabies

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Rabies and rabies-like illness are caused by different species of neurotrophic viruses in the Rhabdoviridae family, genus *Lyssavirus.*

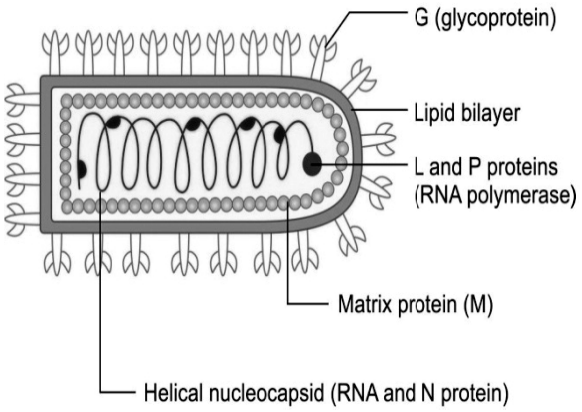


Figure: 1 MORPHOLOGY

- *Bullet-shaped* (75 nm in width &180 nm in length)
- Enveloped- lipid envelope (10 nm long) hem agglutinating peplomer spikes (glycoprotein-G) are embedded.
- The envelope is lined internally by a layer of matrixprotein
- The envelope is susceptible to solvents like ether
- Single-strand RNA virus has a non-segmented, negative-sense (antisense) genome that consists of 11,932 nucleotides and encodes five proteins: nucleocapsid protein, phosphoprotein, matrix protein, glycoprotein, and a large polymerase protein. (Figure 1)

Antigens of Rabies Virus

Glycoprotein G

Peplomers or spikes embedded in envelope. It's Species-specific. It binds to acetylcholine receptors in neural tissues, which is the first step of pathogenesis (attachment). It induces hem agglutination inhibiting antibodies which can be detected in patient's serum by hem agglutination inhibition test. It induces neutralizing antibodies, which are protective in nature & also stimulates cytotoxic T cells.

Role in vaccination- Because it is protective in nature, the purified glycoproteins may provide a safe and effective subunit vaccine.

Nucleoprotein

Capsid proteins associated with viral RNA

Group-specific and cross-reactions are seen with rabies-related viruses.

1. CFT-It induces complement-fixing antibodies, which can be detected in patient's serum by complement fixation test
2. Antiserum prepared against the purified nucleocapsid is used in immunofluorescence test

Animal Susceptibility

- Infects all warm-blooded animals, including humans.
- Susceptibility varies among various animals.
- Very highly susceptible animals- Foxes, jackals, wolves, and cotton rats
- Highly susceptible animals-Rabbits, cattle, cats, hamsters, raccoons, and bats
- Moderately susceptible animals-Dogs, goats, sheep, horses & non-human primates
- Low susceptible animals - Opossums
- Rabies virus also undergoes certain changes when it is serially propagated in animals.

Transmission

- A dog bite is the most common mode

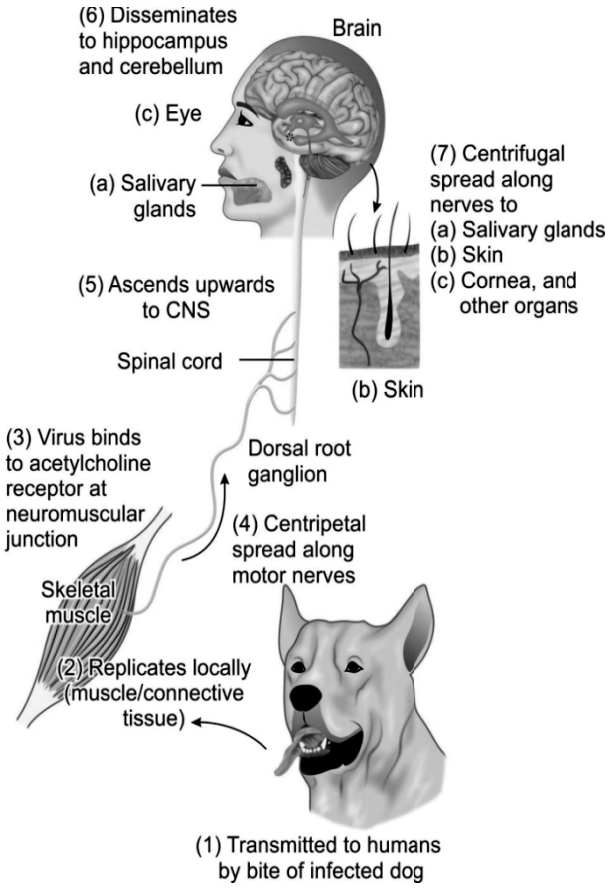
- *Others* - monkey, sheep, goat, cat, buffalo, and horse
- *Bat bite* Migrating fruit-eating bats
- Human-to-human transmission - extremely rare.

