Review Article

Beyond Fear: A Comprehensive Exploration of Rabies Virus and Its Implications

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Abstract | The Rabies virus belongs to the family Rhabdoviridae and genus Lyssavirus. It is a neurological infection that is transmitted through animals and can be lethal. It is a disease that affects all warm-blooded species and is widespread worldwide, except for islands like Australia and Antarctica. Rabies causes more than 60,000 deaths annually, although every year about 15 million people receive Rabies Post-Exposure Prophylaxis (PEP). Wildlife including raccoons, skunks, bats, and foxes are major rabies reservoirs. The disease is primarily spread by the bite of a rabid animal and the saliva of an infected host. The incubation period (average 2-3 months) varies greatly, with periods ranging from 2 weeks to 6 years. Neuropathological lesions are typically modest, despite significant neurologic symptoms and a deadly prognosis. The Rabies virus uses several strategies to circumvent the host's defenses. As a major zoonosis, initial treatment and successful preventive and control efforts depend on a clear and prompt diagnosis. For the diagnosis of Rabies, conventional techniques such as Direct Fluorescent Antibody Test (dFAT) and histopathology are still employed. The OIE and WHO both frequently propose the gold standard test, the Direct Fluorescent Antibody Test (dFAT), for the diagnosis of Rabies in fresh canine brain tissues. The preferred methods for routine diagnosis include PCR (Polymerase Chain Reaction), and Mouse Inoculation Test (MIT). In endemic locations, vaccinations with DNA, recombinant vaccines, and live, attenuated, or inactivated viruses can be administered. This study provides comprehensive information on immunization, treatment techniques, pathophysiology, epidemiology, transmission, and relevant preventive and control measures.

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Introduction

Rabies, derived from the Latin rabere meaning to be mad, has been recognized since civilization's dawn. The earliest formal Rabies record dates to the 23rd century BC in the Babylonian Eshnunna record. Louis Pasteur identified the disease as viral in the 1880s. Despite being preventable by vaccine,

Rabies remains a significant health issue in developing countries, causing over 60,000 deaths annually. Only about 15 million people receive Rabies Post-Exposure Prophylaxis (PEP) each year (Dietzschold and Koprowski, 2004). Despite prevention efforts and awareness campaigns, over 95% of dog rabies deaths occur in Asia and Africa, where the disease is enzootic (WHO, 2013). In India, rabid dog bites cause over 20,000 human deaths annually. Rabies remains fatal in humans despite advanced therapeutic interventions. It ranks sixth among contagious diseases worldwide for its high human fatality rate (Wyatt, 2007). Rabies, a lethal disease in mammals, is caused by a neurotropic, negative-sensing, non-segmented, single-stranded RNA virus from the Lyssavirus genus, Rhabdoviridae family, and Mononegavirale group. This virus, known for its seven unique genotypes, affects the nervous system. The rabies virus (RABV) genome is about 12 kb and includes five structural proteins: RNA-dependent RNA polymerase (L), nucleoprotein (N), matrix protein (M), phosphoprotein (P), and glycoprotein (G) (Albertini *et al*., 2011). The RABV genome's N, P, and L proteins form a ribonucleoprotein complex aiding in the virus's replication in the host cell's cytoplasm. It causes acute encephalomyelitis, affecting mainly carnivores and bats, but also humans, all warm-blooded animals, and various wildlife species serving as infection reservoirs. Rabies is largely lethal and spreads globally without a specialized antiviral treatment. The disease is prevalent on all continents except Antarctica and Australia (Rupprecht et al., 2008). Rabies raises public health concerns across Asia and Africa, with the highest prevalence in Pakistan, Bangladesh, and India. Nepal, Myanmar, Bhutan, Thailand, and Indonesia have moderate prevalence. The disease's frequency in domestic animals ranges from 20% to 50%. Animal susceptibilities vary based on species, age, genetics, strain, biotype, virus dose, and exposure route. Despite rabies' global spread, control efforts have reduced case numbers in countries like the United States (Steele and Fernandez, 2017). In many developing countries, human rabies infection death rates are low due to factors such as underreporting, cultural norms, inadequate or absent rabies diagnostic facilities, and a lack of knowledge about the disease's transmission and control mechanisms.

