



Guarding Against Rabies: The Power of Vaccination in Rabies Disease Management

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Abstract

Rabies, a lethal viral disease caused by the rabies virus, presents a critical public health challenge globally. The disease's etiology involves transmission through the bite or scratch of infected animals, primarily dogs, with the virus targeting the central nervous system and leading to devastating neurological symptoms. Early diagnosis is crucial but challenging due to the disease's non-specific initial manifestations. Vaccination strategies serve as the cornerstone of rabies prevention, encompassing pre-exposure prophylaxis for high-risk individuals and post-exposure prophylaxis for those bitten or scratched by potentially rabid animals. In this article we provide an in-depth exploration of vaccination therapy as a pivotal strategy in the deterrence and management of rabies. The evolution of rabies vaccines, particularly inactivated virus vaccines, is explored, underscoring their role in reducing human rabies cases. The article concludes by emphasizing the significance of continuous research to enhance vaccine effectiveness and promote a comprehensive approach to rabies prevention.

Keywords Rabies, Etiology and its Pathogenesis, Diagnosis, Vaccination Therapy

1. Introduction

Rabies, a lethal viral disease caused by the rabies virus, presents a critical public health challenge globally. Rabies, acquired by the rabies virus, is a severe zoonotic disease that demands effective preventive measures. Rabies (RABV) is a viral disease transmitted between animals and humans, this leads to an estimated annual death toll of approximately 59,000 individuals, along with a loss of over 3.7 million disability-adjusted life years (DALYs) (1). Its presence spans across both rural and urban underserved populations, with a history of over 4,000 years (2). While prevalent worldwide except Antarctica, it's most devastating impact occurs in Asia

and Africa, with children under 15 years constituting about 40% of cases (3). RABV can infect all mammals (4), primarily spreading through dogs, liable for up to 99% of human cases in endemic areas, although some cases stem from wildlife transmission (5). In Pakistan, RABV is endemic, primarily transmitted by dog bites, and clinical diagnoses are common due to limited available data (6). Despite estimated annual fatalities ranging from 2,000 to 5,000, human rabies is not officially reported in Pakistan (7). The absence of a nationally coordinated response to the disease has emerged since the 18th constitutional amendment in 2011, which devolved health responsibilities to provincial levels. While some provinces have initiated

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efforts like dog vaccinations and access to rabies post-exposure prophylaxis (PEP) in public hospitals, there remains an uneven response (8, 9, and 10).

This article provides an in-depth exploration of vaccination therapy as a pivotal strategy in the deterrence and management of rabies. The etiology of rabies, emphasizing its transmission through animal bites and scratches, particularly from dogs. It elucidates the pathogenesis of the virus, highlighting its neurotropic nature and the resulting neurological symptoms. Diagnosis challenges are discussed, with an emphasis on the need for early recognition. The crux of the article revolves around vaccination strategies, including pre-exposure prophylaxis for individuals at heightened threat and post-exposure prophylaxis for those exposed to potentially rabid animals. The evolution of rabies vaccines, particularly inactivated virus vaccines, is explored, underscoring their role in reducing human rabies cases. The article concludes by emphasizing the significance of continuous research to enhance vaccine effectiveness and promote a comprehensive approach to rabies prevention.

1.1 Etiology of Rabies Virus (RABV)

The RNA genome of RABV is negative-stranded and non-segmented, organized into a helical ribonucleoprotein complex (RNP) (11). Within this complex, the viral nucleoprotein firmly binds the linear RNA (12). In a specific sequence, the RABV genome encodes a total of five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and large protein (L or RNA-dependent RNA polymerase, RdRp) (13). Viral transcription and

replication are significantly influenced by the nucleoprotein (N). (14). The surface of the virus is densely covered with approximately 400 trimeric spikes created by the glycoprotein (G) (15). The binding of G to specific receptors is crucial in shaping RABV's neuropathogenicity, and it gains access to the nervous system through the endosomal transport pathway, facilitated by a membrane fusion process triggered by low pH conditions (16). Transcription initiation by the N-L-P polymerase complex results in the creation of a leader RNA molecule that lacks capping and polyadenylation (17). This complex subsequently produces mRNAs for N, P, M, G, and L proteins (18). The shift between genomic RNA transcription and replication is regulated by the N protein level (19). All these processes occur within the cytoplasm in a specialized structure called the Negri body (20). The etiology of rabies is shown in Figure 1. In the initial stages, RABV binds to specific receptors found on the surface of host cells, primarily neurons (21, 22). The cell membrane and the viral envelope undergo fusion, releasing the viral RNA which hijacks host cell's machinery and starts translation (23, 24, 25). New viral nucleocapsids are formed in the host cell's cytoplasm, incorporating the viral RNA genome and associated proteins (23). Once assembled, new viral particles are carried to the cell surface enclosed in vesicles and are then released from the infected cell through a budding mechanism. This process often involves interactions with host cell membrane components (26). After infiltrating the body through a bite or scratch, the virus progresses along peripheral nerves towards the central nervous system (CNS), which includes the brain (27). It can then

