10 Rabies Encephalitis: A Disease Characterized By Complex Neuropathogenic Pathways and Diagnostic Difficulties

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Abstract

Rabies virus belongs to family Rhabdoviridae and Genus Lyssavirus. It is a lethal disease spread by animal bites that carry the virus in their saliva. Rabies encephalitis has the greatest fatality rate among infectious diseases, with an average time gap of 5 to 7 days for furious or encephalitic rabies and 11 to 14 days for paralytic or dumb rabies from the onset of clinical disease to death. The disease is spread through the bites of dogs and other wild animals. Inhalation in bat-infested caves and laboratory settings are two further methods of transmission. Human-to-human transmission has been described in rare cases due to contaminated corneal transplants. Depression and fever are the first symptoms, followed by agitation, increased salivation, and hydrophobia. The presence of the rabies antigen in nuchal skin biopsies and corneal impression smears is required for antemortem diagnosis using the fluorescent antibody approach. The presence of Negri bodies on histological analysis of the brain confirms the postmortem diagnosis of rabies. The vaccine and rabies immunoglobulin are given as a preventative measure. Till date, the disease has no known treatment.

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Keywords

Rabies · Rhabdoviridae · Encephalitis · Negri bodies

10.1 Introduction

The causative agent of rabies disease is a RNA virus, namely rabies virus (RABV). The characteristics of this RNA virus is that it is single-stranded, negative sense, and non-segmented (Rupprecht 1996). The family Rhabdoviridae has three genera Lyssavirus, Ephemerovirus, and Vesiculovirus (Dietzgen et al. 2017). Rabies virus belongs to the genus Lyssavirus and is the only medically important virus of this genus. Rabies virus causes rapidly progressive acute infection of the CNS in humans and animals. Rabies disease is almost exclusively caused by bites of rabid animals, and prevention may be sought for before exposure and also after exposure to RABV, provided the vaccination is administered on time (Rupprecht 1996). Rabies till now is a major public health problem because it is almost always fatal (Koury and Warrington 2023).

10.2 Rabies Virus Structure

The rabies virus is bullet-shaped. The size of virus is 180 nm in length and 75 nm wide (Willoughby 2012). They have a lipid envelope in which 10 nm long peplomers are embedded. The envelope is lined internally by a layer of matrix protein, and the nucleocapsid has a helical symmetry. The rabies genome encodes five proteins (Fig. 10.1): Nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and polymerase (L) (Gérard et al. 2022; King et al. 2012).

10.3 Epidemiology

Rabies has been reported from almost all countries except Australia and Antarctica. Low awareness among people to seek medical advice after a dog bite has claimed lives of more than 55,000 to 60,000 people each year in Asia and Africa (Hampson et al. 2019). India accounts for the most deaths in Asia 65% of human rabies deaths. The age group which is most commonly affected are children less than 15 years and it accounts for about 30–60% of reported rabies cases and deaths in India (Sachdeva et al. 2022). Countries like Middle East, Africa, and Central Asia also have a high burden of the disease.

