

REVIEW ARTICLE

Toward next-generation rabies vaccines: Innovations and challenges

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Abstract

Rabies continues to pose a significant fatal zoonotic threat, with approximately 59,000 human deaths reported annually, despite the disease being mostly preventable through vaccination. Recently, several notable scientific advancements have been made, including the determination of the pre-fusion conformation of the rabies virus glycoprotein (RABV-G), structure-guided antigen design strategies to enhance immunogenicity, and the development of mRNA-lipid nanoparticle (LNPs) delivery platforms encoding RABV-G. Pre-clinical models have shown that these mRNA-LNPs platforms induce neutralizing antibody titers that are approximately ten-fold higher than those elicited by traditional inactivated vaccines. Heterologous prime-boost vaccination regimens integrating inactivated rabies virus with mRNA boosters have demonstrated synergistic efficacy, achieving 100% protection with evidence of potential durability in non-human primate models. However, several critical knowledge gaps remain, such as the structure of the full-length or ectodomain of pre-fusion RABV-G, the mechanisms underlying pH-driven conformational changes of RABV-G and its fusion process, the binding mechanisms of different viral receptors, and the induction of long-term protection. Future breakthroughs will hinge on integrating technological innovation with global accessibility to achieve the “Zero by 30” rabies elimination initiative. This requires coordinated efforts to translate cutting-edge research into scalable, affordable interventions that address both scientific challenges and public health gaps.

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Citation: Li M, Zhang P, Jin X. Toward next-generation rabies vaccines: Innovations and challenges. *Microbes & Immunity*. 2026;3(2):025310068. doi: 10.36922/MI025310068

Received: July 29, 2025

1st revised: September 2, 2025

2nd revised: November 5, 2025

Accepted: November 7, 2025

Published online: February 5, 2026

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Keywords: Rabies vaccine; Rabies virus glycoprotein; mRNA vaccine; Zoonotic disease elimination

1. Introduction

Rabies is a fatal zoonotic disease caused by the rabies virus (RABV), responsible for approximately 59,000 human deaths annually, and nearly 40% of the fatalities occur in children under the age of 15, predominantly in developing countries across Africa and Asia.¹ To eliminate rabies disease and deaths, the World Health Organization (WHO), in collaboration with the Food and Agriculture Organization and the World Organization

for Animal Health, launched the “Zero by 30” global strategic plan, aiming to end human deaths from dog-mediated rabies by 2030.^{2–5}

Rabies virus is an enveloped, single-stranded, negative-sense RNA virus of the *Lyssavirus* genus within the *Rhabdoviridae* family. Its genome encodes five structural proteins, including nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and RNA-dependent RNA polymerase (also known as the large structural protein). The trimeric RABV glycoprotein (RABV-G) plays a critical role in mediating viral attachment to the host cell surface by recognizing host cellular receptor(s) and mediates virus entry through low pH-triggered conformational transition and membrane fusion.^{6,7} As the sole surface protein of the virus, RABV-G is the principal target for virus-neutralizing antibodies and serves as the key immunogen in rabies vaccine development.^{8,9}

Once clinical symptoms manifest, rabies infection remains essentially 100% fatal, with no validated diagnostic tools or curative therapies currently available for clinical use. However, prompt administration of rabies vaccines and human rabies immunoglobulin (Ig) can successfully prevent disease onset.¹⁰ To date, all licensed rabies vaccines are inactivated formulations, produced in various cell substrates, including Vero cells,¹¹ baby hamster kidney cells,¹² and human diploid cells.¹³ While these vaccines have contributed significantly to rabies prevention, limitations remain, including suboptimal immunogenicity, high production costs, and reliance on the propagation of live virus.^{14,15} These challenges underscore the need for innovative vaccine strategies.

mRNA-based vaccines have emerged as a promising platform due to their rapid design, scalable production, and ability to elicit robust immune responses.¹⁶ Following the global success of COVID-19 mRNA vaccines,^{17,18} substantial research efforts have been directed toward developing mRNA vaccines against rabies. Recent pre-clinical studies have demonstrated that the mRNA vaccines targeting RABV can induce strong virus-neutralizing antibody responses and confer complete protection in mice, dogs, and non-human primates against lethal viral challenge.^{19–23}

This review provides a comprehensive overview of recent advances and progress in rabies mRNA vaccines (Table 1), beginning with the biological features of RABV, the structure–function characteristics of RABV-G, and rabies vaccine development, with the aim of informing future directions of next-generation rabies vaccines (Figure 1).