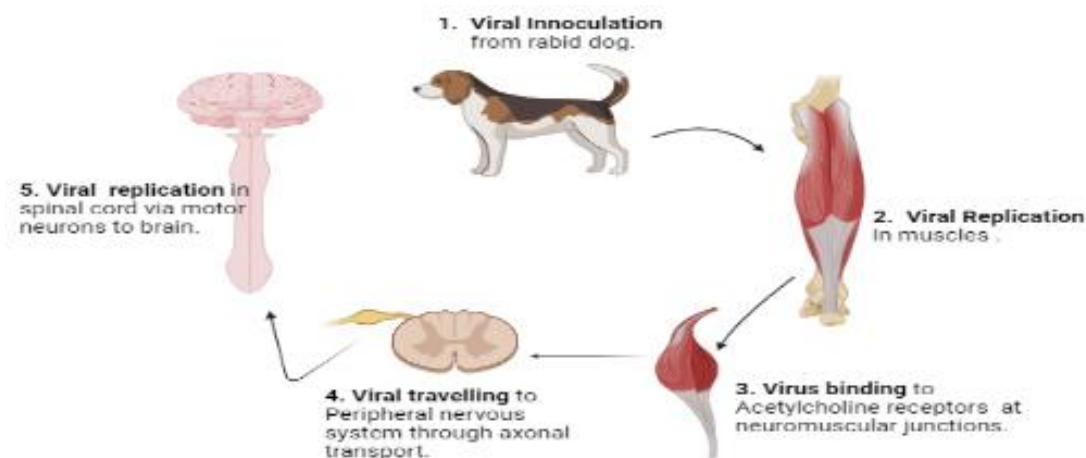


Figure 1: Etiology of Rabies Virus

move centrifugally within the nervous system, reaching various tissues, including salivary glands, from where it can be transmitted through bites to new hosts (28). The virus disrupts normal neural functioning, leading to symptoms such as anxiety, agitation, hallucinations, paralysis, and eventually coma. The hydrophobia and aerophobia seen in infected individuals are linked to the virus's impact on the brainstem (27). RABV belong to the Mononegaviral order, the Rhabdoviridae family and the Lyssavirus genus (29). There exist a minimum of 14 distinct Lyssavirus species, categorized into 2 phylogroups according to genetic variance and serological cross-response (30). According to the World Health Organization (WHO), Asia witnesses about 30,000 RABV-related deaths each year (31). Over 3 billion people in Asia are exposed to dog rabies, resulting in an Asian death every 15 minutes, with children under 15 years constituting around 15% of these casualties (32, 33, and 34). Pakistan experiences around fifty thousand RABV bitten people with about 6000 deaths annually occur (35, 36). Meanwhile, human rabies cases have been declining in many European and American countries, primarily due to enforced pet vaccination programs.

1.2. Pathogenesis of RABV

Although human rabies has a lengthy historical background, its underlying pathogenic processes remain relatively elusive. A significant portion of our understanding about the disease has been acquired through studies conducted using experimental animal models. The outcome of exposure to RABV hinges on several factors, including the specific RABV genotype or variant, its pathogenicity, the virus dose, route of exposure, host species, and its susceptibility, coupled with the host's immune responses (37). Numerous animal model studies underscore that pathogenic wild-type or street RABV and fixed (laboratory-adapted) RABV manifest distinct behavior throughout their life cycles within the host. RABV's G protein induces the production of virus-neutralizing antibodies (VNA) and significantly influences the virus's neurotropism and neuro-invasiveness by binding to neural receptors like acetylcholine receptors and neural cell adhesion molecules. The virus possesses the capacity to enter muscle tissue and undergo replication at the site of inoculation or to directly infiltrate peripheral nerves, avoiding replication in non-neural tissues. On RABV entry in central nervous system (CNS), a fatal disease outcome is typically unavoidable (38). Pathogenic

RABVs employ specific strategies to evade early immune recognition, including limited replication, suppressed interferon response, anti-apoptotic stimulation, and exclusive transportation through neurons. In contrast, fixed RABV triggers inflammation via innate immune responses, initiates apoptosis, attains higher replication levels, and expresses elevated G protein levels. However, the mechanisms through which fixed RABVs incite immune responses and wild-type RABVs evade immune detection remain partially understood. Fixed RABVs induce CNS inflammation and enhanced blood-brain barrier permeability, while street RABVs do not. This divergence highlights the dissimilarity in pathogenic and non-pathogenic rabies biology (39). Further insights into these differences are mentioned in Table-1.

Table 1: Discernible Disparities in Pathogenic and Non-Pathogenic RABV Biology

	<i>Non-Pathogenic Virus (Fixed/Lab adapted)</i>	<i>Pathogenic Virus (Street / Wild-Type)</i>	<i>Ref</i>
<i>Cellular Tropism</i>	Not Neuronal	Highly Neuronal	(40)
<i>Glycoprotein Expression Levels</i>	High	Low	(41)
<i>Replication</i>	High	Low	(42)
<i>Apoptosis</i>	High	Low	(43)
<i>Interferon Sensitivity</i>	Resistant	Highly Sensitive	(44)
<i>Immune System</i>	Shows Innate Adaptive Immunity	Evades Innate Adaptive Immunity	(45)
<i>Blood Brain Barrier Permeability</i>	Enhances	Little/No Change	(46)

1.3. Diagnostic Approaches

Diagnosing rabies in both humans and animals requires a combination of clinical presentation, history of exposure, and laboratory tests. The clinical symptoms of rabies can be divided into two major forms: "furious rabies" and "paralytic rabies." Furious Rabies is characterized by hyperactivity, agitation, aggression, and often "furious" behavior. Hydrophobia, which involves a fear of water,

and aerophobia, characterized by a fear of fresh air or drafts, photophobia (sensitivity to light), paresthesia (tingling or localized pain), and other neurological symptoms may be present. Paralytic Rabies is marked by muscle weakness, paralysis, and a progressive decline in neurological function. It may not exhibit the classic aggression seen in furious rabies. A history of exposure to potentially rabid animals is crucial for considering a diagnosis of rabies. Contact with saliva, bites, or scratches from suspected rabid animals, especially dogs, bats, raccoons, and other wildlife, is significant. Laboratory confirmation of rabies is essential for a definitive diagnosis, which includes Direct Fluorescent Antibody Test (dFA), that is the gold standard for diagnosing rabies and involves examining brain tissue for the presence of rabies virus antigens. It is highly specific and sensitive when performed correctly (47). Reverse Transcription Polymerase Chain Reaction (RT-PCR) test detects the presence of viral RNA in brain tissue or other suitable samples (48). It can provide confirmation of the virus and is particularly useful when brain samples are not suitable for dFA. Immunohistochemistry (IHC) test uses antibodies to detect viral antigens in tissue samples (49). It can be used when fresh brain samples are unavailable. Virus Isolation involves attempting to isolate the live virus from saliva, brain tissue, or other fluids in cell culture. It is a time-consuming method and is less commonly used due to the risk of infection. Ante-mortem diagnosis (before death) in humans is difficult due to the limitations of available tests and the rapid progression of the disease (50). Therefore, post-mortem testing is often the primary method for confirming rabies in cases of human deaths suspected to be due to rabies. The severity of exposure to RABV depends on a variety of factors, including the wound's intensity, its location on the body, the quantity and specific variant (genotype) of the virus introduced into the wound, and the promptness of post-exposure prophylaxis (PEP). Absent PEP, the likelihood of developing rabies subsequent to a bite by a rabid animal is approximately 55% for head bites, 22% for upper extremity bites, 9% for trunk bites, and 12% for lower limb bites (51). The viral load in the saliva of dogs infected with RABV changes throughout the progression of the disease, affecting the likelihood of infection for individuals bitten by these dogs. RABV can be detected in different bodily fluids and nervous tissues of individuals afflicted with rabies, such as saliva, tears, and urine. Consequently, coming into contact with these