Non-bite exposures are rare such as-

- Lick on abrasion or mucosa
- Inhalation of virus-containing aerosols generated from infected bats.
- Corneal transplantation

Pathogenesis

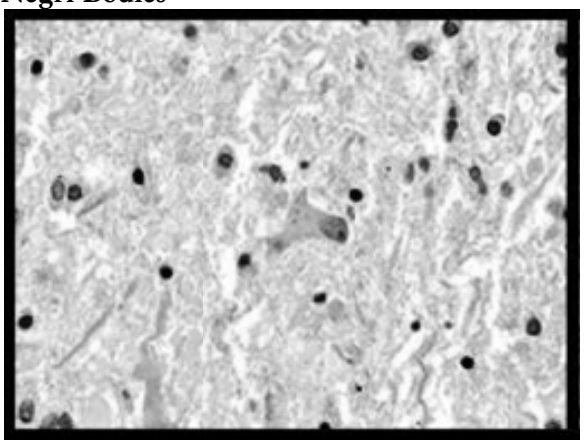
Rabies virus migrates centrally in a retrograde direction within the axoplasm of peripheral nerves at approximately 50 to 100 mm per day (even up to ~250 mm/d), with delays at intervals of ~12 h at each synapse) until reaching the dorsal rootganglia of the spinal cord. Rabies viruses then ascend rapidly up the spinal cord to the brain, initially infecting the diencephalon, hippocampus, and brainstem.



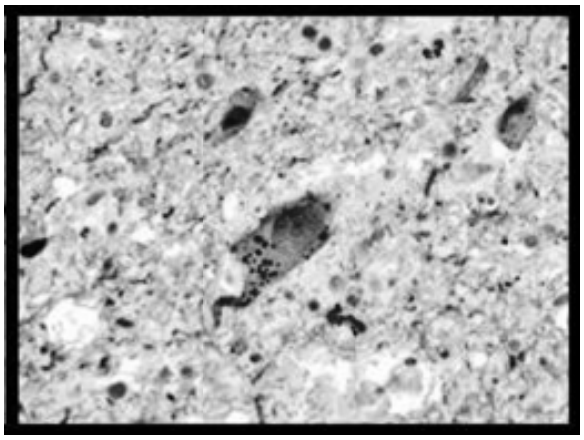
Histopathological Changes

Mononuclear cell infiltration, Perivascular cuffing of lymphocytes, Babes nodules consisting of glial cells

Negri Bodies



A: H &E stain



B: IHC

Intracytoplasmic eosinophilic inclusions in brain neurons

Composed of rabies virus proteins and viral RNA

Site - Purkinje cells of the cerebellum and in pyramidal neurons of the hippocampus are less frequently seen in cortical and brainstem neurons.

Clinical Manifestations

- ***Incubation period*** - prolonged and variable, ranges from 1 week to 19 years

- The incubation period is inversely related to the distance for the virus to travel from the site of inoculation to CNS. Hence it is usually shorter in-
- Children than in adults
- Bites on head, neck & upper limbs than legs
- Short people
- Severe lacerations
- Presence of genetic predisposition
- Low host immunity
- Virus- High dose of inoculum and from the site of inoculation to CNS and ↑virulence of the strain

Prodromal Phase

- It lasts for 2-10 days.
- It is characterized by non-specific symptoms such as fever, malaise, anorexia, nausea, vomiting, photophobia, sore throat, abnormal sensation (paraesthesia, pain, or pruritus) around the wound site.