Materials and Method

Lyssaviruses, neurotropic viruses detectable at clinical disease onset, have distinct zoonotic transmission cycles and global distribution within various mammalian reservoirs. Of the 16 recognized lyssaviruses, most are found in bats. The rabies virus causes all lyssavirus-related human deaths, estimated at 59,000 annually worldwide, and has the highest mortality rate among zoonotic pathogens (Burnet, 1960). Domestic dogs are the primary rabies virus carriers, but over 30 species worldwide are recognized as virus reservoirs. Many more may go unnoticed due to inadequate surveillance (Fernandes *et al*., 2011). Rabies can be eradicated in most high-income countries through sustained dog vaccination and population management programs, and in some wildlife populations, particularly Canadian raccoons, and European red foxes. Lyssaviruses, especially the rabies virus, vary geographically and continue to evolve as global surveillance expands.

Mode of transmission and species affected and reservoirs

Rabies, a lethal disease affecting mammals, is transmitted through the saliva of an infected animal, typically via bites, scratches, or licks on broken skin or mucous membranes. The virus travels to the nervous system once inside the body (Frana *et al*., 2008). It can also spread when infected saliva contacts mucous membranes or fresh wounds. Rarely, transmission occurs through organ transplantation, corneal or skin grafting, and aerosol inhalation. Rabies can infect various mammals, with wild animals, especially carnivores and bats, being common virus reservoirs. Dogs have historically been a significant source of human rabies transmission, but vaccination programs have reduced canine rabies substantially (Xiang *et al*., 2014). Bat species are of concern due to their ability to carry and spread the virus over long distances. Some bats can harbor the virus without showing clinical signs, making them potential silent carriers. Other mammals like cows, horses, and ferrets can also contract and transmit the virus. Species susceptibility varies, with some being more resistant or less likely to show clinical symptoms. Wildlife, particularly certain mammalian species, serve as natural reservoirs, harboring the virus and supporting its circulation within a population without succumbing to the disease (Burnet, 1960). Understanding these reservoirs is crucial for implementing effective control measures. Vaccination campaigns targeting both domesticated and wild animals are key to reducing reservoir populations and preventing rabies spread.

Pathogenesis of rabies

Rabies, a CNS disease, is caused by the negative-stranded RNA rabies virus (RV) from the rhabdovirus family. RV primarily shows neuro-invasiveness, infecting neurons. The RV glycoprotein regulates virus replication and absorption rates and virus propagation across synapses, playing a key role in RV pathogenesis.

Incubation period

The Incubation Period is the time from the bite to when the virus reaches the brain. This phase varies from 2 weeks to 6 years, depending on the virus concentration, inoculation site, and innervation density. The Rabies virus doesn't survive long on inanimate surfaces but can persist in saliva and mucous membranes. The incubation period depends on the wound site and its distance from the brain, the tissue's nerve supply, and the patient's physical condition.

Pathogenicity steps

Virus introduced through a bite.

- A virus slowly moves from nerve cell to nerve cell toward the brain (attaches via G-protein couple receptors).
- Virus moves to CNS using receptors (nicotinic acetylcholine, CD56, NTR75).
- The virus spreads from CNS along parasympathetic nervous system, infecting salivary gland and skin.
- Once the virus in the brain has infected enough brain tissue, it begins to affect the animal's behaviour (apoptosis is inhibited to favour progression) (Center for Disease Control, 1977).

Pathophysiology

The Rabies virus replicates in muscles or connective tissue cells near the bite before attaching to nerve endings and entering peripheral nerves. It spreads centripetally through nerves to the CNS. Once in the CNS, the virus spreads centrifugally in peripheral nerves, including skeletal and myocardial muscles, adrenal glands, and skin. Transmission occurs through salivary gland invasion.

There are no visible changes apart from brain and spinal cord gray matter congestion. Microscopically, there's cell degeneration, phagocytosis of degenerating cells, and inflammatory cell collars around small blood vessels. The pathognomonic feature is Negri bodies or inclusion bodies in ganglionic cells in the hippocampus, medulla, and cerebellum. These are acidophilic bodies with a blue center. The basophilic material represents the virus, while the outer acidophilic material may be a host tissue product. When a dog suspected of rabies has bitten another animal, the dog's brain must be examined for Negri bodies.

Clinical features

I Stage-prodomal phase/ invasive phase: Abnormal sensation around the infection site, general unease, anxiety, depression, irritation, nausea, loss of appetite, and unusual sensitivity to sound, light, and temperature changes.