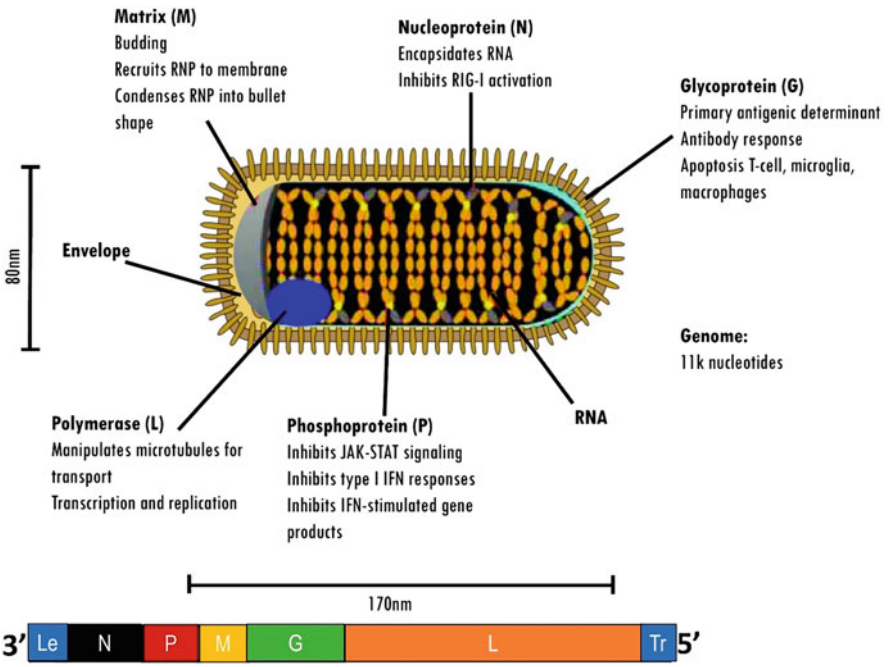

Fig. 10.1 Depiction of structure and genome of rabies virus

10.4 Transmission

The transmission of rabies can be divided into bite and non-bite exposure. In majority of cases, rabies is acquired through bite of dogs and bats. In some case, animals like foxes, raccoons, jackals, and mongooses can also transmit the disease through their bite. Non-bite exposure results from direct saliva contact with open skin or mucous membranes. Other non-bite exposure are transplantation from a donor who was infected with the virus and rarely inhalation of virus-containing aerosols (Hampson et al. 2019; Menezes 2008). Transmission of rabies through fomites or through household contacts has not been reported. During rabies surveillance in the United States, four animals have been identified as reservoir of rabies virus, these are bats, foxes, raccoons, and skunks (Blanton et al. 2012; Zhu et al. 2015).

10.5 Pathogenesis (Fig. 10.2)

Post human transmission via the bite of an infected animal, RABV begins to multiply at the inoculation site. After multiplying, the virus enters local motor and sensory nerves by spreading centripetally (Burrell et al. 2017; Rupprecht 1996). The

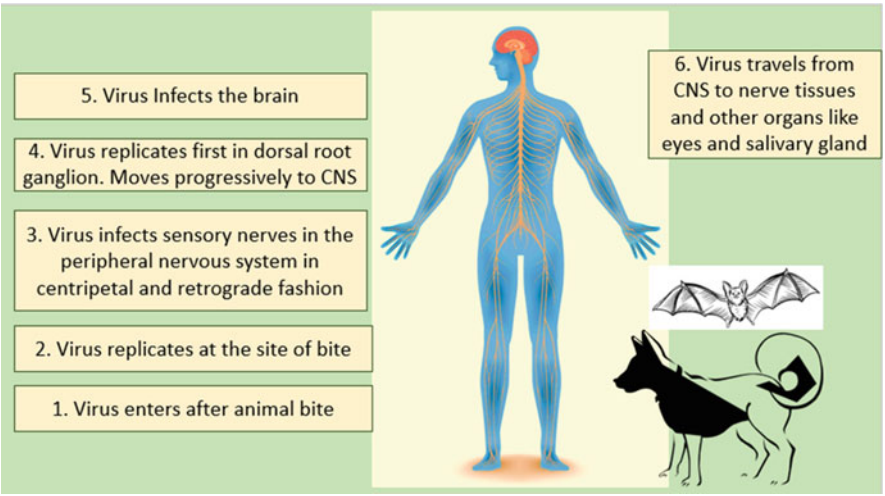

Fig. 10.2 Pathogenesis of rabies

glycoprotein projections on the surface of virus attach to the nicotinic acetylcholine receptors at the neuromuscular junction of the muscle cells. The viruses then enter nerve cells (Lian et al. 2022; Unwin 2013). Lyssaviruses have a predilection for neurons and spread centripetally along motor nerves. Lyssaviruses produce neuronal dysfunction. Malfunctioning of the mitochondria in infected neurons and other cells of the CNS is due to oxidative stress leading to various observed abnormalities (Scott and Nel 2021).

Viruses then travel in a retrograde manner within the axoplasm of nerves at a speed of 50 to 100 mm per day and reaches the dorsal root ganglia of the spinal cord (Tsiang et al. 1989). Rabies virus then reaches up to the spinal cord and the brain, initially infecting the diencephalon, hippocampus, and brainstem (Juntrakul et al. 2005; Mahadevan et al. 2016). Spread of virus occurs centrifugally along the somatic and autonomic nerves resulting in widespread dissemination to salivary glands, cornea, and other organs (Jackson 2011) (Fig. 10.4).

Rabies infection after an exposure is dependent on various factors, including the site of exposure (Rupprecht 1996; Yibrah and Damtie 2015). Exposures of the head or neck area are more likely to result in a productive infection than an exposure to distal part of the body. Host immunity, size of inoculum, and increase virulence of strain are also important factors for developing full blown disease (Grill 2009). In order to evade adaptive or innate immune response and subsequently promote replication and dissemination, rabies virus upregulates a few host pathways (Scott and Nel 2016).