Acute Neurologic Phase

- Either encephalitic (80%) or paralytic type(20%)
- **Encephalitic rabies (Furious rabies)** (2-7days):
- Hyperexcitability
- Lucid interval
- Autonomic (sympathetic) dysfunction features may be seen, such as ↑ lacrimation,
- ↑salivation, ↑ perspiration, gooseflesh, cardiac arrhythmia, and priapism.

Hydrophobia **(fear of water)**- sudden spasm of muscles of the mouth, pharynx, larynx, and whole of the respiratory musculature, particularly the diaphragm and muscles of inspiration. The attack can be induced by offering water to patients.

or *aerophobia* (fear of air)

- Paralytic or dumb rabies (20% of cases)
- Especially in people who are partially vaccinated or infected with the bat rabies virus and characterized by flaccid paralysis → quadripares is with facial paralysis.

These cases are commonly misdiagnosed as Guillain-Barré syndrome

Coma and Death

- The patient develops a coma that eventually leads to death within 14 days
- Patients with paralytic rabies may survive longer, up to 30 days.
- Death is almost certain. Recovery and survival are extremely rare.

Diagnosis

Most routine laboratory tests in rabies yield normal results or show nonspecific abnormalities. Complete blood counts are usually normal.

Examination of cerebrospinal fluid (CSF) - mild mononuclear-cell pleocytosis with a mildly elevated protein level

- **Direct immunofluorescence test** (direct-IF); also called as direct fluorescent antibody (DFA)test:
- Performed to detect rabies nucleoprotein antigens in specimens by using specific monoclonal antibodies tagged with fluorescent dye
- High sensitivity and specificity
- "Gold standard"
- Best specimen - hair follicle (at least 10) of the nape of neck just above hairline (most sensitive)
- A corneal impression smear can also be used. It is usually positive in the latest age with a sensitivity of 30%.
- Immunohistochemistry

Isolation of Virus

- Mouse inoculation: Intracerebral inoculation into suckling mice can cause encephalitis and death.

- Brain biopsies - Examined for Negri bodies and rabies antigen
- Cell lines: Mouse neuroblastoma cell lines and baby hamster kidney (BHK) cell line
- Yield virus (2–4 days) much faster than that of mice inoculation
- Viral growth - detected by direct-IF test using specific antiserum.
- Antibody Detection
- Detection of CSF antibodies is more significant than serum antibodies.
- CSF antibodies appear early and are produced only in rabies-infected individuals but not in response to vaccination.

Various antibody detection tests include:

- Mouse neutralization test (MNT)
- Rapid fluorescent focus inhibition test (RFFIT)
- Fluorescent antibody virus neutralization (FAVN)
- Indirect fluorescence assay (IFA)
- Hem agglutination inhibition test (HAI)
- Complement fixation test (CFT)
- Immunoperoxidase inhibition assay

Viral RNA Detection

- Reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify genes of rabies virus RNA from fixed or unfixed brain tissue.
- Most sensitive and specific assay available

Negri bodies

- Useful for post mortem diagnosis of rabies.
- They are intracytoplasmic eosinophilic inclusions with characteristic basophilic inner granules.
- Sharply demarcated, spherical to oval, and about 2-10 μm in size.
- The most common sites of Negri bodies are neurons of the cerebellum and hippocampus.
- Commonly used stains are histological stains such as H&E and Sellers stains (basic fuchsin and methylene blue inmethanol).
- Immunohistochemistry- Peroxidase labeled specific antibodies are used to detect the viral inclusions in formalin-fixed tissues.
- Negri body detection is pathognomonic of rabies. Seen in two-third of the patients

Treatment

- No specific treatment for rabies.
- Symptomatic treatment may prolong life, but the outcome is almost always fatal.
- Isolation- The patient should be isolated in a quiet room, protected as far as possible from external stimuli such as bright light, noise, water, or cold air, which can precipitate spasms.
- Sedative and anti-anxiety drugs such as morphine can be used.
- Hydration and urination should be properly maintained

Antiviral Therapy

Three agents (interferon-alfa, ribavirin, and amantadine) have been used to treat adults with rabies.