2. Rabies epidemiology and transmission

The primary rabies reservoir host varies significantly by region.⁴² Over 95% of the deaths occur in Africa and Asia, where dogs are the primary reservoir in endemic areas, and even healthy dogs can carry the virus.^{43,44} Across animal vaccination programs, Europe, North America, Australia, Japan, and parts of the Middle East have eliminated dog-mediated rabies.⁴⁵ In North America and Latin America, wildlife species, such as skunks, raccoons, insectivorous bats, or vampire bats are the dominant reservoir.^{46–48} Terrestrial wildlife animals (e.g., foxes, raccoons, skunks, and red foxes) serve as the wild reservoir in Africa and Asia.^{49,50} Although rabies incidence has dramatically decreased in these regions, it remains a significant public health crisis in developing countries, primarily in impoverished rural communities, due to a lack of vaccination, awareness, and effective surveillance.

The viral lifecycle begins with viral attachment, endocytosis, fusion, replication, assembly, and release. These processes ensure efficient propagation of RABV within the host and its transmission to new susceptible cells. Rabies virus is transmitted to humans via bites, scratches, or saliva contact with mucous membranes.^{5,51} After entry, the virus travels retrogradely along peripheral nerves to the central nervous system (CNS), a process that can typically take 1–2 months, on average, but may vary from a few days to years, depending on factors, such as the distance from the entry site to the CNS.^{52–54} As the virus progresses to the CNS, patients enter the acute neurologic phase, which can manifest in two distinct forms—furious rabies and paralytic rabies. Furious rabies is more common, accounting for approximately 80% of human cases.⁵⁵ It is characterized by hyperactivity, excitable behavior, hydrophobia (fear of water), aerophobia (fear of drafts or fresh air), hallucinations, and insomnia. Paralytic rabies accounts for about 20% of cases and presents with muscle weakness starting at the site of exposure, gradual paralysis, and coma.^{56,57} Currently, there are no WHO-approved diagnostic tools capable of detecting rabies infection before the onset of clinical symptoms.⁵⁸ Once clinical symptoms appear, rabies is almost invariably fatal, and treatment options are limited to palliative care.

3. Biological characteristics of the rabies virus

Rabies virus is a non-segmented, single-stranded, negative-sense RNA virus belonging to the *Lyssavirus* genus in the *Rhabdoviridae* family.⁴⁸ The virion possesses a characteristic bullet-shaped morphology, enclosed within a lipid envelope. Its genome is approximately 12 kb in length and encodes

Table 1. Rabies mRNA vaccines in development

No.	Year	Affiliation(s)	RNA type	Delivery system	Vaccine design	Modified nucleotide	Animals	Characteristic	References
1	2009	Indian Veterinary Research Institute	Self-amplifying RNA	N/A	CVS strain RABV-G	N/A	Mice	The self-amplifying RNA vaccine Sin-Rab-G induced an immune response in mice, but offered less protection than inactivated vaccines	24
2	2016	CureVac, Friedrich-Loeffler-Institut	mRNA	Protamine	Pasteur strain RABV-G	No	Mice, pigs	An unmodified nucleoside mRNA vaccine encoding the glycoprotein protected mice and pigs against virus challenge	25
3	2017	CureVac, Friedrich-Loeffler-Institut	mRNA	Protamine	Pasteur strain RABV-G	No	Mice	A lyophilized rabies mRNA vaccine maintained 100% challenge survival after 3 months of storage at 70°C	26
4	2020	GSK	Self-amplifying RNA	CNE	Flury-LEP strain RABV-G	N/A	Rat	A self-replicating rabies RNA vaccine, mixed with CNE, demonstrated good tolerability and safety upon repeated administration in rats	27
5	2022	Liverna; National Institutes for Food and Drug Control, China	mRNA	LNPs	CTN-1 strain RABV-G	Yes	Mice, dogs	LNP-formulated rabies mRNA vaccine LVRNA001 provided protection in dogs against fixed and street rabies virus challenge, effective both pre- and post-exposure	20
6	2023	CureVac, Karolinska University Hospital	mRNA	LNPs	Pasteur strain RABV-G	No	NHPs	LNP-formulated mRNA vaccines elicited superior and more durable rabies virus neutralization in NHPs than the inactivated vaccine	23
7	2023	GSK	Self-amplifying RNA	LNPs or CNE	Flury-LEP strain RABV-G	N/A	Rat	Subsequent studies comparing the safety of LNP-based and CNE-based rabies self-amplifying RNA vaccines revealed that LNP formulations activated innate immune responses earlier than CNE	28
8	2023	Changchun Institute of Biological Products; National Institutes for Food and Drug Control, China	mRNA	LNPs	CTN-1 strain RABV-G	Yes	Mice	A 0.032 µg/dose ED50 was observed for the rabies mRNA vaccine RV021 with two doses in pre-exposure prophylaxis	21
9	2023	CSPC; China Center for Disease Control and Prevention	mRNA	LNPs	CTN-1 strain RABV-G	Yes	Mice, NHPs	A rabies mRNA vaccine offered more effective post-exposure protection than inactivated vaccines across diverse rabies virus clade challenge experiments	29