10.6 Immune Evasion

Within the central nervous system, RABV is shown to evade the adaptive immunological response (Fig. 10.3) and also interfere with innate immune response (Fig. 10.4). When RABV infects neurons, it sequesters Toll-like receptor 3 (i.e., TLR3) into Negri bodies, preventing TLR3 from being activated as an innate sensor. Immune sensors (i.e., RIG-I and MDA5) detect RABV-RNA in neuronal cytoplasm, and this recognition of RABV-RNA triggers the expression of innate antiviral type-I interferon (IFN-alpha/beta) through the generation of a complex of proteins that phosphorylate interferon regulatory factors 3 and 7 (IRF3/7). Along with activator protein, AP-1, and nuclear factor B, i.e., NF-B, phosphorylated-IRF3/7 is transported to nuclei in order to stimulate IFN-α/β transcription. Rabies virus phosphoprotein alters IFN-α/β gene transcription by preventing IRF3/7's phosphorylation and nuclear import while leaving activator protein-1 and nuclear factor-B

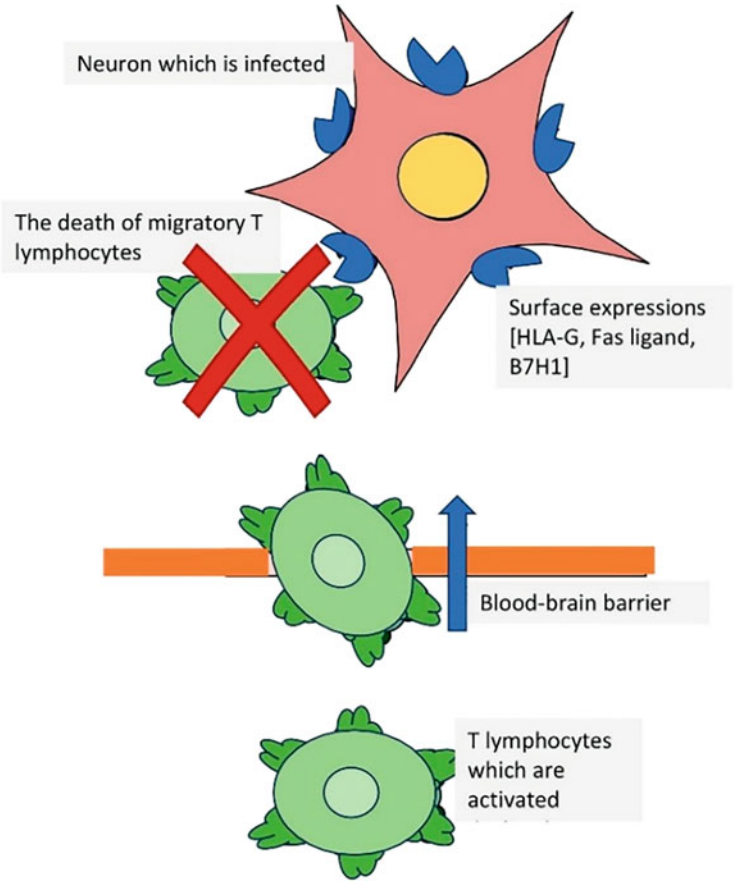

Fig. 10.3 (Adaptive) immune response evasion

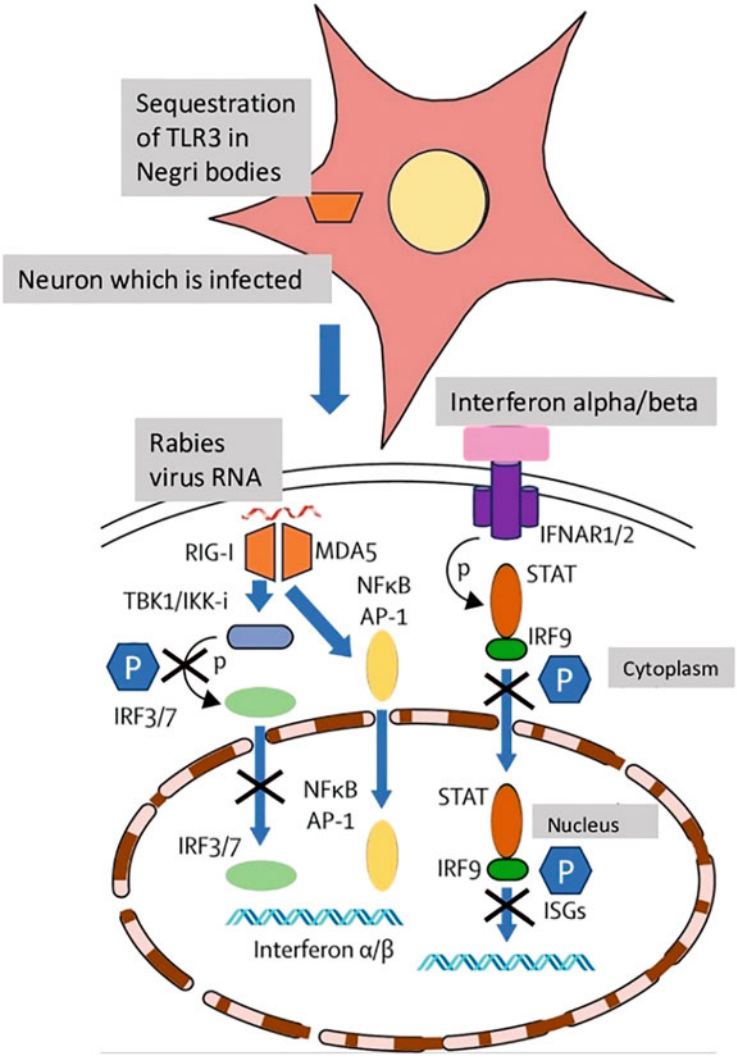

Fig. 10.4 (Innate) immune response inhibition

activation unaffected. The signal transducer and activator of transcription (STAT) signaling pathway is also suppressed by viral phosphoproteins. Viral phosphoprotein inhibits nuclear import of (phosphorylated) STAT, binds to intranuclear STAT-IRF9 complex, and binds to intracytoplasmic (phosphorylated) STAT to suppress interferon stimulated gene (ISG) transcription. Even though the blood–brain barrier is preserved in RABV-infected individuals, activated T lymphocytes and monocytes can penetrate it and enter the central nervous system. RABV-infected neurons that exhibit the immunosubversive components, namely, B7-H1 (B7-homolog 1, also

known as programmed death-ligand 1), Fas ligand (FasL), and human leukocyte antigen G (HLA-G) on their surfaces, cause T cells that express the appropriate ligands to bind, which is promptly accompanied by the death of migratory T cells.

10.7 Clinical Presentation

The incubation period of rabies is between 4 weeks to 12 weeks days but can go up to several weeks to many years after an exposure (Rupprecht 1996).

The clinical manifestation has three stages (Table 10.1), which are

- Prodromal phase.
- Acute neurological phase- can either be encephalitic (80% cases) or paralytic (20% cases).
- Coma and death- after the neurological phase the patient goes into coma and eventually dies within 2 weeks. Death is almost certain and recovery and survival is rare.