Interferon-alfa – Rabies virus triggers an innate immune response after entering the nervous system, including a type I interferon (interferon-alpha/beta) response limiting viral spread.

Ribavirin is a broad-spectrum antiviral agent that is a purine analog and RNA mutagen. Ribavirin has in vitro activity against rabies virus infection but no demonstrated efficacy in mouse models

Ribavirin is known to shift the immune response toward a Th1-type inflammatory response. Because of the importance of a

balanced Th1/Th2 immune response for the clearance of rabies virus infection, there are concerns that ribavirin could suppress antibody production essential for recovery from rabies.

Amantadine is a synthetic antiviral agent that can inhibit in vitro replication of certain viruses, including rabies. However, amantadine did not show efficacy when inoculated at daily intervals into the site of intramuscular inoculation of rabies virus in a mouse model

Favipiravar (T-705) is a broad-spectrum viral RNA polymerase inhibitor that has not yet been approved for human use in most parts of the world but has shown promise in vitro and in vivo studies in a variety of viral infection.

Prognosis

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Prevention of Rabies

- Post-exposure prophylaxis (PEP) includes local wound care and both active and passive immunization.
- *Local wound care* can greatly decrease the risk of rabies if initiated immediately.
- Physical cleansing
- Chemical inactivation- Antiseptics such as povidone-iodine or alcohol can be used to inactivate the residual viruses.
- Biological neutralization of the virus by giving anti-rabies immunoglobulin
- Devitalized tissues should be debrided
- Tetanus prophylaxis should be given
- Antibiotic treatment is initiated to prevent secondary bacterial infection
- *Suturing is contraindicated*

Passive Immunisation

- **Rabies Immunoglobulins (RIG)** - causes neutralization and loss of infectivity of the virus. Hence RIGs are usually administered locally at the site ofexposure.
- RIG should be administered as soon as possible after initiation of post-exposure prophylaxis but not after the 7th day from the first dose of ARV.
- *Two types* of RIGs are available:
- Equine rabies immunoglobulin (ERIG)- It is given a dose of 40 IU/kg. Being heterologous in origin (horse), it is associated with serum sickness.
- Human rabies immunoglobulin (HRIG)- It is given in a dose of 20IU/kg. It is devoid of side effects.
- *Maximum volume* of RIGs should be infiltrated into and around the wound(s); remaining volume, if any, should be administered by deep IM injection at a site distant from the vaccine injection site.
- *RIG should not be given in excess as it may reduce antibody production.*

Rabishield

Rabies human monoclonal antibody is indicated as passive antibody component of post-exposure prophylaxis of rabies infection

- *The maximum volume* of Rabishield should be infiltrated into and around the wound(s); the remaining volume, if any, should be administered by deep IM injection at a site distant from the vaccine injection site.
- *Dose 3.33IU/kg*

Active immunisation

- *Neural vaccines* - derived from the nervous tissues of animals infected with the fixed rabies virus.
- No longer in use since 2004 - Encephalitogenic, poorly immunogenic, and are associated with serious risk of neurological complications.
- Examples-
- Semple vaccine: Derived from infected sheep brain, inactivated with phenol.
- Beta propiolactone (BPL) vaccine: Modified Semple vaccine, which is inactivated with beta propiolactone instead of phenol
- Infant mouse brain vaccines: Derived from infected neural tissue of new born mice.

Non-Neural Vaccine

- Cell culture vaccines (CCVs) and purified duck embryo vaccines (PDEV) are currently used in India.
- **Egg-derived vaccines**- Allantoic cavity of embryonated eggs provide an excellent site for the preparation of the rabies vaccine.
- ***Purified duck embryo vaccine (PDEV)***- Duck eggs are larger, giving a higher yield than a hen. Unlike the neural vaccine, it is less reactogenic, but it is less antigenic, too; hence multiple (16–25) doses must be given to obtaining a satisfactory antibody response. It is no longer manufactured.
- *Live attenuated chick embryo vaccine* such as Flury strain was in use before for vaccinating animals, but now it is obsolete.
- Recombinant viral vaccine -Vaccinia virus carrying the rabies surface glycoprotein gene has been developed.
- Given orally, it has been successful for immunizing animals, but it is still in the experimental stage for human use.
- Cell culture-derived vaccines are the most recommended vaccine for the prevention of rabies. They are highly immunogenic and devoid of neurological complications. Three vaccines are available in India -
- **Purified chick embryo cell (PCEC) vaccine** is prepared from chicken fibroblast cell line
- **Purified Vero cell (PVC) vaccine** is prepared from Vero cell line
- **The human diploid cell (HDC) vaccine** is derived from WI-38 (human embryonic lung fibroblast cell line).

