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From virus to vaccines: A critical review of rabies prevention

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Abstract
Rabies, a fatal zoonotic disease caused by the Lyssavirus, remains a major global health concern. The review critically examines the virus's origin, transmission, and the immune response triggered in the host following infection. It also evaluates the current state of rabies prevention and control strategies, with a focus on vaccination approaches, diagnostic techniques, and preventive measures. By highlighting key developments and challenges in rabies research, this review aims to contribute valuable insights into combatting the disease and safeguarding human and animal populations.

Keywords: Rabies, lyssavirus, vaccination stratagies, pathogenesis, post exposure prophylaxis, diagnosis

Introduction
Rabies is an acute & fatal zoonotic viral encephalitis disease with 100% case fatality rate. This disease is known to humankind since time immoral, and is primarily associated with the bite of rabid dog. The word “rabies” is derived from “rabhas” which means “violence”. The medical condition induced by the rabies virus in humans is referred to as "hydrophobia," owing to the pronounced aversion to water exhibited by patients. This aversion stems from the inability to consume fluids despite experiencing profound thirst.

Historically, the etiological agent responsible for rabies had been linked to the saliva of infected dogs. Beginning in 1881, Louis Pasteur conducted a series of seminal investigations, elucidating that the rabies virus predominantly resides within the central nervous system of afflicted animals. Through a sequence of intracerebral passages in rabbits, Pasteur managed to isolate a stable form of the virus, known as the fixed virus. Subsequently, he demonstrated the capacity to confer immunity upon dogs through a graduated regimen of injections utilizing this fixed virus of varying degrees of infectivity.

The rabies vaccine was developed utilizing a process involving desiccation of segments of the spinal cord obtained from rabbits previously infected with the fixed virus. A pivotal moment in the advancement of medical science occurred in July 1885, when Joseph Meister, a nine-year-old boy who had been bitten by a rabid dog, received a regimen comprising 13 inoculations of the vaccine derived from infected spinal cord. Remarkably, the young boy not only survived this treatment but thrived thereafter, representing a significant milestone in the annals of medical progress. Every year, rabies infection kills tens thousands of people, mostly in Asia and Africa (95%). Rabies has a huge economic impact accounting economic losses of about $8.6 billion annually in the form of deaths and the cost of vaccination and control programme (WHO-2018). Children below the age of 15 constitute 40% of individuals who have been bitten by animals suspected of carrying the rabies virus. The duration of the initial stage of rabies infection, known as the eclipse phase, displays significant variation, spanning from two weeks to six years. This variability is influenced by factors such as the concentration of the virus, the site of inoculation, and the density of nerve connections (Abera *et al*., 2015) [1]. The rabies virus, a neurotropic agent, exhibits a bullet-shaped morphology measuring 180nm x 75nm. It belongs to the family Rhabdoviridae and the genus Lyssavirus (Madhusudana *et al*., 2012) [2]. This single-stranded, negative-sense, non-segmented RNA virus possesses an enveloped structure and linear genome of approximately 12 kb in size. The Lyssavirus genus, including rabies, encodes five highly conserved viral proteins: arranged as 3'-N-P-M-G-L-5'. Within the viral particle, genomic RNA is tightly encapsulated by the N and P proteins, forming a ribonucleoprotein RNP) complex that supports viral replication in the host(cell cytoplasm (Tojinbara *et al*., 2016) [3].

The virion's outer surface is adorned with protruding spike structures comprised of trimeric viral glycoproteins. Notably, these glycoproteins play a pivotal role in viral pathogenicity and stimulate the development of protective immunity against rabies (Blanton *et al*., 2008) [4].