- The classical symptom of rabies is **hydrophobia**. It occurs in 33 to 50% of patients (Tongavelona et al. 2018). There is discomfort in throat and difficulty in swallowing followed by sudden development of fear for water. The terror of

Table 10.1 Clinical phases of rabies disease (Rupprecht 1996)

Phase	Clinical manifestations
Prodromal phase	Iideally 2 to10 days; marked with nonspecific symptoms: • Fever • Malaise • Anorexia • Nausea and vomiting • Photophobia • Abnormal sensation • Sore throat • Pain at the site of bite
Excitation phase	• Restlessness and tremor • Pharyngeal and laryngeal spasm • Aerophobia • Hydrophobia • Respiratory distress and cardiac arrythmias • Hallucination • Hypertension and autonomic dysfunction • Change in tone and pitch of voice • Death
Paralytic phase	It mostly occurs in persons who are partly vaccinated or infected with bat rabies virus • Flaccid paralysis • Facial paralysis • Paralysis of all the four limbs • Unconsciousness • Death

water leads to involuntary spasms of the muscles of pharynx during attempts to drink (Koury and Warrington 2023; Tongavelona et al. 2018).

- **Aerophobia** is also pathognomonic of rabies (Mahadevan et al. 2016). Painful inspiratory spasms of the diaphragm and accessory inspiratory muscles can lead to aspiration, coughing, choking, vomiting, and hiccups; when severe, these spasms can lead to asphyxiation and respiratory arrest (Kietdumrongwong and Hemachudha 2005).
- **Autonomic hyperactivation** occurs in 25% of cases (Mahadevan et al. 2016). As a result of overactivity excess salivation, lacrimation, sweating, goose flesh, and mydriasis occur. Increased heart rate and cardiac arrhythmias are common and it may be related to myocarditis which results from direct viral injury (Alexander et al. 2021; Park et al. 2019).
- Difficulty in speaking, difficulty in swallowing, and double vision may occur. Dysphagia was reported in approximately half of all cases in one retrospective series (Kietdumrongwong and Hemachudha 2005).
- Aggression and excitation are also common (approximately 50% of patients). During the aggression phase patient is restless, agitated, and disoriented. Hallucination is a common symptom in this phase. After a period marked by agitation, the patient often goes into a period of calmness (Koury and Warrington 2023).
- Respiratory and cardiac complication is the leading cause of death in rabies patients (Alexander et al. 2021).