(Cont'd...)

Table 1. (Continued)

No.	Year	Affiliation(s)	RNA type	Delivery system	Vaccine design	Modified nucleotide	Animals	Characteristic	References
10	2023	Fudan University	mRNA	LNPs	Pitman-Moore strain RABV-G	Yes	Mice	A single dose of the rabies mRNA vaccine induced higher neutralizing antibody levels in mice than three doses of the inactivated vaccine	30
11	2023	Chengdu Qingbaijiang District People's Hospital, China	mRNA	LNPs	CTN-1 strain RABV-G, nucleic immunostimulators	Yes	Mice	Adding CpG adjuvant to LNP-delivered rabies mRNA vaccines enhanced both humoral and cellular immune responses	31
12	2023	Academy of Military Medical Sciences, China	mRNA	LNPs	RABV-G	N/A	Mice, dogs	A single 50 µg dose of the mRNA vaccine induced protective neutralizing antibody levels in all dogs within 2 days, sustained for at least 20 weeks	32
13	2023	Huazhong Agricultural University; Stemirna Therapeutics	mRNA	LPPs	Flury-LEP strain RABV-G	Yes	Mice, dogs	A single low dose of LPP-mRNA-glycoprotein fully protected mice from lethal rabies virus and elicited strong antibodies in dogs	33
14	2024	Liverna; China Center for Disease Control and Prevention	mRNA	LNPs	CTN-1 strain RABV-G	Yes	mice, dogs, NHPs	Subsequent studies validated the efficacy and safety of the rabies mRNA vaccine LVRNA001 in dogs, mice, and macaques	34
15	2024	Peking Union Medical College	mRNA	LNPs	FY4 strain RABV-G (H270P)	Yes	Mice	Incorporating the H270P mutation into rabies glycoprotein boosts the humoral and cellular immune responses of mRNA vaccines	35
16	2024	Jilin University	mRNA	LNPs	Pasteur strain, Full-length RABV-G, RABV-tG (ectodomain with a MTQ motif), RABV-preG (H261L, H270P), RABV-VLP	Yes	Mice	Rabies VLP mRNA vaccines, incorporating matrix protein, nucleoprotein, and glycoprotein, elicited high levels of neutralizing antibodies in mice	36
17	2024	China Pharmaceutical University	mRNA	LNPs	CTN-1 strain, linear design algorithm-designed RABV-G	Yes	Mice, NHPs	Rabies mRNA vaccines demonstrated superiority over approved inactivated vaccines in mice and monkeys, generating durable neutralizing antibodies and memory B cells	37
18	2024	Huazhong Agricultural University	Circular RNA	Mannose-modified LNPs	SAD-L16 strain RABV-G	Yes	Mice	Mannose-modified LNPs enhanced rabies circular RNA's lymph node targeting, prolonging antigen retention, and promoting germinal center formation	38

(Cont'd...)

Table 1. (Continued)

No.	Year	Affiliation(s)	RNA type	Delivery system	Vaccine design	Modified nucleotide	Animals	Characteristic	References
19	2025	CSPC; Jilin University	mRNA	LNPs	Full-length RABV-G, ectodomain of RABV-G, ectodomain and transmembrane domain of RABV-G	Yes	Mice	A prime-boost strategy with inactivated and mRNA vaccines yielded higher neutralizing antibodies in mice	39
20	2025	Starna Therapeutics Co., Ltd	mRNA	Muscle-targeting LNPs	CTN-1 strain RABV-G	No	Mice	Muscle-targeting LNPs induced higher neutralizing antibody levels and provided more effective post-exposure protection than liver-targeting LNPs	40
21	2025	Fudan University	mRNA	LNPs	RABV-G + RABV-L	Yes	Mice, NHPs	The dual-antigen RABV-G-LT vaccine provided more comprehensive and durable protection by simultaneously stimulating both T cell and antibody immunity	41