fluids and tissues poses a potential risk of transmission. Blood, however, does not contain RABV (52).

1.4. Vaccination as the Key Strategy for Rabies Prevention and Treatment

The prevention of rabies heavily depends on educating at-risk populations about the disease. Efforts to increase awareness should involve educational initiatives, partnering with relevant sectors to prevent animal bites, and encouraging prompt first aid following exposure (53). Vaccination is effective in preventing rabies in both humans and animals. Human rabies vaccination is predominantly employed for pre- and post-exposure prophylaxis among populations with a heightened risk of exposure. The World Health Organization (WHO) and its collaborators have endorsed the "Zero by 30" initiative, aiming to eliminate human deaths from dog-transmitted rabies by the year 2030 (54, 55). Educational initiatives aimed at rabies prevention play a pivotal role in augmenting the impact of rabies vaccination programs, reducing the occurrence of human rabies and alleviate the economic strain associated with treating dog bites (56, 57). Preventive immunization through human rabies vaccines as given in Figure 2, is a crucial strategy for safeguarding individuals from potential exposure to the RABV(58). This type of vaccination is particularly advised for individuals involved in high-risk occupations. This includes laboratory workers who handle live rabies and related viruses, as well as personnel responsible for animal disease control and wildlife rangers, children residing in or visiting high-risk remote areas and travelers to remote areas affected by rabies, are also encouraged to consider pre-exposure immunization (59). Expatriates and long-term travelers in regions with limited access to rabies biology should contemplate getting immunized. The origin of rabies vaccines can be traced back to 1885 when Louis Pasteur and Emile Roux pioneered the development of the initial injectable live attenuated RABV vaccine. The early vaccine was created using inactivated homogenates of rabbit nerve tissue infected with RABV (60). However, the World Health Organization (WHO) has since recommended the discontinuation of nerve tissue vaccines due to their increased adverse reactions and decreased immunogenicity compared to modern vaccines, such as concentrated, purified cell culture and embryonated egg-based rabies vaccines (CCEEVs). These CCEEVs have been administered globally since the 1960s and are recommended for both pre-exposure



prophylaxis (PrEP) and post-exposure prophylaxis (PEP) (61). Rabies Vaccine BP is designed to protect individuals, both adults and children, against rabies. It functions in two keyways: pre-exposure vaccination before potential contact with the virus and post-exposure vaccination after contact has occurred. The vaccine contains a modified form of the rabies virus, prompting the body's immune system to develop protection against rabies.

Rabies Immunoglobulin (RIG) as given in serves as a form of passive immunization against rabies and is administered as a single dose. Its administration is crucially time-sensitive, initiated after PEP and not extending beyond the seventh day after the initial vaccine dose (62). When administered correctly, RIG functions to neutralize the virus existing at the wound site within a matter of hours. There are two types of RIG - equine rabies immunoglobulin (eRIG) and human rabies immunoglobulin (hRIG)(63). World Health Organization (WHO) provides recommendations on maximum dose of RIG to be 20 IU (hRIG) and 40 IU (eRIG) per kilogram of body weight(64). It's not always necessary to inject the remaining calculated dose of RIG intramuscularly at a distance from the wound; instead, it can be divided into smaller, individual syringes for other patients, with aseptic retention in mind. In situations where RIG is unavailable, a rigorous and immediate cleansing of the wound, combined with the prompt administration of the initial vaccine dose, followed by a complete course of

rabies vaccine, has been proven highly effective in preventing rabies (65).

2. Current Rabies Vaccination Approaches

2.1. Vaccine Therapies

Concentrated Cell Culture and Embryonated Egg-based Vaccines (CCEEVs) are manufactured using RABV grown in either embryonated eggs (such as duck or chicken eggs) or cell cultures (including primary chick embryo cells, Vero cells, or human diploid cells) (66). After the virus is harvested, it undergoes a process of concentration, purification, inactivation, and lyophilization. In certain CCEEVs, stabilizers like human albumin or processed gelatine are incorporated. Adhering to WHO recommendations for both manufacturing and clinical evaluation is crucial for the production of RABV vaccines for human use (67). Before administration, the lyophilized vaccine is reconstituted using a suitable diluent. CCEEVs injection site is typically the deltoid muscle of the upper arm for adults and the anterolateral thigh for children (68). Proper injection technique and site selection are important to ensure the vaccine is delivered into muscle tissue for optimal immune response. The recommended dose for CCEEVs is at least 2.5 international units (IU) per injection. Depending on the specific vaccine type, the reconstituted volume can be either 0.5 mL or 1.0 mL(69). Following reconstitution,