II Stage- excitement stage: Alternating periods of excitement and calm. Intolerance to noise, bright light, cold drafts, aerophobia, increased reflexes, muscle spasms, dilated pupils, increased salivation, lacrimation, fear of death. Hydrophobia is absent in animals. Illness lasts 2-3 days, up to 5-6 days. Death usually occurs from cardiac or respiratory failure, or progression to paralysis and coma.

III Stage- paralytic stage: Muscle paralysis causing paraplegia, quadriplegia. The patient may go into a coma and death occurs (Gastka *et al.*, 1996).

Symptoms

Rabies symptoms may include unexplained aggression, impaired locomotion, varying degrees of paralysis, and extreme depression or viciousness. Animals may display the vicious or dumb form, appearing sick, dazed, or very lethargic. Once symptoms appear, death usually occurs within 5 days.

Clinical samples for testing include:

- Saliva for nested RTPCR and/or virus isolation.
- Nuchal skin biopsy for antigen detection.
- Serum and CSF for IgM and IgG antibody determination.

The standard test for rabies virus antigen detection in CNS tissues is the DFA test. DRIT is an alternate procedure.

Complications may include general symptoms like headache and giddiness, local symptoms like swelling and infection, allergic reactions, and neuro-paralysis.

Immunopathology

The CNS shows perivascular cuffing, localized gliosis, and neuronophagia, indicating a near-total lack of an inflammatory response. The brainstem experiences more severe lesions than other CNS parts. These findings suggest that rabies' deadly consequence is

due to neural malfunction rather than neuronal death. The virus may be stimulated to replicate and spread by components of the immune response. Immune cells may help the virus move from poorly to highly innervated locations, such as lymph nodes, facilitating RV migration to the CNS.

This involves cytokine secretion, cytokine response, and antigen presentation. These properties allow physical contact between microglia and/or astrocytes and cytokine communication. IFN-g secretion by infiltrating activated T cells initially induces astrocytes and microglia to express class I and II major histocompatibility complex (MHC) antigens and primes these antigens for cytokine production. Astrocyte and microglia activation may contribute to the initiation and/or propagation of intracerebral immune and inflammatory responses. Many mediators, including prostaglandin, the cytokines IFN-a, IFN-b, and IFN-g, and endogenous neuropeptides such as Vaso intestinal peptides and norepinephrine, can decrease these responses by preventing the development of class I and II MHC and the production of cytokines by glial cells.

Apoptosis can be caused in two ways: cell-dependent and virus-dependent. Various viral strains and inoculation techniques can implicate numerous pathways in neuron death or survival during RV infection, both in vitro and in vivo. Observations suggest that apoptosis may be a protective mechanism in RV infections, as fewer pathogenic viruses induced more apoptosis than a greater number of pathogenic viruses. Defective neurotransmission involving neurotransmitters like acetylcholine, serotonin, GABA, could play a significant role in the pathogenesis of rabies (Alvarez *et al*., 1994; Baloul and Lafon, 2003; GM *et al*., 1990).

Evasion of RABV from host immune response

Rabies, a deadly disease, poses a high zoonotic risk. Its spread depends on human-mediated dispersion and host animal migration. Factors like population growth, income increases, and the COVID-19 pandemic have led to a significant rise in animal populations, intensifying the need for precautionary measures like vaccination, surveillance, and animal control programs (Chupin *et al*., 2023). Despite the global distribution of the rabies virus, 95% of cases are reported from Africa and Asia, which are developing regions. There's no effective treatment after clinical symptoms appear, highlighting the importance of understanding rabies pathophysiology to identify potential therapeutic targets (Knobel *et al*., 2005).

Replication of RABV

The most important feature of RABV is its tendency to replicate in the CNS which help it to increase its number on the immunologically exempted area of host (Carson *et al*., 2006). First the virus enters the host muscle tissue via bite and RABV glycoprotein binds to specific receptors (nicotinic acetylcholine receptor) of muscle tissue. This pathway of entry into host tissue indicate that RABV get exposed to immune responses after getting entry into host muscle tissue the RABV replicate slowly showing that virus goes to different periods of incubation at the initial stage of infection (Hemachudha *et al*., 2013; Siniscalchi *et al*., 2010; Yamaoka *et al*., 2013). It was revealed that virus got entry into peripheral nervous system through the neuromuscular junction (Lafon, 2005, 2011).