Etiology & Classification

Rabies, a viral disease, is primarily caused by the rabies virus (RABV) belonging to the Lyssavirus genus within the Rhabdoviridae family, situated in the Mononegavirales order as per the ICTV-Virus Taxonomy of 2017. The Lyssavirus species can be classified into two distinct phylogroups. The classical rabies virus (RABV) is categorized within phylogroup-1. This genus, Lyssavirus, is among the seven genera constituting the Rhabdoviridae family, which is a component of the Mononegavirales order. It encompasses various viruses including the classical rabies virus (RABV; genotype 1), Lagos bat virus (LBV; genotype 2), Mokola virus (MKV; genotype 3), Duvenhage virus (DV; genotype 4), European bat lyssavirus 1 (EBLV-1; genotype 5), European bat lyssavirus 2 (EBLV-2; genotype 6), and Australian bat lyssavirus (ABLV; genotype 7) as described by Ceccaldi *et al*. in 1989 [6]. Recent additions to the Lyssavirus genus from insectivorous bats comprise Irkut virus (IV), Khujand virus (KV), Aravan virus (AV), and West Caucasian bat virus. Despite variations in antigenic properties, these lyssaviruses maintain identical morphological and neurotropic characteristics as outlined by Ruprecht *et al*. in 2002 [7].

Epidemiology & Geographical Distribution

Rabies, an extensively widespread animal disease, is documented across all continents with the exception of Antarctica. However, the distribution of this disease is notably disproportionate, as more than 95% of fatalities transpire in Asia and Africa. This unsettling trend underscores the uneven prevalence of the disease. Rabies continues to maintain endemic status in over 100 countries globally, encompassing regions inhabited by 2.5 billion individuals as of 2010.

The geographic areas where rabies is prevalent can be classified into three primary categories. First, there are nations grappling with enzootic canine rabies, a classification encompassing the entirety of Asia, Africa, and Latin America. The second classification pertains to countries that have achieved control over canine rabies and where wildlife-driven rabies predominates; this category includes Western Europe, Canada, and the United States. The third grouping comprises countries free from rabies, predominantly comprised of islands such as England, Australia, and Japan. Notably, India is categorized within the high-risk first zone, characterized by a prominent prevalence of the disease.

In general, most of the developing countries face problem of urban rabies (dogs are responsible for maintenance and spread of virus to human) and developed countries face problem of wildlife rabies (sylvatic rabies: disease is maintained and transmitted by wildlife). The most common mode of rabies virus transmission is bite of infected animals. Other possible modes of transmission include aerosol contamination of mucous membrane (eyes, nose, mouth), corneal and organ transplantation, but such cases are rare. In India, human rabies is mainly transmitted through dog bite (96.2%) and majority of these were stray dogs (75.2%) (Sudharshan 2007). Transmission through other wild animal bite and mongoose should not be neglected.

Reservoir hosts are important to maintain virus in nature. Important reservoir hosts are species of family Canidae (dogs, jackals, coyotes, wolves, foxes and Raccons), Mustelidae (skunks, martens, weasels, and stoats), Viveridae (moongose and meerkats) and Procyonidae (raccoons) and order Chiropetra (bats). Raccoons, skunks, foxes, coyotes and several species of insectivorous bats are important reservoir in major portion of America and Europe; whereas, stray dogs are the common reservoirs in India.

Biosafety Measures

The lyssavirus are enveloped, bullet shaped virions containing proteins, lipids and single stranded RNA such viruses are sensitive to detergents and lipid solvents(soap solutions, ether, chloroform, acetone), alcohols and iodine preparations. However care should be taken while handling acetone -fixed brain impression slides because acetone may not completely inactivate virus. Other properties of rabies virus include resistance to drying, repeated freezing and thawing. Virus is relatively stable at pH 5-10 but inactivated by extremely low pH (below 3). Virus is sensitive to pasteurization temperature, ultraviolet light and formalin fixation and inactivated by beta-propiolactone (BPL) and gamma irradiation. Quaternary ammonium disinfectants in 1:256 dilution,70% alcohol(ethanol or isopropanol),1% soap solution,5-7%iodine solutions & oxidizing agents inactivate lyssaviruses within few minutes. Additional time is required to inactivate virus on materials, instruments or surfaces contaminated with proteins found in cell culture media & tissues. So, carcasses and animal tissues are best disposed within biohazard bags and then incinerated. Virus is swiftly deactivated by sunshine and drying, and it does not persist in the environment for lengthy periods of time (in dried blood and secretions) (Blanton *et al*., 2007) [9].