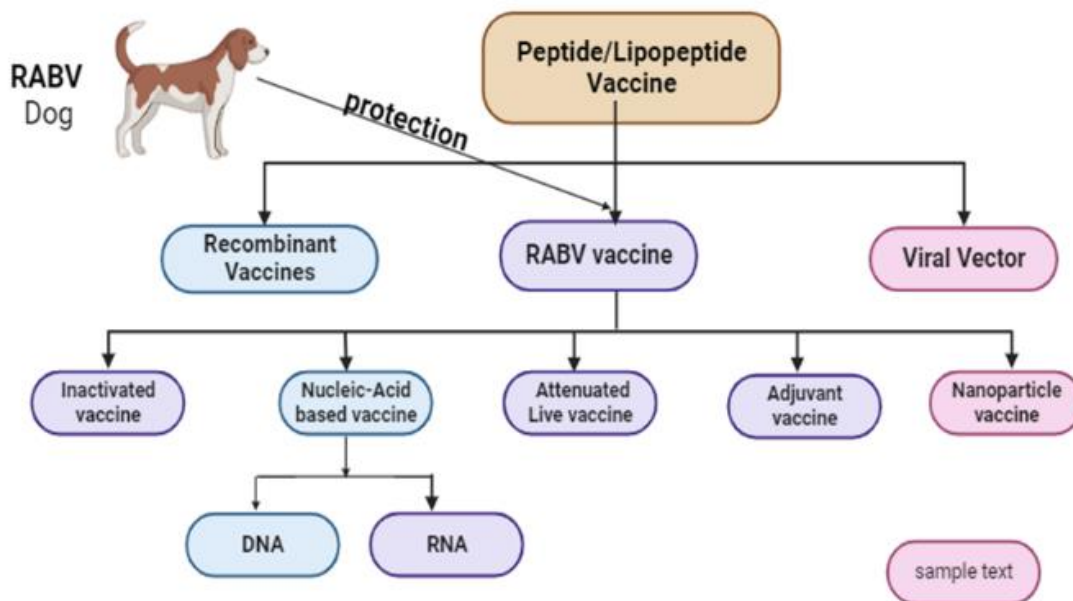


Figure 2: Comprehensive view of RABV vaccine

the vaccine is drawn into a syringe and administered using a sterile needle. CCEEVs are available in single-dose vials, which eliminates the need for multi-dose vials and minimizes the risk of contamination. Proper storage and handling are critical to maintaining the vaccine's efficacy. Vaccines that have been compromised due to improper storage should not be administered (70). After administration, individuals receiving the vaccine should be monitored for any immediate adverse reactions. Healthcare providers should document the vaccine administration, including the type of vaccine, lot number, date, and injection site. Modifying the route of administration or the vaccine product in a PEP or PrEP

regimen is both safe and effective in maintaining immunogenicity. When stored within the temperature range of 2–8 °C and shielded from sunlight, these vaccines remain viable for a minimum of 3 years. After reconstitution with a sterile diluent, it's recommended to use the vaccines within 6–8 hours. For most individuals, regardless of age or nutritional status, this threshold is achieved within 7 to 14 days of PEP regimen (71). The duration of immunity induced by RABV vaccines can vary based on multiple factors, including the type of vaccine used, the individual's immune response, and the specific circumstances of vaccination.

2.2 Limitations and Challenges of traditional Approaches

Table 2: Constraints and difficulties in traditional RABV approaches

Traditional Approach	Limitations and Challenges	Ref
Clinical Diagnosis based on symptoms	<ul style="list-style-type: none"> • Overlapping symptoms can lead to delays in accurate diagnosis and treatment. • Lack of specific symptoms early in the disease can hinder early intervention. • Misdiagnosis may occur due to symptom similarities with other neurological conditions. 	(72)
History of animal exposure for diagnosis	<ul style="list-style-type: none"> • Incomplete exposure history (in young/unconscious patients) • Lack of awareness about rabies risk and proper reporting of exposure. • Exposure history might not be available for cases with dog bites and scratches. 	(73)
Fluorescent antibody testing for diagnosis	<ul style="list-style-type: none"> • Limited access to specialized testing facilities, especially in remote or resource-constrained areas. • Variability in test sensitivity due to differences in disease stage, sample quality, and technical proficiency. • False negatives can occur if samples are improperly collected or handled. 	(74)
Use of Rabies Immunoglobulin (RIG)	<ul style="list-style-type: none"> • Shortage of RIG supply in some regions, limiting its availability for post-exposure prophylaxis. • Potential adverse reactions, including allergies or side effects, associated with RIG administration. • Ethical concerns related to sourcing and production of RIG from animal sources 	(64)
Nerve tissue vaccines	<ul style="list-style-type: none"> • Lower immunogenicity and higher risk of adverse reactions compared to modern vaccines. • Production involves animal tissues, raising concerns about disease transmission and ethical considerations. • Availability of nerve tissue vaccines may be limited in some areas due to modern alternatives 	(66)
Multiple doses for vaccination	<ul style="list-style-type: none"> • Compliance issues with multi-dose schedules due to logistical challenges, forgetfulness, or inability to access healthcare facilities regularly. • Incomplete vaccination series can leave individuals partially protected or vulnerable to rabies infection. • High number of required doses may deter some individuals from seeking vaccination 	(75)

3. Novel Vaccination Therapies

The origins of first-generation vaccines are attributed to Louis Pasteur, who pioneered the development of the initial rabies vaccine given in Table 3. His approach involved using the spinal cords of infected rabbits, which were subjected to physical inactivation of the rabies virus through sun drying. Through a sequence of passages and the process of adapting the street (wild type) rabies virus to laboratory animals, Pasteur was able to modify the virus's attributes, influencing its virulence and the duration of its incubation period. By conducting over 50 successive passages, Pasteur's continuous observations revealed that the incubation period, spanning from the initial inoculation to the onset of rabies symptoms, consistently remained at 7 days. This observation led him to term the virus as a "Fixed" virus (38). He conducted a range of experiments on dogs, which are natural hosts. On July 6, 1885, Pasteur administered his experimental rabies vaccine to a 9-year-old boy named Joseph Meister. The boy had experienced multiple severe bites from a dog that was rabid. Meister was given a series of 13 injections containing air-dried suspensions of rabbit spinal cord infected with rabies virus, with the virus's virulence increasing progressively over 11 days. Pasteur's strategic vaccination saved Meister from succumbing to rabies. The Pasteur vaccine had its limitations. It contained progressively virulent rabies virus, raising concerns about the consistency of inactivation. Instances were reported where individuals developed rabies even after vaccination. Furthermore, the challenge of producing sufficient quantities of the vaccine to meet demand posed a significant obstacle to large-scale production. Pasteur's method remained in use for over half a century before substantial modifications were introduced to the rabies vaccine preparation process. Louis Pasteur's initial vaccine underwent enhancements through relatively simple chemical modifications, pioneered by Fermi in 1908 and further refined by Semple in 1911. Sir David Semple, based at the Central Research Institute (CRI) in Kasauli, India, contributed to the development of newer nerve tissue vaccines (NTVs) using adult sheep (66). Termed the "Semple vaccine," This method included using chemical agents, specifically phenol, to inactivate infected sheep or goat brain tissue. While the addition of phenol aimed to inactivate the virus, it inadvertently altered the protein structure and disrupted the antigenic properties of the rabies virus, as previously observed with the Pasteur vaccine. Unfortunately, this modification was