Relationship between pathogenicity and neuroinvasiveness

The specificity of viral strain affects the replication and entry of virus into PNS. Strain showing the greater pathogenicity will be more efficient in neuroinvasiveness (Siniscalchi *et al*., 2010). Pathogenic and non-pathogenic strains show the similarity in replication efficiency and higher neuron infecting ability, so it was seen that.

RABV protein's role in evasion from immune response Rabies Virus (RABV) proteins, specifically phosphoprotein (P), nucleoprotein (N), and the G protein, are crucial in evading the immune response. The P protein is particularly important as it affects the Ifn-β, Mx1, and Oas1 genes, which are vital for eliciting the immune response against virus infection. It's concluded that sequestration from specific immune responses in host muscle tissues is essential for the effective reproduction of RABV, necessary for its neuroinvasion (Scott and Nel, 2016; Siniscalchi *et al*., 2010; Srithayakumar *et al*., 2014).

Aspects of RABV that contribute to neuroinvasiveness The entry of any virus into the host's Central Nervous System (CNS) is typically restricted by two mechanisms: Apoptosis of infected neurons and immune response. However, both these mechanisms are deceived by the Rabies Virus (RABV), allowing the spread of the virus in the CNS. RABV has evolved two main strategies: killing protective

migratory T cells, and infiltrating the CNS without triggering apoptosis of the infected neurons, thereby maintaining neuronal integrity (Lafon, 2011). Two aspects contribute to RABV's neuroinvasiveness: It avoids causing neuronal cell death, and protective T lymphocytes migrating into the infected nervous system are either killed by apoptosis or rendered inactive due to the overexpression of immune halting molecules in the diseased neural system, such as FasL, HLA-G, or B7-H1. This makes the virus particularly difficult to combat. RABV Evasion from Host Immune Response

Previous researches showed that the wild-type (wt) rabies virus (RABV) suppresses the expression of glycoprotein (G), which prevents dendritic cells (DCs) from activating and stimulates the production of virus-neutralizing antibodies (VNAs) to overcome immune response of host (Li *et al.*, 2019). Key events for rabies virus neuroinvasion and CNS transmission include preserving the neuronal network and killing T cells that infiltrate the nervous system due to infection. The blood-brain barrier may prevent immune effectors-antibodies and plasma B cells from reaching relevant sites. Alternatively, the virus may inhibit immune function or there may be insufficient antigen exposure following a natural infection to mount a successful response. Understanding how immunity to RABV develops and operates is crucial to define protection parameters, which could aid in curing this deadly virus (Johnson and Cunningham, 2015). The increase in G protein levels doesn't induce a pathogenesis and similarly, the decline in G protein levels doesn't induces it (Scott and Nel, 2016). Moreover, specific PDZ area restricting locales on the cytoplasmic space of the RABV G protein communicate with microtubule related serine/threonine kinase 2 (MAST2) and protein tyrosine phosphatase, non-receptor type 4 (PTPN4) bringing about the control of neuronal apoptosis (Préhaud *et al.*, 2010). The RABV G protein from pathogenic and weakened strains has different circulation designs in neuronal cells , coming about in the anti-and pro-apoptotic abilities of the different tstrains, respectively (Scott *et al.*, 2008).

Apoptosis as method of RABV evasion

Apoptosis, a method to prevent viral replication or a means for viruses to evade immunity, is influenced by more than just the RABV G protein level. Contrary to the idea of apoptosis as a host defense mechanism, RABV targets cerebellum cells, specifically Purkinje cells, for replication. While neuronal apoptosis is not significant in RABV infections in humans and bats, it might occur in mice due to immature neuron cells (Jackson et al., 2010).

Interestingly, pathogenic RABV doesn't induce neuronal apoptosis, but causes axonal and dendrite swelling and neuronal dysfunction, leading to clinical signs. However, apoptosis occurs in brain cells infected by attenuated cells. During natural RABV infection, apoptosis of macrophages and infiltrating T cells, potential targets of RABV-induced apoptosis, were observed. The key difference between virulent and non-virulent RABV strains is that the non-virulent strain causes neuronal apoptosis, while the virulent strain induces apoptosis in infiltrating T cells (Fernandes *et al.*, 2011; Lafon, 2005, 2008; Li *et al.*, 2019).