10.8 Diagnosis

The diagnosis of rabies requires a detailed history taking from the patient and a high level of suspicion. Rabies should be kept in mind as a differential diagnosis in all patients presenting with acute progressive encephalitis irrespective of history of animal bite or exposure (Chacko et al. 2017; Madhusudana and Sukumaran 2008).

There are two methods—direct tests and indirect tests.

Direct tests:

- Antigen detection- sellers staining, direct fluorescence antibody test (DFAT), direct rapid immunohistochemistry test (DRIT), rapid antigen detection test (RADT) (Lembo et al. 2006; Torquato et al. 2020).
- Molecular methods- nucleic acid amplification test (NAAT).
- Virus isolation- rabies tissue culture inoculation tests (RTCIT), mouse inoculation test/biological test (MIT/BT).

Indirect tests:

- Serology—Enzyme linked immunosorbent assay (ELISA), Rapid fluorescent focus inhibition test (RFFIT), indirect fluorescence antibody testing (IFA) (Debnath et al. 2019).

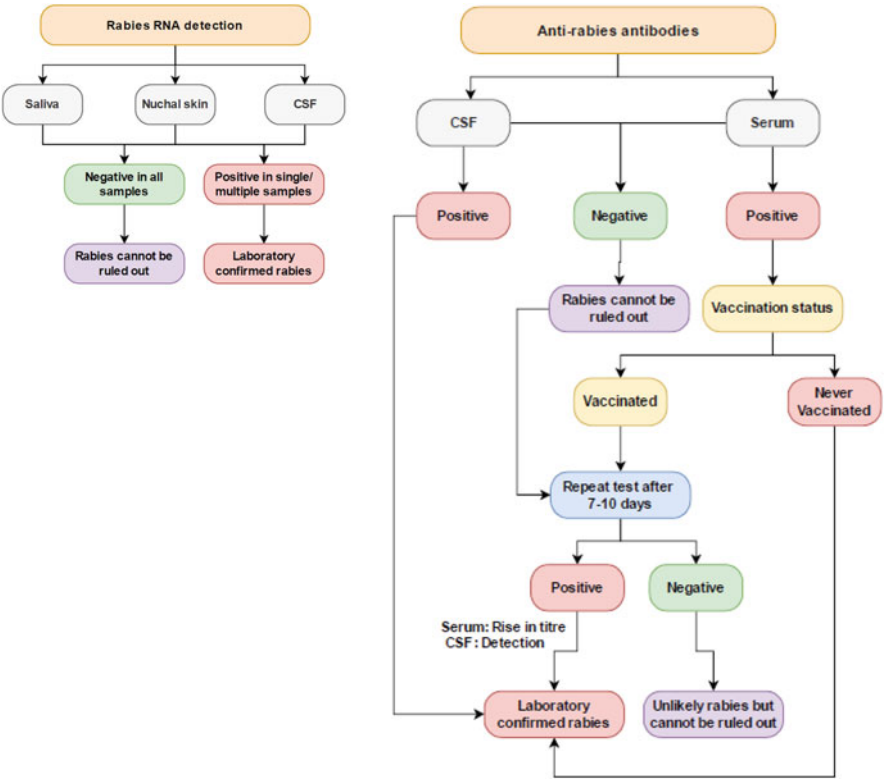

Fig. 10.5 Antemortem laboratory confirmation of rabies

The diagnosis of rabies can be made both antemortem and postmortem. All samples from suspected cases of rabies should be considered as highly infectious and must be properly sealed and labeled in leak-proof containers. Antemortem samples include serum, saliva, CSF, biopsy from skin and corneal impression (Fig. 10.5). Postmortem samples which can be collected are brain, spinal cord, and salivary gland (Fig. 10.6) (Jackson 2011; Madhusudana and Sukumaran 2008).

10.8.1 Sample Collection

10.8.1.1 Saliva

Around 500 μL saliva is collected in a sterile container and sealed securely. No preservative is required. Laboratory tests to be performed include detection of rabies RNA by RT-PCR technique (Madhusudana and Sukumaran 2008; Singh and Ahmad 2018).