Laboratory facilities should be designed with quality standards, biosafety and biosecurity in mind. Lyssaviruses are defined as risk Group2 or depending on jurisdiction, local risk assessments, they may be handled in 2 or 3 facilities. Use of class II biosafety cabinet (BSC) is recommended for any procedures that may produce an aerosol or for techniques employed to produce lyssaviruses within cell culture. There should be restricted entry or entry for those only who are fully vaccinated against rabies. The essential components of requisite personal protective equipment (PPE) encompass a laboratory coat or gown, protective gloves, ocular safeguarding through safety glasses or goggles, facial protection via face shields, and respiratory defense utilizing N95 masks, among other pertinent items.

Rabies Virus And Genome Organization

Rabies virus is neurotropic, bullet shaped (180nm x 75nm), single-stranded, negative sense, linear, non-segmented, enveloped RNA virus with ~12 kb Genome with helical symmetry (Madhusudana *et al*., 2012) [2]. It has 7 numbers of genotypes; more than 16 different lyssaviruses have been described. RV- Genotype 1 is the main disease-causing genotype in mammals. Lyssaviruses encodes highly conserved and monocistronic five viral proteins: the nucleoprotein (N- 1334 bp, 57 KDa), phosphoprotein (P- 978 bp, 38.5 KDa), matrix protein (M- 840 bp, 25 KDa), glycoprotein (G- 1647 bp, 67 KDa) and large RNA dependent RNA polymerase protein (L- 6381bp, 200 KDa). The five viral proteins are listed in the following order: 3'-N-P-M-G-L-

5'.There are intergenic elements between the coding regions sections of the genome; N–NS, NS–M, M–G, and G–L, where the lengthy intergenic element between G and L is known as the pseudo gene and it has been utilized as a reference for discriminating distinct rabies genotypes (Blanton *et al*., 2008) [4]. The ribonucleoprotein (RNP) structure assumes a helical conformation, which is enveloped by the M protein. This interaction serves to tether the RNP to the lipid bilayer membrane envelope, thereby establishing a vital connection between the RNP and the membranous architecture. (Rupprecht *et al*., 1992). The exterior of the virion is enclosed with projecting spikes made up of viral G protein trimers (10nm long).

Propagation & assay in in-vitro & in-vivo laboratory models

Animal inoculation: Viral isolation through intracerebral inoculation in juvenile mice (3-4 weeks of age) can be viably pursued utilizing specimens derived from cerebral matter(brain), cerebrospinal fluid, saliva, and urine. This endeavor demonstrates higher prospects of success during the initial stages of the disease. Subsequent to the disease's commencement, typically within a few days, the presence of neutralizing antibodies becomes evident, subsequently leading to sporadic instances of viral isolation.

Tissue culture: The isolation of the virus can be pursued through intracerebral inoculation in young mice aged 3 to 4 weeks. This endeavor involves utilizing samples from various sources such as brain tissue, spinal fluid, saliva, and urine. The optimal chances of successful isolation are observed during the initial phases of the disease. However, it is worth noting that after a few days from disease onset, the presence of neutralizing antibodies becomes evident, occasionally hampering the isolation process.

For a more expeditious and highly sensitive isolation method, reliance on tissue culture cell lines proves advantageous. Prominent examples of these cell lines include the mouse neuroblastoma cell line Neuro2a/CCL 131, baby hamster kidney-21 (BHK-21), Vero cells, McCoy cells, and WI-38. Notably, the manifestation of cytopathic effects is either minimal or absent within these cell lines, prompting the identification of virus propagation through immunofluorescence (IF) techniques. The positive IF outcome serves as an indicator of viral presence as swiftly as 2-4 days post inoculation.

Of particular significance is the CCL 131 (ATCC) neuroblastoma cell line, which exhibits a remarkable susceptibility to the street rabies virus without necessitating any preliminary adaptation measures. Consequently, this cell line has become a routine choice for the isolation of the rabies virus.

Pathogenesis

The incubation period typically spans 1 to 3 months; however, it displays variability ranging from several weeks to numerous years, contingent upon factors such as the distance between the site of the bite and the central nervous system, the severity of the bite, and the magnitude of the inoculum. Within the initial 48 to 72 hours post-inoculation, the virus undergoes replication at the point of entry, residing within muscle, connective tissue, or nerve tissues. Subsequently, it gains access to peripheral nerve cells by utilizing nicotinic acetylcholine receptors as entry points, as documented by Lentz *et al*. in 1982 [48].