Role of nitric oxide in apoptosis

Both the pathogenesis of RABV and the immune response to it have been linked to nitric oxide. The artificial growth of NO showed that NO had the ability to stop RABV replication and viral protein formation *in vitro*) (Scott and Nel, 2016). On the other hand, a number of other studies have shown that RABV causes the production of iNOS in neurons and macrophages, resulting in increased pathogenesis and the hypothesized explanation of symptoms in other non-neuronal organs, such as encephalitis (Jackson *et al.*, 2010; Kammouni *et al.*, 2012; Liao *et al.*, 2012). The development of NO by iNOS s in certain examinations recommending that neuronal apoptosis was either the reason for the pathology in RABV diseases or because of pure RABV replication (Scott and Nel, 2016). However, subsequent research has demonstrated that neuronal dysfunction rather than neuronal death is the cause of rabies symptoms (Fernandes *et al.*, 2011; Fu and Jackson, 2005; Lafon, 2008; Li *et al.*, 2005).

Axonal swelling, a disease that has been fervently linked to the beginning of symptoms, including poor axonal transport of proteins and ultimate axonal degeneration, is triggered by high levels of iNOS synthesis by neuronal cells and microglia (Jackson *et al.*, 2010). Moreover, experiments conducted *in vitro* on dorsal root ganglion (DRG) cells have demonstrated that CVS was able to prevent the formation of axonal processes but was not able to cause a loss of viability

in DRG neurons (Jackson *et al*., 2010). In neurons, apoptotic cell death and axonal swelling are probably caused by mitochondrial malfunction (Gholami *et al*., 2008). It was suggested that RABV's mechanisms keep low levels of chemokines and/or their cognate receptors in the infected CNS, suppress inflammation and virus clearance, leading to a lethal outcome. Further, the data indicated a correlation between pathogenicity and RABV's ability to suppress inflammatory responses in the CNS (Wang *et al*., 2005).

Diagnosis of rabies

Rabies symptoms can be mistaken for other neurotropic etiological agents. The incubation period for rabies can extend up to 90 days, with early symptoms evolving to paralysis in 75% of dogs (Hemachudha *et al*., 2013). The early symptoms, which last for two to five days, evolve to paralysed or dumb forms in 75% of dogs.This is quite dangerous when handling simple scenarios. In both clinical forms, paralysis and mortality occur 4–8 days following the onset of clinical symptoms. An approximate diagnosis can be established using a few common clinical symptoms seen in dogs along with various animal species (Blanton *et al*., 2009). For ten days, the animals exhibiting strange behaviour should be housed in isolation and are not allowed to bite other animals. During the prodromal phase of rabies, there are noticeable behavioural abnormalities. These traits vary depending on the species and can include being agitated, more sensitive to light and noise, more aware, restless, and friendly, aggressive (particularly in cats), and attacking without provocation. They can also become depressed, hide in dark areas, have mild pyrexia, have impaired corneal reflex, and mutilate themselves at the bite site. Nervous symptoms such as anger, a fierce bite or assault, shaking of the muscles, flaccidity or incoordination, pica, spasms, and rigidity of deglutination, voice changes, difficulty swallowing, drooling along with frothy saliva, dropping of the jaw, paralysis, coma, and death occur during the excitative phase. There are no discernible gross brain lesions. Nonetheless, injuries from the bite and the possibility of foreign objects in the stomach because of pica may be considered. It is important to distinguish this illness from canine distemper, encephalitis in dogs, horses, and cows, hepatic encephalopathy, thiamine deficiency in cats, lead and organochloride compound poisoning, benzoic acid poisoning, strychnine poisoning, pseudorabies, spongiform encephalopathy, and listeriosis (Charlton, 1988). The clinical signs of bat-acquired rabies can differ from those of dog-acquired rabies. These results could enhance the accuracy of rabies early diagnosis (Udow *et al*., 2013). Human rabies can present as either encephalitic (mad) or paralytic (dumb), with the brainstem being involved in both clinical manifestations (Hemachudha *et al*., 2002). At the point of the bite wound, rabies might cause discomfort, itching, or paraesthesia. The pathognomonic symptom of hydrophobia, which is caused by a triad of inspiratory muscular spasm, painful spasm of, terror (the dread of swallowing), aerophobia, and widespread flaccid paralysis, includes irritability, agitation, hyperaesthesia, and autonomic abnormalities in cases of furious rabies. While paralytic rabies cannot be distinguished from Guillain-Barre syndrome, polio, and herpesvirus simiae due to its slower pace and more travel neurological features (Leung *et al*., 2007), furious form of rabies in humans should be distinguished from delirium tremens, botulism, diphtheria, drug consumption (phenothiazines and amphetamines), and cultivate ingestion. Brain involvement is typically characterised by spasms in reaction to touch, olfactory, visual, and auditory inputs, interspersed with intervals of clarity, confusion, agitation, and autonomic dysfunction symptoms. Only 50% of patients with the paralytic sort of rabies experience phobic spasms (Consales and Bolzan, 2007), and excitement is less common in these cases. Patients infected with bat related RABVs may have non-classical symptoms such as radicular pain, neural pain, objective muscular and sensory impairments, and choreiform movements in the bitten limb during the prodromal phase.Since rabies is a serious zoonosis, prompt and accurate diagnosis is necessary for prompt treatment and successful preventive/control actions in suspected cases. The way that different species exhibit the clinical indications of rabies depends on how the virus damages the limbic system in each species. Because of the way that this regulates conduct, the severe lesions are likewise not pathognomonic (Frana *et al*., 2008). The results of the MRI, or magnetic resonance imaging, approach was similar in humans with both furious and paralytic forms of rabies; however, they were more noticeable in cases of paralysis.