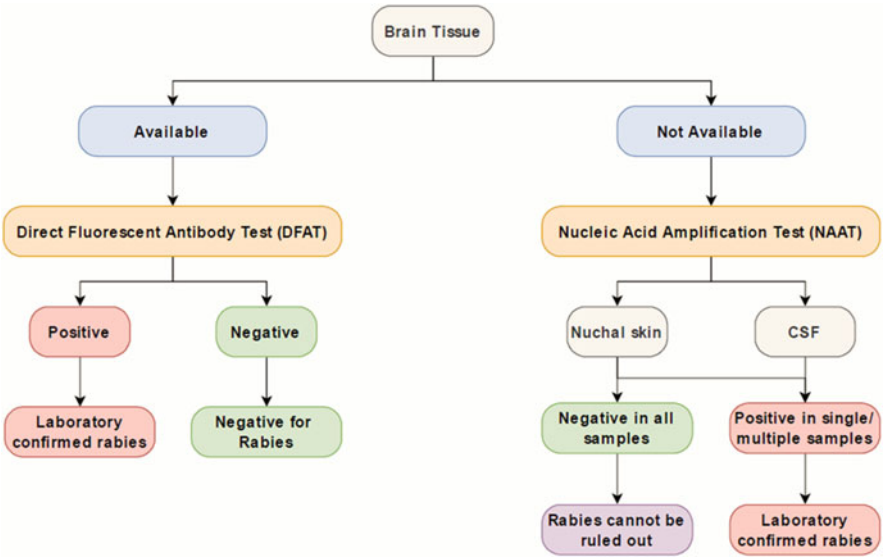

Fig. 10.6 Postmortem laboratory confirmation of rabies

10.8.1.2 Cornea

Eyelids are retracted using thumb and finger. A clean slide is pressed against the cornea. Excess pressure is avoided to prevent any damage to the eye. Corneal impression is air-dried for 10–15 min at room temperature. Then it is treated with chilled acetone and processed further for Direct Fluorescent Antibody Test (DFAT) (Zaidman and Billingsley 1998).

10.8.1.3 Skin Biopsy

From the nape of the neck, 5 to 6 mm of skin tissue sample, with an approximate depth of 5 to 7 mm, is procured. The specimen should contain hair follicles and cutaneous nerves at the base of hair follicles. An excision or punch biopsy may be collected (Madhusudana and Sukumaran 2008; Singh and Ahmad 2018). The skin biopsy is placed on the slide and covered with a piece of sterile moistened gauze to prevent specimen from drying. No fixative is required. The sample can be used for DFAT/DRIT for detecting viral antigens. RT-PCR for detecting viral nucleic acid, and RTCIT for virus isolation.

10.8.1.4 CSF

Two to three millilitre each of serum and CSF is collected in a sterile vial with all aseptic precautions. If vaccine or immunoglobulin has not been given, the presence of antibody to rabies virus in the serum can be diagnosed. Antibody to rabies virus in the CSF suggests a rabies virus infection regardless of the immunization history. Laboratory tests for antibodies include ELISA, indirect immunofluorescence, and

virus neutralization. CSF samples can also be processed for RTCIT and molecular technique (NAAT-PCR) (Mani and Madhusudana 2013; Torquato et al. 2020).

10.8.2 Laboratory Testing

10.8.2.1 Negri Bodies

Some viral infections are linked with the production of inclusion bodies in the affected cells. These inclusion bodies are of two types- intranuclear and intracytoplasmic. In Rabies infection, intracytoplasmic inclusion bodies commonly known as Negri bodies which are acidophilic in nature can be demonstrated. Negri body-staining is carried out by the seller's staining test (SST) (Lahaye et al. 2009).

10.8.2.2 DFAT

It is considered as gold standard test. It works on the following principle: Rabies-specific antibodies and antigens combine to form an antigen–-antibody complex when mixed and kept under optimum condition The complex formed is not visible to the naked eye. Fluorescent dyes, like fluorescein isothiocyanate (FITC), can be used to visualize this antigen–antibody complex under a fluorescence microscope. To achieve this, the FITC is tagged with an anti-rabies antibody to form the conjugate. As the conjugate directly binds to the antigen, the process is called the direct fluorescent antibody test (DFAT) (Realegeno et al. 2018; Vengatesan et al. 2006).

10.8.2.3 DRIT

Detects rabies antigen in the sample based on specific antigen–antibody reaction followed by detection using a compound microscope. The formaldehyde fixed smears are incubated with polyclonal or monoclonal anti-rabies antibodies which are labeled with biotin moiety. The unbound antibodies are washed away. The biotin moiety has a significant affinity toward streptavidin. The slide is then incubated with streptavidin conjugated with horseradish peroxidase (HRP). After removing unbound reagent, the slide is incubated with chromogen substrate (amino-ethyl carbazole, AEC) in the presence of Hydrogen Peroxide (H_2O_2). The substrate is converted into an insoluble red precipitate which is visible under compound microscope (Debnath et al. 2019).