associated with severe side effects, notably including Guillain-Barre Syndrome (GBS), and raised concerns about the potential transmission of Transmissible Spongiform Encephalopathies (TSE) (76). Despite its wide adoption in various regions, the use of this vaccine was ultimately suspended by the World Health Organization (WHO) in nearly all countries due to these concerns. The Fermi and Semple vaccines achieved success, but they were not without drawbacks. Some vaccinated individuals experienced sensitization, and a few cases even saw heightened risk of fatal encephalitis due to elevated myelin levels. To address these concerns, researchers sought a less reactogenic alternative. During the 1940s, significant attention was directed towards clinical research centered on allergic encephalomyelitis and demyelinating diseases of the central nervous system (CNS) related to vaccination. The utilization of embryonated eggs, specifically embryos of chicks or ducks, as well as neonatal rodent brains, as a medium for producing rabies vaccines significantly improved safety during vaccine development. Clinical investigations highlighted that embryonic and newborn animal nerve tissues lacked the components responsible for vaccine side effects. A neonatal rodent brain vaccine was developed by researchers from the former Soviet Union using rats. Subsequently, in 1964, Fuenzalida and his team produced an inactivated rabies vaccine that lacked myelin components, termed Suckling mouse brain (SMB) vaccine, derived from a suckling mouse's brain (77). This was achieved through phenolic inactivation followed by partial purification. Compared to the Semple vaccine, the SMB vaccine, derived from tissues of newborn animals devoid of myelin, exhibited reduced reactogenicity. However, it was discovered that the SMB vaccine still contained trace amounts of myelin and other undesired components, leading to critical adverse reactions. Consequently, in alignment with WHO recommendations, regulatory authorities worldwide collectively determined to discontinue the SMB vaccine after its extended usage over several decades. In 1931, Ernest W. Goodpasture introduced a novel approach by adapting various human viruses using embryonated eggs (78). Around 1940, chicks were subjected to the Flury strain of the rabies virus, merely one day old. After this application, the strain found a stable environment in chick embryos after nearly 40-50 passages. The resulting Flury low egg passage (LEP) vaccine consisted of live attenuated virus, which underwent extensive



lyophilization from a 33% whole-embryo suspension (79). This LEP vaccine was extensively employed for large-scale dog vaccinations. However, residual virulence persisted, particularly evident in kittens, cats, and cattle. Following this, the Flury high egg passage (HEP) vaccine was produced through a series of approximately 180 egg passages or more. Despite being tested on humans during the 1950s and 1960s, the HEP vaccine's potency did not meet satisfactory standards, leading to its eventual discontinuation. Transitioning to the late 1950s, duck embryos emerged as an alternative medium for vaccine production, giving rise to the development of a duck embryo vaccine (DEV) for rabies which contained 10% suspension of whole embryos, that was inactivated using β -propiolactone and was used until 1980s (80). However, due to adverse reactions and subpar antigenicity, these vaccine formulations were subsequently discontinued. The successful adaptation of rabies virus to embryonic environments offered hope for substitutes to the brain tissue-based vaccines. Despite certain improvements in the quality of these vaccines through different strategic approaches, concerns about their safety, efficacy, and immunogenicity remained unresolved. Consequently, these vaccines were gradually phased out in various regions worldwide. The introduction of cell culture systems for virus propagation has inaugurated a fresh phase in the development of rabies vaccines, leading to the creation of second-generation cell culture vaccines. This method presents numerous benefits compared to nerve tissue vaccines and egg-based systems. These advantages include proven safety and effectiveness, shortened development time, and enhanced process adaptability. The journey began in the 1930s when the rabies virus was successfully cultivated in primary chick embryo brain cells and maintained through serial passages. The following studies concentrated on cultivating stabilized RABV in mouse embryo brain tissues. In 1942, Plotz and Reagan accomplished the initial direct *in vitro* isolation and growth of street rabies virus from rabid cases utilizing primary chick embryo cells (81). The notion of cultivating the rabies virus in non-neuronal tissues arose in 1958, resulting in the development of the initial tissue culture rabies vaccine using hamster kidney cells. Further advancements included the propagation of fixed RABV in human diploid cell strains, such as the WI-38 and MRC-5 cell lines. However, while human diploid cell vaccines like HDCV exhibited reduced adverse effects

and earned WHO recommendations, they faced limitations in virus yield and cost-effectiveness. As a result, alternatives like purified duck embryo cell vaccine (PDECV) and purified chick embryo cell vaccine (PCECV) were developed. These vaccines demonstrated superiority over their predecessors, being devoid of allergenic components and showing enhanced immunogenicity and tolerability (82). Continuous cell lines like Vero cells derived from African green monkey kidney cells presented a breakthrough. They allowed for higher virus titers, scalability, and lower production costs. Vero cells supported the replication of various lyssaviruses, including rabies virus, and significantly improved vaccine accessibility in developing countries. The purified Vero cell rabies vaccine (PVRV) gained widespread use, replacing nerve tissue vaccines and earning approval from the WHO. Taking the vaccine production process further, an enhanced serum-free version known as PVRV-Next Generation (PVRV-NG) was developed from the inactivated PM strain of RABV. This next-generation vaccine exhibited strong immunogenicity and safety profiles, offering a promising alternative for rabies prophylaxis. The integration of cell culture techniques into rabies vaccine development has revolutionized the field, paving the way for more efficient, effective, and accessible preventive measures against rabies. Over the years, the development of RABV vaccines has remained focused on ensuring safety and immunogenicity and main goal has been to improve the candidate vaccines by modifying or inactivating the virus.