Therapeutic approaches

Despite post-exposure prophylaxis (PEP), rabies is typically fatal once central nervous system symptoms appear. Injecting plasmid vectors encoding a RABV glycoprotein can trigger an antiviral immune response. However, combining this with a plasmid expressing interferon-g (IFNg) reduces this response (Xiang *et al*.,

2014). Small interfering RNA (siRNA), delivered by a replication-defective adenoviral vector (rAdV), shows strong antiviral properties against rabies. This siRNA targets the DNA polymerase (L) and nucleotide (N) genes. When introduced into BHK-21 cells in vitro, this method proves successful and serves as an effective complementary therapy (Sonwane *et al*., 2012).

Rabies (Vaccination and Prevention)

Rabies diagnosis requires quick transmission of refrigerated specimens to the lab due to the virus's rapid inactivation. The Fluorescent Antibody Test (FAT) is widely used and recommended by WHO and OIE. Immunochemical tests, using an enzyme like peroxidase instead of fluorescein isothiocyanate (FITC), have similar sensitivity to FAT but require extra incubation and risk false positives.

Rabies control requires a comprehensive approach, considering its two transmission cycles: Sylvatic and non-sylvatic. The one health approach combines cross-sectoral coordination for effective prevention/management.

Key steps include systematic data collection and compilation, coordination and data sharing, efficient surveillance (including wildlife), pre-exposure prophylaxis for high-risk individuals, prioritizing local care of bite wounds and scratches in post-exposure prophylaxis, and using virucidal chemicals for wound cleaning. These measures aim to prevent human rabies and control its spread in animal populations (Shankar, 2009). Chemical tests use virucidal chemicals like alcohol, tincture, or aqueous iodine solution to inactivate any remaining virus post-washing. Cauterization with carbolic or nitric acid is not recommended due to scarring. Anti-rabies serum can be applied locally or infiltrated around wounds to prevent rabies, with patient sensitivity checked beforehand (Acharya *et al*., 2020). Raising awareness through public campaigns is key in combating rabies. Educating on dog vaccination, PEP availability, and dog bite prevention can help improve the situation. The WHO advises on rabies prevention by addressing the disparity between prior guidelines and current PEP and PrEP usage in endemic areas, aiming to improve access to life-saving care (Basavanthappa, 1998).

WHO advice on rabies prevention

By addressing the disparity between prior WHO guidelines and current PEP and PrEP usage in endemic areas, the update attempts to consider the most recent evidence available to improve vulnerable people's access to life-saving care (Table 1).

Booster vaccination

Booster immunization after PrEP is recommended for those at frequent or ongoing elevated risk of rabies exposure, such as laboratory and animal workers. It is not necessary to maintain antibody concentrations ≥0.5 IU/mL over time post-PrEP. Boosters should be elicited quickly and strongly during post-exposure treatment. The relationship between the interval from primary immunization to PEP booster doses and the pace and magnitude of booster responses is unclear. Few studies on booster administration after extended periods (>2 years post-original series) limit comprehensive insight into long-term boost ability. Data on long-term persistent immunity are sparse (Langedijk *et al*., 2018).