10.8.2.4 RADT

It is based on immunochromatographic principle in lateral flow format. The assay employs a lateral flow device which has an anti-rabies antibody immobilized on a nitrocellulose membrane. The reagent has a secondary anti-rabies antibody which forms a complex with the virus present in the sample. The secondary antibody is usually tagged with a reporter dye or colloidal gold. Formed complexes in the prepared samples chromatographically move along the lateral flow device till they are captured by immobilized anti-rabies antibodies present at designated bands. This results in formation of a colored band at the site of immobilized anti-rabies antibody in lateral flow device. The band can be observed visibly. A second immobilized

antibody captures a control protein from the sample. This control should be clearly visible to interpret the assay results (Wang et al. 2010).

10.8.2.5 NAAT

It is based on the detection of genomic material of the target organism in the sample. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) can be used for Rabies RNA detection in suspected samples. Therefrom, extracted samples are tested following pan-lyssavirus nested-PCR protocols. Initial reactive processes involve degenerate primers to amplify RNA of all lyssaviruses followed by a second reaction using both pan-lyssavirus degenerate primers and rabies-specific primers. The protocol confirms the presence of rabies and non-rabies lyssavirus RNA in the suspected sample (Biswal et al. 2012).

10.8.2.6 ELISA

It is used to demonstrate rabies antibodies in human and animal (dogs, cats, fox) serum. The assay is a quantitative indirect ELISA which uses WHO's (human samples) and WOAH's (animal samples) traceable standards for quantification of antibodies. The microwells are coated with rabies glycoprotein extracted from inactivated and purified rabies membrane. Therefore, the antibodies detected are specific to rabies glycoprotein (Piza et al. 1999; Servat et al. 2007).

10.8.2.7 RFFIT

It is a type of rabies virus neutralization test. It is carried out in cell culture to demonstrate the rabies virus antibody level in human or animal sera. Immunofluorescence staining is used as an indicator of viral growth (Burgado et al. 2018).

10.8.2.8 RTCIT

It is based on the ability of rabies virus to infect some cell lines in vitro. Murine neuroblastoma 2A (MNA) has been shown to be the most sensitive cell line for this method. However, Baby Hamester Kidney (BHK) cell line is easy to maintain and commonly employed for RTCIT. The growth of the virus in the cell culture can be ascertained by a sensitive technique such as DFAT (Chhabra et al. 2007).

10.8.2.9 MIT/BT

The growth of rabies virus takes place only in living tissues (Rupprecht 1996). These living tissue could be animals, chick embryo, or tissue culture (Koprowski et al. 1954). Rabies virus is pathogenic to all animals when introduced via intracerebral route. Animals for experimental studies are mouse, rat, guinea pigs, hamster, rabbit, and dog (Jackson 2011). RABV-positivity in any sample may be determined by intracerebral inoculation of the specimen into mice, and also by keeping the animal under observation to check for any sickness development or RABV-induced death (Madhusudana and Sukumaran 2008; Rupprecht 1996).

Table 10.2 Differential diagnosis of rabies: different phases of rabies resembles closely to conditions as given in clinical manifestation

Phase	Clinical manifestation
Prodromal phase	• Nonspecific viral illness • Mononucleosis • Meningitis • Bacteremia • Hysteria • Encephalitis
Encephalitis phase	• Herpes simplex virus encephalitis • West Nile virus encephalitis • Central nervous system vasculitis • Toxic or metabolic encephalopathy • Auto immune encephalitis • Delirium tremens • Belladonna poisoning
Paralytic phase	• Acute polyneuritis • Acute transverse myelitis • Poliomyelitis • Guillain Barre syndrome • Neuromuscular junction disorders

10.9 Differential Diagnosis

The nonspecific prodromal phase of rabies may be confused with a wide range of disorders (Table 10.2). In patients with signs and symptoms of encephalitis, more common infection such as herpes simplex virus, west Nile virus and other non-infectious disorders of the CNS should be ruled out. Other causes of muscular rigidity that can be seen with rabies includes: Tetanus, dystonia, and strychnine poisoning.

10.10 Management and Prevention

In rabies endemic countries, there is continuous animal to animal transmission and therefore any animal bite should be considered as bite by rabid animal and postexposure prophylaxis (PEP) should be started as early (Menezes 2008; Radhakrishnan et al. 2020). To maintain uniformity, WHO has classified type of exposure into three classes and has recommended appropriate PEP (Table 10.3) (O'Brien and Nolan 2019).