Modified live vaccines are produced by subjecting the virus to successive passages in various cell types to ensure their safety. The SAD strain of rabies virus serves as the source for attenuated vaccines, which have undergone varying degrees of attenuation through multiple cell culture passages (83). In certain Asian countries, the Flury strain is utilized for manufacturing modified live vaccines derived from chicken embryos. However, residual virulence remains a challenge for MLV, and temperature sensitivity and accidental self-inoculation are concerns. The WHO discontinued recommending MLV RABV vaccines for parenteral injection in 2004 due to these limitations. Inactivated vaccines have a long history and use complete, inactivated viruses with similar antigenic properties to wild-type viruses. Immunization using these vaccines triggers the production of virus-neutralizing antibodies

by activating T cells, offering protection against rabies virus challenges. Inactivated rabies vaccines have been produced using various virus strains through different methods, including cell culture and embryonated egg systems. Beta propiolactone (BPL) is the most common inactivating agent. However, its limitations have driven research into alternative agents like binary ethylenimine (BEI). Drawbacks of inactivated vaccines include lower immunogenicity and the need for multiple vaccination regimens. Adjuvants enhance immune responses to weak immunogens, like inactivated vaccines. Aluminum salts are commonly used as adjuvants, though they have limitations. Research into alternative adjuvants like synthetic derivatives, liposomes, and other compounds aims to improve immunogenicity and effectiveness (84). Compounds such as, Isatisindigotica root polysaccharides, CpG oligo deoxynucleotides and monophosphoryl lipid A (MPLA) show potential for enhancing immune responses and reducing adverse effects in future adjuvanted rabies vaccines.

3.1 DNA and RNA-based vaccines

The need for safer and more effective RABV vaccines has driven the exploration of advanced technologies like recombinant DNA and reverse genetics with improved stability and immunogenicity by targeting recombinant RABV virus strains or specific antigenic glycoproteins. RABV vaccines often attenuate or weaken viruses to eliminate virulence (85). Genetic manipulations involving site-specific mutations or modified glycoprotein insertions can eliminate residual pathogenicity and enhance safety. These vaccines play a crucial role in large-scale immunization efforts, especially among stray dogs and wildlife. For instance, a genetically modified ERA vaccine strain (rERA) with specific mutations induced potent and lasting immune responses in dogs and mice. Other strains like ERAG3G and SPBN GASGAS, developed through reverse genetics, showed high efficacy against pathogenic RABV in mice (86). These genetically modified vaccines represent a significant advancement in RABV prevention. These advances suggest the potential of recombinant RABV strains as inactivated vaccine candidates. Nucleic acid vaccines, including DNA and RNA vaccines, offer a cost-effective, safe, and efficient approach to triggering protective immune responses against viruses. DNA vaccines involve cloning the glycoprotein gene into expression vectors, inducing specific immune responses in animal models. While

effective, their slower immune responses limit their post-exposure prophylactic use. Combining DNA vaccines with adjuvants or co-delivery techniques has shown promise in enhancing their efficacy (87). Similarly, RNA vaccines, translated in the cytoplasm, provide rapid antigen expression without integration into the host genome. Their capabilities are demonstrated by non-amplifying mRNA vaccines and self-amplifying mRNA vaccines, with the latter showing effective immunogenicity in animal models. Despite their potential, challenges like RNA instability need addressing. Protein subunit vaccines use peptides or proteins as antigens to stimulate immune responses. The RABV G protein is a key target for protective immunity. Yeast and insect cell-produced G protein showed immunogenicity, but issues with folding and purification were encountered. Genetically altered plants have been explored for producing edible vaccines, but challenges in stability and degradation remain. Synthetic peptides mimicking G protein epitopes have also been investigated. However, challenges in proper protein folding and trimer formation hinder the success of protein-based vaccines. Viral vector rabies vaccines involve inserting the target antigen into a non-pathogenic virus that carries the gene into host cells for expression. Various viruses like adenoviruses, poxviruses, and lentiviruses have been manipulated to serve as carriers, triggering immune responses against the encoded antigens. These vaccines have proven effective due to their adjuvant properties, promoting cellular immunity and sustained antibody levels. Recombinant adeno-associated viruses (AAVs) and adenoviruses have been employed to express rabies glycoprotein, inducing strong immune responses. Chimpanzee adenovirus vectors, such as AdC68, have shown promise in stimulating protective responses, especially via oral administration (88). The creation of oral rabies vaccines (ORVs) has turned into a pivotal strategy for managing rabies in wildlife populations and free-roaming animals. These vaccines are administered orally through bait containing vaccine suspension. Modified live vaccines (MLVs) derived from attenuated strains of the rabies virus have been used in ORVs. First-generation MLVs like SAD-Bern and ERA played a significant role in controlling wildlife rabies in various regions. Concerns about reversion to virulence prompted the development of third-generation MLVs, like SPBN GAS GAS and ERA G333, which maintain safety and improved

immunogenicity (89). Oral vaccination campaigns using these vaccines have demonstrated success in immunizing free-roaming dogs and wildlife populations in developing countries. However, challenges such as pre-existing immunity to the vector and potential infections caused by the vector itself need to be addressed. Despite these challenges, the success of ORVs in controlling wildlife RABV and their potential to reach large populations make them important tools for rabies eradication efforts in developing countries.