The virus commences its passive migration within the axoplasm of nerve endings, progressing at an approximate velocity of 3 millimeters per hour towards the central nervous system. Upon reaching this pivotal system, the infection disseminates in a centripetal manner from axons to neuronal cell bodies. The ensuing impact of viral infection upon multiple nerve cells within the central nervous system contributes to the manifestation of distinct rabies-specific symptomatic expressions.

Once established in the central nervous system, the virus adopts a centrifugal trajectory, traversing neuronal pathways, notably implicating the parasympathetic nervous network. This intricate pathway engagement facilitates the infection of diverse bodily organs, including but not limited to the salivary glands, skin, and heart, as elucidated by Jackson and Wunner in 2007. This intricate course culminates in the excretion of infectious rabies virus into saliva, a pivotal mechanism for its transmission to susceptible hosts.

Replication And Transcription

The transmission of the infection is facilitated through the bite of an afflicted animal carrying the rabies virus. Enhanced interaction between the G protein and acetylcholine receptors within the peripheral nervous system indicates the virus's attachment (Albertini *et al*., 2011) [11]. Subsequently, the virus employs endocytosis to gain access to the central nervous system. Within the endosome, a decrease in pH triggers conformational changes in the G protein, leading to the fusion of the viral membrane with the endosomal membrane. This process culminates in the release of the viral nucleocapsid (Albertini *et al*., 2008) [12].

Given that mammalian cells lack the capacity to transcribe negative-strand RNA, the Rabies virus encodes its own RNA-dependent RNA polymerase. The monocistronic mRNAs encode the five virion proteins: nucleocapsid (N), matrix (M), polymerase proteins (L), phosphoprotein (P), and glycoprotein (G). While G protein synthesis initiates on free ribosomes, it concludes within the endoplasmic reticulum and Golgi apparatus, where glycosylation occurs. The virion-associated RNA polymerase transcribes the single-stranded RNA genome into positive-sense mRNA. The genome ribonucleoprotein (RNP) functions as a template for complementary positive-sense RNA, generating negative-sense progeny RNA (Abera *et al*., 2015) [1].

The L-P polymerase complex penetrates the nucleocapsid's 3' end, yielding brief RNA molecules known as leader RNA. Post-transcription, the polymerase recommences transcription of nucleoprotein mRNA, which is capped and polyadenylated by the viral polymerase complex (Ogino and Banerjee, 2007) [14]. In the later stages of infection, L activity transitions to replication, producing full-length positive-strand RNA copies lacking caps or poly (A) tails. These complementary RNAs (cRNAs) are encapsidated by nucleoprotein and linked to the L-P complex, serving as templates for generating new negative-strand RNA genomes, enclosed by N to form new nucleocapsids (Arnheiter *et al*., 1985) [15].

During the assembly phase, the N-P-L complex envelops negative-stranded genomic RNA to construct the ribonucleoprotein (RNP) core, while the M protein generates a protective capsule around this core.

Clinical Signs

The rabies related viruses cause illness in distinguishable from classical rabies (Smith, 1996) [16].

In humans

Prodrome Period: During the prodrome period, individuals typically experience symptoms such as fever, headache, malaise, anorexia, sore throat, myalgia, and in some cases, an unfamiliar or unexplained tingling, pricking, or burning sensation at the site of a wound.

Acute Encephalitic Phase: The acute neurological phase is characterized by intermittent hyperactivity followed by episodes of abnormal behavior and agitation interspersed with periods of normalcy. A distinctive hallmark of human rabies is the manifestation of "Hydrophobia." While patients might retain the ability to ingest dry solids, the consumption of liquids becomes challenging.

Coma: A subset of patients progresses to a comatose state during the course of the disease.

Death: The fatality usually occurs within a span of 1 to 6 days due to respiratory arrest in conjunction with convulsions.