Abbreviations: CNE: Cationic nanoemulsion; CVS: Challenge virus standard; G: Glycoprotein; L: Large structural protein; LEP: Low egg passage; LNP: Lipid nanoparticle; LPP: Lipopolyplex; NHP: Non-human primate; RABV: Rabies virus; VLP: Virus-like particle.

five viral proteins: Nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and the large structural protein. Nucleoprotein forms a helical ribonucleoprotein (RNP) complex encapsulating the genomic RNA and forms a stable RNA-nucleoprotein complex to protect viral RNA from host nuclease degradation. Phosphoprotein prevents nucleoprotein from binding host RNA and facilitates RNA synthesis by interacting with the large structural protein. Matrix protein mediates virion assembly and budding, condenses RNP cores, and links them to the envelope via RABV-G cytoplasmic tails (CT). RABV-G mediates receptor binding and membrane fusion.⁵⁹ The large structural protein catalyzes RNA synthesis to replicate genomic RNA and generates mRNAs.

Rabies virus enters host cells primarily through interactions between its envelope RABV-G and specific cellular receptors. The major host receptors include the nicotinic acetylcholine receptor,⁶⁰ the neural cell adhesion molecule,⁶¹ and the p75 neurotrophin receptor (p75^{NTR}).⁶² Other extracellular components may also contribute to virion-host interactions, such as heparan sulfate⁶³ and the metabotropic glutamate receptor subtype 2.⁶⁴ Specific regions within RABV-G are responsible for receptor interaction. For instance, the residue Arg333 has been shown to play a crucial role in binding to p75^{NTR} and is closely associated with viral neurovirulence.^{62,65} In addition, RABV-G contains a “neurotoxin-like” motif (residues 189–214) that mediates binding to neuron receptors.⁵⁹

Following receptor binding, the virus enters host cells via receptor-mediated endocytosis, leading

to internalization of the virus within endocytic vesicles.^{59,66,67} The acidic environment of the endosome triggers a pH-dependent conformational rearrangement of RABV-G, from a pre-fusion hairpin-like structure to a post-fusion extended conformation.^{6,68,69} This structural transition facilitates the fusion of the viral envelope with the endosomal membrane, allowing release of the viral RNP core into the host cytoplasm to initiate replication.^{60,62,64,70,71} The released RNP complex uses dynein motors to travel along microtubules toward the cell nucleus. Upon reaching the perinuclear region, viral RNA polymerase (large structural protein), along with its cofactor phosphoprotein, initiates transcription to express viral proteins. The genes encoding the five proteins—nucleoprotein, phosphoprotein, matrix protein, glycoprotein, and large structural protein—are arranged in order along the genome, and transcriptional termination and re-initiation occur at conserved gene junctions, which follow a sequential start-stop mechanism. As a result, a transcriptional gradient is established in which the upstream genes (the nucleoprotein-encoding gene has the highest mRNA abundance) are expressed at a higher level than the downstream genes.^{72,73}

Viral replication begins once sufficient nucleoprotein has accumulated, allowing the RNA-dependent RNA polymerase to shift from transcription to replication and synthesize full-length positive-sense RNA. This RNA then serves as a template for producing new negative-sense genomic RNAs. These nascent genomes are immediately encapsulated by nucleoproteins to form new nucleocapsids.