3.2 Nanotechnology-based vaccine delivery systems

Recent progress in nanotechnology and colorimetric analytical methods present encouraging paths for accurate and dependable diagnosis of the rabies virus. These methods typically rely on a single-mode signal presentation, which can prove inadequate for accurate diagnoses requiring high sensitivity. The progress in both inorganic quantum dots (QDs) and organic fluorophores has been substantial and are extensively employed in bio-recognition applications known for their heightened sensitivity, particularly in colorimetric techniques (90). A recent study detailed an exact immunoassay approach for detecting the RABV, utilizing a dual-modal detection mode that combines colorimetric and fluorescent signals. This method is based on utilizing pomegranate-shaped silica nanospheres (PSS) with densely packed QDs linked to horseradish peroxidase-labeled antibodies (HRP-Ab2). The process began with the fabrication of uniform and mono-dispersed dendritic silica nanospheres (DSN), primarily intended for encapsulating QDs. DSN, featuring well-defined and manageable mesoporous pathways, and enabled the efficient encapsulation of a high concentration of QDs through robust thiol-metal coordination (91). This led to enhanced fluorescence emission, significantly elevating the sensitivity of fluorescence-based bio-recognition compared to conventional QDs that are coated externally on silica nanospheres through electrostatic interactions. The introduction of a substantial silica shell on DSN aimed to functionalize and safeguard the particles, which diverges from the approach of coating silica nanospheres with QDs on their surface (92). This differentiation in the method resulted in lower fluorescence due to the limited presence of quenching biomolecules. The nucleoprotein found in RABV is exceptionally conserved, and the clinical diagnosis of RABV requires the identification of this protein (93). The elevated surface-to-volume ratio of PSS facilitates a stronger conjugation of HRP-Ab2 and

enhances interaction with RABV antigens from various directions, leading to an amplified colorimetric signal within a controlled region. By introducing RABV antigens to anti-RABV antibodies in a controlled environment, sandwich-like immuno-complexes are formed. Detection of RABV and PSS's uniform fluorescent emissions can be achieved by gauging the fluorescence intensity of the underlying sandwich structure (94). This approach was further confirmed using brain tissue models and has been acknowledged as a dependable method for diagnosing the virus with both precision and sensitivity. In a novel study, for the first time, researchers are working on an improved double-mode biosensing technique that employs Y-shaped DNA nanostructures combined with enzyme pairs within the detection system (95). To this end, a specific segment: 5'-TGGACTAATAACTGAACTTATGT-3' (24 bases, X03673.1) was chosen as the reference analyte due to the fact that rabies is the sole disease with a one hundred percent fatality rate (96). Drawing from prior research, this oligonucleotide is recognized as a distinctive rabies diagnostic marker and is pivotal for the development of a swift, robust, and sensitive detection tool for RABV. This investigation employs three DNA probes with metastable hairpin structures (HP-A, HP-B, and HP-C). Each probe consists of an 18-base pair stem and an additional 6-nucleotide binding end at the 5' terminus. Essentially, HP-A bears a fluorophore (FAM) at the 5' end and a quencher (BHQ1) at the 3' end, forming a hairpin configuration through the Watson-Crick interactions between complementary sequences. Subsequently, HP-B and HP-C were respectively modified with glucose oxidase (GOx) and HRP at their 3' ends. Due to intrinsic intramolecular fluorescence resonance energy transfer (FRET), HP-A exhibited weak fluorescence when the target was absent. The introduction of the RABV target triggered a conformational change in HP-A, GOx-B, and HRP-C, leading to the formation of Y-shaped DNA nanostructures containing three hairpin probes. This rearrangement resulted in the revival of HP-A's fluorescence and the catalytic processes of GOx and HRP, facilitated by their close proximity. Following the addition of glucose and 3,3',5,5'-Tetra methyl benzidine (TMB), the enzyme glucose oxidase (GOx) catalyzed the oxidation of glucose, resulting in the production of gluconic acid and hydrogen peroxide (H₂O₂). This H₂O₂ acted as a substrate for horseradish peroxidase (HRP), facilitating the efficient conversion of TMB to

Table 3: Roadmap of Discoveries of RABV vaccine

Year	Development	Ref
1198	Moses Maimoides @potrayed incubation in Rabies infected ones	(100)
1769	Giouanni Battista Morgan Identified RAB virus in nerves	(101)
1809	George Gottfried Ziule established dog saliva as a source	(102)
1852	Apollinaire Bouchardat established inoculations against RAB virus	(103)
1881	Pierre Victor Galtier made 1st RABV Immunization in sheep	(103)
1885	Roux maintained medullary parts of embryonic chicken for several days	(104)
1907	Harrison cultivated nerve fibers in vitro	(105)
1908-1911	Fermi and Semple developed Nerve Tissue Vaccine (NTV's)	(106)
1931	Ernest W. Goodpasture developed infected chick virus by Varicella Virus	(107)
1940	Flury Low Egg Passage (FLP) vaccine was developed.	(108)
1942	Plotz and Reagen did in vitro isolation, cultivation of explants of chick embryo	(109)
1951	Gey developed Hela cell line.	(110)
1958	First culture of RABV from Primary hamster kidney cell (PHKCV)	(111)
1960	Fenje caused adaptations in PHKC culture using Street Alabama	(112)
1961	Hayflick et al, developed Human diploid cell strain WI-38	(113)
1962	Y. Yasurmura and Y. Kawakita developed vero cell line.	(114)
1964	Fuenzalida et al developed neonantal rodent brain vaccine using rats.	(115)
1971	PHCKV was produced using Vnukovo-32 strain.	(116)
1974	HDCV rabies vaccine WI-38, MRC-5 was licensed, recommended by WHO	(117)
1975-1978	Fetal bovine kidney bovine cell rabies vaccine and Canine kidney cell rabies vaccine was developed	(118)
1985	PVRV licensed using vero cells	(119)
Late 1900's	Inactivated rabies vaccine was made using, Isatisindigotica root polysaccharide, CPG oligodeoxy nucleotide etc.	(120)

oxTMB, a colored product with a wavelength of 65 nm. The restored fluorescence and the formation of the colored product served as the basis for a simplified dual-mode signal presentation (97). Each step of the assembly process involved a subsequent disassembly, with HP-C causing the separation of the target from the composite. This disassembly allowed the target to be reassembled, amplifying the signal. Notably, the increased absorbance and fluorescence of oxTMB upon reassembly exhibited an inverse relationship with the concentration of the target. Compared to single-mode detection, the dual-mode recognition technique using Y-shaped DNA structures is superior due to its ability to eliminate external interfaces, resulting in qualitative and quantitative identification. This approach boasts advantages such as simplicity of operation, remarkable sensitivity, rapidity, quantitative visualization, and an exceptional capacity for detecting target nucleic acid. Phage display technology serves as an efficient system for generating antibodies in vitro by mimicking the collection methods of the immune system.