In Canines: In dogs, the incubation period generally ranges from 3 to 6 weeks, with potential variations spanning from 10 days up to a year. There are two prevalent forms of rabies in dogs:

Furious Form: This form is characterized by heightened excitability, spasms, hydrophobia, and at times, aerophobia. Death typically ensues due to cardiorespiratory arrest.

Paralytic (Dumb) Form: Rabies in this form is characterized by a progressive paralysis that ascends through the body. This variation follows a less dramatic and generally extended course compared to the furious form. The afflicted animal gradually enters a comatose state, culminating in eventual demise.

Anti-Rabies Vaccines

Anti-rabies vaccines are standardized formulations containing specified quantities of immunogens. These vaccines are primarily derived from Pasteur's original strain (1882), its derivative strains (Pasteur Virus, Challenge Virus Standard, Pitman-Moore), and strains isolated during the 20th century (Flury: low and high egg passage; Street alabama-Dufferin: Vnukovo). Live vaccines are attenuated for the target species through techniques such as passaging in heterologous hosts. Inactivated vaccines are produced by subjecting the antigen to chemical or physical methods of inactivation. Biotechnology-based vaccines encompass a range of genetic engineering approaches, including chimera, marker, mutant, reverse genetic-based, edible, and oral vaccines.

Categorization of Anti-Rabies Vaccines: Anti-rabies vaccines can be classified into two primary categories:

Neural Vaccines: Neural vaccines consist of suspensions of nervous tissues sourced from animals infected with the fixed rabies virus. An early example is Pasteur's cord vaccine, which involved the desiccation of infected rabbit spinal cord over caustic potash for varying durations, followed by subsequent procedures.

The formulation of the Fermi vaccine involves treating an infected brain utilizing phenol, whereas the Semple vaccine is prepared by generating a 5% suspension of sheep brain that has been infected with a fixed virus. This suspension is subsequently inactivated through the application of phenol at a temperature of 37 ℃. However, neural vaccines exhibit limitations in terms of their immunogenicity due to their predominant nucleocapsid antigen composition, with only minimal quantities of the glycoprotein G, which serves as the primary protective antigen.

Non-neural vaccines

Egg-based vaccines: A vaccine sourced from duck eggs, wherein a fixed virus is adapted for proliferation within these eggs and then rendered inactive using beta propiolactone. This approach faced discontinuation due to its inadequate ability to provoke an immune response.

Vaccines cultivated in live attenuated chick embryos: Two vaccine variants were developed utilizing the flurry strain. The low egg passage (LEP) vaccine, propagated through 40-50 passages, was designed for canine immunization. Conversely, the High Egg Passage (HEP) vaccine, subjected to 180 passages, was tailored for use in cattle and cats.

Tissue culture-derived vaccines: The inaugural cell culture vaccine, known as the human diploid cell (HDC) vaccine, was conceived by Koprowsky, Wiktor, and Plotkin. It involves a concentrated and refined preparation of a fixed rabies virus (Pitman-Moore Strain) cultivated on human diploid cells (WI-38). The virus is subsequently inactivated using beta propiolactone or tri-n-butyl phosphate. Other cost-effective and efficacious vaccines include those propagated on primary chick embryo cell cultures and the Vero cell line.

Subunit vaccines: A recently devised approach encompasses the recombinant vaccinia virus/G protein vaccine, currently employed for fox rabies eradication in Europe.

Diagnosis

Clinical manifestations such as pruritus, discomfort, or paraesthesia at the bite site, along with gastrointestinal disturbances, are utilized for initial diagnostic considerations (Hemachudha *et al*., 2002) [20]. Historically, diagnostic procedures primarily relied on histopathological methodologies involving various staining techniques to identify cytoplasmic virus inclusions, specifically Negri bodies (Tierkel and Atanasiu, 1966) [21]. However, contemporary diagnostic techniques encompass nucleic acid amplification, reverse transcriptase-polymerase chain reaction, rapid fluorescent focus inhibition test (Nadin-Davis *et al*., 2009) [22], fluorescent antibody virus neutralization test, enzyme-linked immunosorbent assay, direct rabid immunohistochemistry test, histological detection of Negri bodies, mouse inoculation test, direct fluorescent antibody test (gold standard test), and the Rabies tissue culture infection test (Hemachudha *et al*., 2002) [20].