Requirements for booster injections

Those who have completed a primary series of pre-exposure or post-exposure prophylaxis with a CCEEV do not need rabies vaccine boosters unless they're at constant or frequent risk of exposure. Antibody surveillance is preferred over routine boosters. Antibody testing should be done every 6 months for those exposed to excessive amounts of live rabies virus, and every two years for professionals like certain veterinarians and animal health officers. Boosters are advised only if rabies virus neutralizing antibody titres fall below 0.5 IU/, as vaccine-induced protection typically lasts years (Phanuphak *et al*., 1987; Rupprecht *et al*., 2009).

Table 1: *WHO categorizes rabies exposure and indications for PEP.*

WHO category of rabies exposure	PEP indications
Category I (no exposure): Handling or feeding an animal, licks on unbroken skin.	PEP is not indicated.
Category II (i.e., exposure): Minor scratches or abrasions without bleeding	PEP is indicated (just wound cleaning and vaccination). If exposed to a bat, treat as category III, and follow PEP
Category III (severe exposure): Single or many transdermal bites or scratches, contamination of mucous membranes or damaged skin with saliva from animal licks, and exposures due to direct contact with bats.	Recommendations (wound cleansing, vaccination, and RIG). (O'Brien and Nolan, 2019)

Alternative rabies vaccine development

While current rabies vaccines are successful, efforts have been made to develop alternatives using genetic manipulation. Antibodies, crucial for protection against RABV, primarily target the viral glycoprotein. Cloning the RABV glycoprotein into bacterial plasmids has led to alternative techniques for developing new rabies vaccines. These include:

RABV glycoprotein expressed on the vaccinia virus surface.

- Canary pox virus expressing the RABV glycoprotein.
- Canine adenovirus expressing the RABV glycoprotein.
- DNA immunization using a plasmid vector with the RABV glycoprotein.

These methods rapidly elicit high titres of RABV neutralizing antibodies and prevent infection in small animal models. However, they haven't challenged existing vaccines due to cost and acceptance for human use (Sudarshan *et al*., 2007).

Novelty Statement

The review article provides a thorough examination of the Rabies virus, which is a member of the Lyssavirus genus and family of Rhabdoviridae and is responsible for a fatal brain infection. Even while rabies is quite common worldwide, except for remote areas like Australia as well as Antarctica, it nevertheless poses a serious threat to public health, accounting for over 60,000 deaths annually. Nonetheless, the fact that 15 million people receive Rabies Post-Exposure Prophylaxis (PEP) each year highlights the continued efforts to lessen its effects. The rabies virus uses a variety of tactics to get past host defenses, which highlights the importance of an accurate and timely diagnosis. OIE and WHO-endorsed conventional methods like histopathology and the Direct Fluorescent Antibody Test (dFAT) continue to be essential. The study does, however, emphasize developments in diagnostic techniques, such as PCR as well as the Mouse Inoculation Test (MIT), providing information about their utility and effectiveness. Various vaccine techniques, including DNA, reconstituted, live, attenuated, or eliminated viruses, become essential components of control and prevention efforts in endemic areas. The research offers a comprehensive analysis of vaccination, therapeutic approaches, pathophysiology and epidemiology, and propagation dynamics, offering insightful information to improve our comprehension of rabies and guide successful preventive and control measures. This thorough investigation fills in important knowledge gaps and compiles current knowledge, making it an invaluable tool for scientists, physicians, and legislators who are committed to addressing this serious zoonotic issue.

Author's Contribution

Muhammad Wasif Gulzar: Devised and developed the study strategy, created the search query, conducted the literature search, gathered the data, proofread the files, uploaded them, and sent them.
Jawad Hussain: Before submitting the work, they screened the articles, authored, evaluated, prepared, and cleared the final draft.
Riffat Maqsood: Researched data and shared her reviews about pathogenesis of rabies and immunopathology.
Muhammad Zain: Shared his reviews about vaccination and prevention of Rabies.
Muhammad Suleman: Made his findings about evasion of rabies virus from host immune responses.
Tayyab Ur Reman: Described brief history and highlighted epidemiology of rabies.
Abdul Wadood: Made a comprehensive review about mode of transmission and species affected and reservoirs of rabies.
Sana Asif: Makes a comprehensive review of the final draft of article and analyzes the final data before submission.

Data availability

No data was used for the research described in this article, as it is a review article.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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