To achieve this, antibody fragments are fused with capsid proteins on the surface of filamentous bacteriophage particles, measuring 7 nm in width and 900–2000 nm in length (98). As a result, this approach directly links the phenotype (specificity and affinity of phage-displayed antibody) with the antibody's genotype (the DNA sequences of the phage particle). In this context, libraries of phage display single-chain fragments of variation (scFv) antibodies were harnessed to gather single-chain fragments of variation (scFv) targeting the rabies virus. These scFvs can be smaller in size compared to human antibodies like IgGs (25 vs. 150 kDa), yet they retain the ability to strongly bind to their target antigens (with dissociation constants ranging between 5 μ M to 10 nM). They also present mechanically minimized versions of full-length human IgGs. Moreover, when linked to antigen-binding fragments (Fabs) of approximately 50 kDa, scFvs tend to resist aggregation and exhibit a size two times smaller than Fabs. Consequently, the scFv antibody emerges as a valuable nanostructure with diverse therapeutic applications (99).

3.3 Advantages and Potential Challenges

Table 4, showing advantages and potential challenges in areas of cost effectiveness, regulatory hurdles, education regarding Rabies vaccine and finances.

Table 4: Advantages and Potential Challenges

Aspect	Advantages	Potential Challenges	Ref
Cost effectiveness	➤ Reduces health care costs.	➤ Ensuring affordable access for all populations	(121)
	➤ Prevents high medical expenses.	➤ Addressing disparities in vaccine distribution	
	➤ Reduces rabies-related economic burden	➤ Sustaining funding for vaccination programs	
Logistic Challenges	➤ Expands vaccine coverage.	➤ Cold chain maintenance in remote areas	(122)
	➤ Reaches remote populations.	➤ Infrastructure limitations in some regions	
	➤ Enables mass vaccination campaigns	➤ Supply chain disruptions during emergencies	
Regulatory aspects	➤ Ensures safety and efficacy.	➤ Complex and evolving regulatory requirements.	(123)
	➤ Validates vaccine quality.	➤ Regulatory harmonization	
	➤ Builds public trust in vaccines	➤ Expedited approvals without compromising safety	
Public awareness and education	➤ Improved effectiveness as compared to traditional methods.	➤ Lack of awareness among public and health care providers.	(124)
		➤ Misconceptions and resistance due to lack of education	
		➤ Limited financial resources for research and implementation	
Funding and Resources	➤ Research advancement	➤ Challenges in securing sustained funding	(125)
	➤ Global collaborations for shared knowledge and innovation		

humans, safeguarding the well-being of all three components – humans, animals, and the environment. Animal-Assisted Interventions (AAIs), particularly Animal-Assisted Therapy (AAT) and Animal-Assisted

4. Conclusion and Future Directions

The development of RABV vaccines is essential to stop the spread of fatal RABV disease. The importance of RABV vaccine treatment resides in its capacity to safeguard both human and animal communities, hence lowering the number of fatalities attributable to rabies. The effectiveness of RABV vaccination programs reinforces the significance of continued study and collaboration within fields including virology, veterinary medicine, and public health. For expanding RABV control, enhancing vaccine effectiveness, and eventually working towards the global eradication of RABV, ongoing efforts in advancement and international cooperation remain crucial.

The collaboration among veterinarians, public health experts, and epidemiologists plays a crucial role in preventing the transmission of diseases from animals to

Activities (AAA) in healthcare, embody the practical manifestation of the One Health approach. Each professional, leveraging their respective expertise, contributes to a specialized team dedicated to preventing and managing zoonotic diseases, ensuring the health and welfare of both individuals and animals, as well as the environment. There is a plan to collaborate with international experts in the field of AAIs to develop standardized protocols encompassing hygiene, health, and behavior. The goal is to establish consistent health and behavioral certifications for animals engaged in AAT and AAA within the healthcare sector. As vaccination programs expand, tracking adverse events, rare side effects, and long-term protection provided by the vaccines is important. Tailoring RABV vaccination strategies based on factors such as age, genetics, and previous exposures could enhance vaccine effectiveness and minimize unnecessary vaccinations. Personalized approaches might involve adjusting dosage, booster

schedules, or vaccine formulations to optimize immune responses for different individuals. Research should continue to explore new vaccine technologies, adjuvants, and delivery methods to enhance the safety, immunogenicity, and duration of protection offered by rabies vaccines. This includes investigating novel platforms like mRNA vaccines, vector-based vaccines, and other innovative formulations that could improve vaccine efficacy and simplify administration. Collaborative efforts involving governments, international organizations, and NGOs should continue to strengthen vaccination programs, especially in regions with high rabies burden. This includes prioritizing mass dog vaccination campaigns and addressing challenges related to vaccine distribution and accessibility. Expanding rabies vaccination efforts beyond domestic animals to target wildlife reservoirs is critical for sustained control. Developing safe and effective vaccines for wildlife, such as oral rabies vaccines for wild carnivores, can help break the transmission cycle and reduce spillover into domestic animals and humans. Incorporating rabies vaccination into existing health programs, such as routine veterinary care or primary healthcare services, can enhance coverage and accessibility. This integrated approach can lead to more efficient and cost-effective vaccination strategies. Public education and awareness campaigns should be ongoing to inform communities about the importance of rabies vaccination for both animals and humans. Encouraging responsible pet ownership, reporting of animal bites, and seeking prompt medical attention after potential exposure to rabies is vital for preventing human cases. Rabies is a global concern that requires international cooperation. Collaboration between countries, organizations, and experts is essential for sharing practices, coordinating research efforts, and addressing challenges that cross borders. By addressing these considerations, we can work towards more effective and comprehensive rabies control and prevention strategies on a global scale.

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