Detection of Rabies Antigen

Mouse Inoculation Test (MIT) and Fluorescent Antibody Test (FAT)

The Mouse Inoculation Test (MIT) is capable of detecting minute viral quantities within samples. Notably, it remains

applicable even with partially decomposed specimens, demonstrating practicality, sensitivity, reliability, and technical feasibility. However, ethical and environmental concerns surrounding the use of live animals in laboratory settings pose inherent challenges to MIT's viability. Additionally, MIT entails a considerable time frame, typically requiring 7-8 days for interpretable outcomes. Adequate maintenance of an animal facility for a continuous supply of mice and a substantial number of mice per sample is imperative, rendering MIT labor-intensive. In contrast, the Fluorescent Antibody Test (FAT), pioneered by Goldwasser and Kissling in 1958, stands out as a primary and highly dependable diagnostic tool for routine rabies. Its refinement has been to the extent that several laboratories have transitioned away from employing MIT (Rudd and Trimarchi, 1989). Demonstrating a sensitivity of 100%, FAT aligns completely with MIT results; however, MIT confirmation is recommended for FAT-negative samples to preclude false negatives. Direct FAT has gained prominence as a diagnostic approach due to its endorsed sensitivity, precision, and expeditiousness by organizations such as WHO and OIE (Meslin *et al*., 1996) [26].

PCR and real-Time PCR techniques

Polymerase Chain Reaction (PCR) stands out as a highly sensitive, specific, and rapid diagnostic tool for a range of viral ailments. Employing PCR to amplify rabies cDNA proves invaluable in characterizing isolates and conducting molecular epidemiology studies on the virus. The utility of RNA detection methods lies in their applicability to diverse sample types, particularly those unsuitable for direct Fluorescent Antibody Testing (FAT), such as saliva and cerebrospinal fluid (Crepin *et al*., 1998) [27].

Notably, the N-gene is a prime target in PCR due to its conserved nature, substantial abundance, and comprehensive sequenced data related to this gene. In situations where conventional testing methods are impractical, PCR serves as a confirmatory tool for human rabies diagnosis (Black *et al*., 2002) [28]. Leveraging real-time PCR has proven successful in detecting rabies within infected and decomposed brain tissue, addressing challenges associated with FAT sensitivity and tissue degradation. This adaptation of quantitative Reverse Transcription PCR (qRT-PCR) has been pivotal.

The scientific community has seen the emergence of alternative diagnostic and epidemiological tools for rabies. Sacramento *et al*. (1991) [29-33] pioneered the application of PCR as an alternative method for diagnosis and molecular epidemiology of the rabies virus. Heaton *et al*. (1997) [30] introduced semi-nested PCR assays, catering to the detection of six genotypes of rabies and related viruses. Hughes (2004) [31] devised a Taqman PCR-based approach to identify rabies virus in tissue samples. Recent advancements include in situ PCR, demonstrating its efficacy in detecting rabies virus RNA within cell cultures (specifically neuroblastoma cells) and brain tissue (Jayakumar *et al*., 2003; Praveena *et al*., 2007) [49, 32].

Detection and quantification of rabies antibodies

The primary objective of detecting and measuring rabies antibodies is to assess immunity against rabies and the efficacy of rabies vaccines. This process is grounded in the principle of employing known viral neutralization by antibodies present in serum samples collected from the field. These antibodies demonstrate the capacity to hinder rabies infection both in living animals (*in vivo*) and within cell cultures (*in vitro*), as outlined by Bourhy and Sureau in 1991 [33]. The quantification of results is expressed in international units, referencing a universally recognized standard antiserum.

Several techniques have been established to fulfill this purpose, including the Mouse Neutralization Test (MNT), Rapid Fluorescent Focus Inhibition Test (RFFIT), and the Fluorescence Antibody Virus Neutralization Test (FAVN). Throughout its history, the evaluation of rabies virus neutralizing antibodies has predominantly employed the Mouse Neutralization Test (MNT), initially introduced by Webster and Dawson in 1935 [35].