Regimen for Post-Exposure Prophylaxis-

- IM regimen or Essen regimen (1-1-1-1-1): Five doses of intramuscular injections; one dose (0.5 or 1ml) each given on days 0, 3, 7, 14 and 28. Day 0 indicates the date of administration of a first dose of vaccine and may not be the date of rabies exposure/animal bite.
- **ID Regimen (or Thai Red Cross Schedule)** (2-2-2-0-2)-This involves the injection of 0.1ml of the reconstituted vaccine on two sites per visit on days **0, 3, 7, and 28.**
- The principle that allows intra-dermal vaccination to be effective at a lower dose is the better response to an equal volume of antigen when placed in contact with Langerhans cells of the epidermis and use of multiple sites of vaccination at a time to obtain maximum drainage of antigen-presenting cells to lymphnodes
- Potency- Single intramuscular dose should have a minimum potency of 2.5IU.
- Site of injection: Deltoid region is ideal site (Gluteal region - not recommended) Infants and young children-Antero-lateral part of the thigh is the preferred site.

Intra-dermal vaccination is contraindicated in patients with immuno-compromised states or those on immunosuppressants like chloroquine or prednisolone. They should take the full dose of IM injections.

They should receive RIG in addition to ARV

Category of risk	Type of exposure	Recommended prophylaxis (WHO)
Categoy I (No risk)	Touching, or feeding of animal Licks on intact skin	No treatment needed if history is reliable
Category II (Minor risk)	Minor scratches or abrasions without bleeding or nibbling of uncovered skin	• Wound management • Rabies vaccine+ (RIG in immune compromised) • Observe the dog for 10 days
Category III (Major risk)	Single or multiple transdermal bites with oozing of blood, Licks on broken skin (fresh wounds) or mucous membrane Bite by wild animals /bat	• Wound management • Rabies immunoglobulin/Monoclonal antibody • Rabies vaccine • Observe the dog for 10days

Pre-Exposure Prophylaxis

- **Recommended for high-risk groups:**
- Laboratory staff handling the virus and infected material
- Clinicians or any person attending to human rabies cases
- Veterinarians
- Animal handlers and travelers from rabies-free areas to rabies endemic areas.
- Three doses are given at day - 0, 7, and 21 or 28 days either by IM (0.5ml) or ID (0.1ml) schedule.
- Antibody titre-checked every six months for two years and after that every two yearly. A booster dose is given if it is less than 0.5IU/ml.

Previously Vaccinated Individuals

Schedule 1:

- One dose to be injected intramuscularly or intradermally on days 0 and3
- The dose is either one single immunizing intramuscular (IM) dose (1 ml or 0.5 ml, depending on vaccine type) or one intradermal (ID) dose of 0.1 ml per site

Schedule 2:

- A "4-site" intradermal (ID) PEP can be used
- It consists of 4 injections of 0.1 mL equally distributed over left and right deltoids, thigh or suprascapular areas during a single visit

Immunoglobulins are usually not needed.

HIV and Rabies Vaccine

Intradermal vaccine is contraindicated. The victim should receive the full course of IM vaccination and RIG (CAT II and III).

Rabies Prophylaxis during Covid-19 Pandemic

1. COVID-19 vaccine and ARV can be given on the same day if circumstances necessitate, but at different sites
2. If a person is exposed to the animal after the first dose of COVID vaccination, 2nd dose should be scheduled at a minimum gap of 2 weeks from the last dose of anti-rabies vaccination.
3. If an animal bit victim receiving the course of ARV gets infected with COVID 19, he/she should continue and complete the full course of vaccination.

4. Rabishield can be given for passive immunization in a patient who has received monoclonal antibodies for COVID-19

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