10.10.1 Wound Toilet

Mostly the virus enters the body through a bite or scratch, so it is important to remove the infected saliva from the wound (Di Quinzio and McCarthy 2008). The wound should be washed with soap and water thoroughly (Scholand et al. 2022). Antiseptic with virucidal activity can be used. Rabies virus multiply locally at the

Table 10.3 WHO guidelines for recommended postexposure treatment against rabies based on categories of exposure

Category	Type of exposure	Recommended postexposure prophylaxis
I	Touching or feeding of animals Licking on unbroken skin Exposure of unbroken skin with secretions/excretions of infected animal/human case	None if reliable case history is available Wash exposed area with water and soap
II	Nibbling of uncovered skin Small scratches or abrasions with no bleeding	Wound cleaning Rabies vaccination
III	One or many transdermal bites or scratches Lick on non-intact skin Staining of mucous membrane with saliva	Wound cleaning Rabies immunoglobulin Rabies vaccination

bitten for a long period and hence wound cleaning must be done even if the patient comes late. Tetanus prophylaxis should be given as per guidelines. Local treatment along with proper instillation of ERIG or HRIG in the wound is also given (Liu et al. 2017; Pounder 2005).

10.10.2 Passive Immunization

10.10.2.1 Equine Rabies Immunoglobulin (ERIG)

Immunoglobulin gives passive immunity in the form of ready-made anti-rabies antibody to overcome the acute phase of the infection (Terryn et al. 2016). RIG binds with the rabies virus, and makes the virus noninfective (Bharti et al. 2017). Immunoglobulin should be started as soon as possible, but can be given up to 7 days.

10.10.2.2 Human Rabies Immunoglobulins (HRIG)

HRIG do not possess the side effects encountered with ERIG. HRIG has a longer half-life, and so their dose is half of ERIG. The Immunoglobulin should be brought to room temperature (20–25 °C) before use (Haradanhalli et al. 2022).

10.10.2.3 Dose of Rabies Immunoglobulins (RIG)

The dose of ERIG is 40 IU per kg body weight and is given after testing of sensitivity, up to a maximum of 3000 IU can be given. (Bharti et al. 2017). The dose of HRIG is 20 IU per kg body weight, maximum of 1500 IU can be given (Madhusudana et al. 2013).

10.10.3 Active Immunization

Administering potent and safe Cell Culture Vaccines (CCVs) bring about active immunization. There is a Rabies immunization schedule (Table 10.4) that has been issued by the World Health Organization (WHO) (O'Brien and Nolan 2019).

Table 10.4 Vaccination routes and their respective schedules for postexposure prophylaxis (PEP) and pre-exposure prophylaxis (PrEP) for rabies virus

Types of prophylaxis	Route of administration*	Dose of vaccine	Day of dose	Injection per visit	No. of visits
Postexposure prophylaxis	ID	0.1 mL per dose	0, 3, 7, and 28	2	4
	IM	1 entire vaccine vial	0, 3, 7, 14, and 28	1	5
Pre-exposure prophylaxis	ID	0.1 mL per dose	0, 7, [plus 21 or 28 in previous regimen]	1	2 [3 in previous regimen]
	IM	1 entire vaccine vial	0, 7 [plus 21 or 28 in previous regimen]	1	2 [3 in previous regimen]
Re-exposure	ID	0.1 mL per dose	0 and 3	1	2
	IM	1 entire vaccine vial	0 and 3	1	2

Immunization with live attenuated vaccines produce humoral and cell mediated immunity within a week and provides protection (Overduin et al. 2019). Immunoglobulin gives instant passive immunity with proven effectiveness over the initial few weeks after exposure in humans (Haradanhalli et al. 2022). The antibodies help in managing the growth of rabies virus infection as they are capable of neutralizing the virus. The induction of a potent cytotoxic T lymphocyte (CTL) response appears to be related to both the effectiveness of postexposure immunization and the long-term benefits of vaccine-induced prophylaxis against rabies (Venkataswamy et al. 2015).

Intramuscular Regime for Rabies Postexposure Prophylaxis—There are three intramuscular schedules for category 2 and 3 exposures:

- **The 5-dose regimen (1–1–1-1-1):**
  Vaccination is given on day 0, 3, 7, 14, and 28.
  Site: Deltoid region for adults and anterolateral aspect of thigh in children.
- **The 2–1-1 regimen (2–0–1-0-1).**
  Vaccination is given on: 2 doses on day 0, one dose on day,7 and one on day 21.
- The 4-dose regimen with RIG in both category 2 and 3.

Intradermal Regime for Rabies Postexposure Prophylaxis:

- 2 Site intradermal method (2–2–2–0–2) given on day 0, 3, 7, 28.
- One dose of vaccine 0.1 mL intradermally at two different sites.

Site of injection:

- **Adult**- deltoid muscle.
- **Infants** and **children**- anterolateral thigh.

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