The prevailing techniques utilized to assess the immune response in vaccinated animals post rabies vaccination predominantly involve serum neutralization methods employing mice and cell cultures, as outlined by Smith in 1996 [16]. The World Health Organization (WHO) recommends employing both the *in vivo* Virus Neutralization Test in Mice (VNT) and the *in vitro* Rapid Fluorescent Focus Inhibition Test (RFFIT), established by Smith *et al*. in 1973 [50], to determine rabies virus neutralizing antibody titers.

In recent times, Enzyme-Linked Immunosorbent Assay (ELISA)-based methodologies are emerging as promising alternatives to the Rapid Fluorescent Focus Inhibition Test (RFFIT). Despite primarily focusing on antigen-binding interactions rather than the functional aspects of neutralizing antibodies like RFFIT, ELISA assays offer a streamlined, straightforward, safer, and expedited replacement for RFFIT. A noteworthy advantage of these assays is their independence from live viruses or high-containment environments, leading to swift results. Validated research has demonstrated a robust correlation between these assays and RFFIT, as shown by Servat *et al*. in 2007 and Muhamuda *et al*. in 2007 [36].

A subsequent advancement in the ELISA technique, namely Platelia Rabies II, has been specifically designed for detecting glycoprotein antibodies within human serum and cerebrospinal fluid (CSF) samples. This second-generation ELISA has undergone comprehensive evaluation across various research centers. The outcomes of this evaluation strongly align with RFFIT results, substantiating its effectiveness. Notably, this particular variant of ELISA presents notable advantages for laboratories lacking access to virus and cell culture facilities, as emphasized by Feyssaguet *et al*. in 2007.

Therapeutics

Because dog bites cause nearly all human rabies deaths, mass vaccination of dogs remains the primary technique for eliminating urban rabies in endemic regions. However, in most countries, the main preventive approaches for limiting rabies in reservoirs are different means of culling free-roaming dogs (Mustafa *et al*., 2015) [38]. Because the reduction in the vector population was minimal, it didn't seem to stop the spread of rabies. Furthermore, mass vaccination of dogs must be supplemented by bait immunization of wild animals to avoid disease spread from wild animals (Yale *et al*., 2022) [39]. Distributing bait rabies vaccination to high-risk areas is an effective means of reducing wildlife rabies. In general, vaccination coverage of around 70% of the vector population is thought to be adequate to prevent rabies transmission. However, present rabies vaccinations for humans and animals have intrinsic limitations, thus attempts have been undertaken to improve traditional rabies vaccines

in light of technological innovation (Dean *et al*., 1963) [40]. Passive immunization with human and horse rabies immunoglobulins is presently accessible. However, cleaning the bite or scratch site with large amounts of water or detergent within 15 minutes of exposure will lower the virus load. Apply a topical iodine-containing or similarly virucidal treatment (Gautret *et al*., 2014) [41]. Ingredients such as chilli, salt, lemon, herbs, and so on should never be used. After categorizing the bite or scratch location, treatment should be carried out. An animal-like on the intact skin, touching and feeding animals are deemed categories I (No exposure), small scratches lacking bleeding and nibbling of uncovered skin are regarded categories II (Exposure), single or numerous transdermal bites or scratches, saliva infectivity of mucous membranes or broken skin, and contact with infected wild mammals are considered categories III (Exposure) (Severe exposure) (WHO-2018). The closer the virus is exposed to the head, the faster it infects the brain. Pre- and post-exposure prophylactic measures are employed for rabies vaccination tactics. Pre-exposure prophylaxis is suggested for those at high risks, such as isolated populations, travelers, and individuals at occupational risk in highly endemic environments with inadequate access to effective post-exposure prophylaxis (Mustafa *et al*., 2015) [38]. The pre-exposure immunization strategy includes vaccinations from 0, 7, and 28 days. Serological antibody estimate should be performed 1 to 3 weeks following the last vaccination. Laboratory professionals should be re-examined every 6 months, and other diagnosticians should be re-examined every 2 years. Booster immunization is strongly advised even if the titer falls below 0.5 International Units (IU) per ml. When serological surveillance is not available, a one-year booster immunization is advised, followed by vaccinations every one to three years (Stankov *et al*., 2021) [42]. The regimen for post-exposure immunization includes vaccinations at 0, 3, 7, 14, and 28 days. Vaccines and rabies immunoglobulins are required for post-exposure prophylaxis. In category I, wound cleaning is sufficient; in category II, wound washing with vaccinations is necessary; and in category III, rabies immunoglobulins are administered in addition to vaccines and wound treatment. VaxiRab-N, Verorab, Rabivax-S, and Rabipur are rabies vaccinations that have been pre- qualified by the WHO. The immunization is administered by intradermal and intramuscular methods; the injection sites for the intradermal and intramuscular routes are the same (Tojinbara *et al*., 2016) [3]. WHO recommends a vaccination efficacy of 2.5 IU per single intramuscular injection; a minimum titer of 0.5 IU/ml of serum is seemed protective. Rabies immunoglobulins are indicated for category III exposure and are not advised if one have had rabies vaccination as pre-exposure prophylaxis and first post-exposure prophylaxis dosage was more than 7 days ago. Rabies immunoglobulins must be injected deep into and surrounding all wounds; it can be diluted for large and many wounds if the need exceeds the maximum dose (Yousaf *et al*., 2012) [43]. It is made up of either human (HRIG) or equine (ERIG) rabies antibodies. Because immunoglobulins cannot traverse intact blood-brain barriers, they are rendered useless once the virus has reached the brain and clinical signs have started (Kallel *et al*., 2006) [44]. The immunoglobulin, HRIG dosage 20 IU/kg body weight; ERIG dosage 40 IU/kg body weight, is injected locally around the bite location, with the remainder administered intramuscularlyfar from the vaccination site (Mustafa *et al*., 2015) [38].

Prevention & Control

There is currently no specific therapeutic intervention for rabies, a fatal disease that is both preventable and incurable. Limited understanding exists regarding the treatment and prevention of rabies in both humans and animals. Despite awareness of the disease's grave consequences, many individuals opt for various traditional and religious remedies rather than seeking appropriate post-exposure prophylaxis (Consales & Bolzan, 2007) [45]. A diverse array of conventional treatments is commonly employed following incidents of bites from rabid animals, primarily dogs. The continued presence of practitioners of traditional rabies treatments within communities underscores the significance of the disease. Implementing well-structured strategies encompassing adequate planning, health education, achieving 70% vaccination coverage for dog populations, and implementing robust epidemiological surveillance can lead to effective rabies control in resource-limited countries. Notably, mass vaccination of dogs emerges as the most pragmatic and cost-effective approach to eradicating Canine Rabies, thus safeguarding the lives of both canines and humans (WHO, 2013) [51]. During mass vaccination campaigns, universal immunization of canines, irrespective of age (above 3 months), weight, or health condition, is recommended. Primary immunization can be achieved through a single or double administration of vaccines, spaced one month apart. Subsequently, periodic vaccine administration, whether annual, biannual, or triennial, can be tailored to bolster immunity, contingent upon vaccine efficacy (Abera *et al*., 2015) [1].

A seminal instance of a successful large-scale immunization effort in canines occurred in 1948 in Memphis and Shelby County, Tennessee, resulting in the complete elimination of both animal and human rabies cases (Warrell, 2012) [46]. Animals encompassing dogs, cats, and ferrets that lack prior vaccination and encounter exposure to rabies-infected animals necessitate euthanasia (Srinivasan *et al*., 2005) [47]. Should owners dissent, strict isolation for a duration of six months is prescribed. Adhering to the pre-exposure vaccination recommendation mandates rabies vaccination upon admission to isolation or up to 28 days prior to release (Mustafa *et al*., 2015) [38]. Preventing human rabies hinges on a) evading contact with the rabies virus, b) administering suitable rabies pre-exposure prophylaxis, and c) promptly administering local wound treatment in conjunction with appropriate rabies post-exposure prophylaxis. Inactivated human vaccines are available for individuals at risk, including veterinary personnel, animal handlers, wildlife officers, and laboratory workers (Yousaf *et al*., 2012) [43].

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