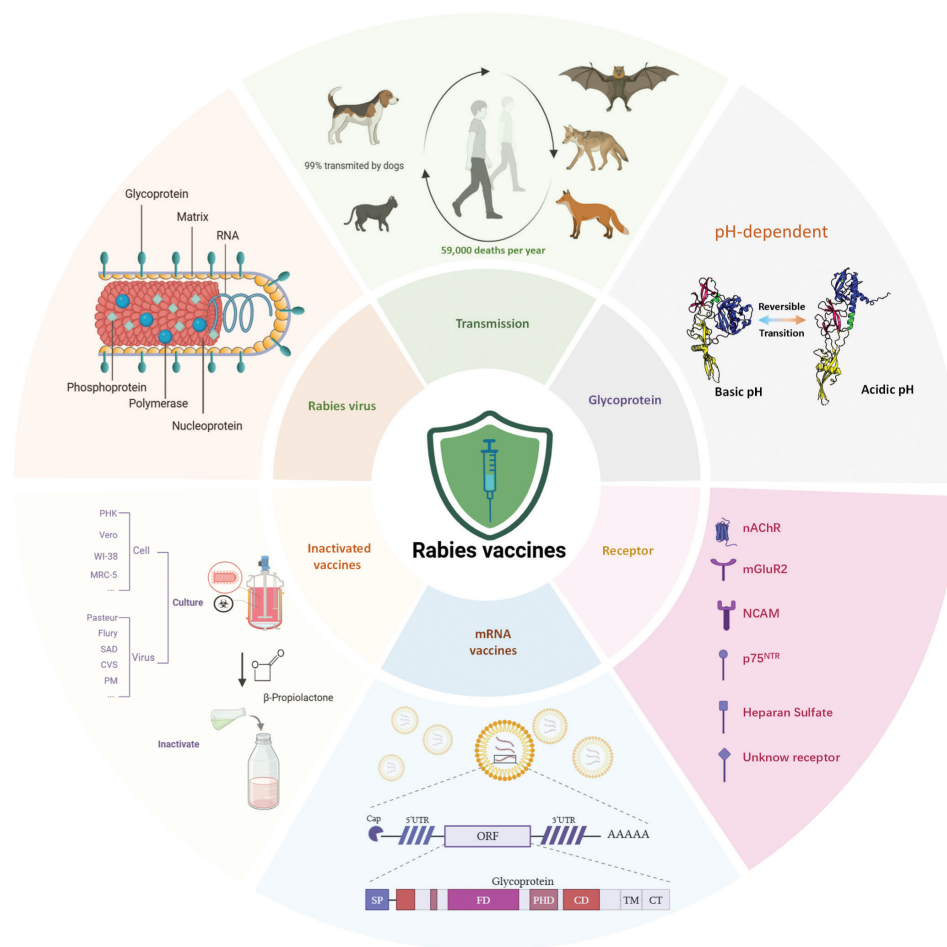


Figure 1. Overview of the rabies virus vaccine. Rabies virus exhibits a characteristic bullet-shaped morphology, consisting of a glycoprotein, matrix protein, phosphoprotein, polymerase, nucleoprotein, and a single-stranded RNA genome. As a zoonotic pathogen, the rabies virus is transmitted between animals and humans, causing an estimated 59,000 deaths annually worldwide. Rabies virus glycoprotein undergoes reversible, pH-dependent conformational changes and can utilize multiple host cell receptors for entry, including nAChR, mGluR2, NCAM, p75^{NTR}, and heparan sulfate. The structure of mRNA vaccines includes a 5' cap, 5' UTR, ORF, 3' UTR, and a poly-A tail. In rabies mRNA vaccines, the ORF typically encodes the glycoprotein. The production of inactivated rabies vaccines involves virus cultivation followed by chemical or physical inactivation processes. Created with BioRender. Jin, X. (2026) <https://BioRender.com/lqgcgwy>. Abbreviations: CD: Central domain; FD: Fold domain; nAChR: Nicotinic acetylcholine receptor; NCAM: Neural cell adhesion molecule; mGluR2: Metabotropic glutamate receptor 2; ORF: Open reading frame; p75^{NTR}: p75 neurotrophin receptor; PHD: Pleckstrin homology domain; SP: Signal peptide; TM: Transmembrane domain; UTR: Untranslated region.

The matrix protein also plays a regulatory role in this process. As matrix protein accumulates late in infection and binds to the RNP complex, it halts transcription to prioritize genome replication and virion packaging transition by modulating polymerase activity.⁷⁴ The matrix protein associates with mature nucleocapsids in the cytoplasm and acts as a structural scaffold, directing them to RABV-G-enriched regions of the plasma membrane and initiating viral budding. During this process, the nucleocapsid becomes enveloped and acquires surface glycoprotein spikes, ultimately releasing mature virions from the host cell.^{74,75}

4. Rabies virus fusion machinery: RABV-G

Fully formed RABV particles exhibit a characteristic bullet-shaped morphology, with a helically symmetrical nucleocapsid encased in a lipid envelope studded with densely packed trimeric RABV-G.⁵⁹ Within the infected host, progeny virions are capable of retrograde axonal transport and trans-synaptic spread, allowing the virus to reach the CNS, where it causes fatal encephalitis.^{76,77} As a member of class III fusion machinery, RABV-G is a homo-trimeric type I transmembrane glycoprotein.^{7,78-81} As the sole glycoprotein on the viral envelope, RABV-G is essential for viral entry into host cells and represents

the primary target for virus-neutralizing antibodies.^{6,80,82} A thorough understanding of the biological functions and antigenic epitopes of RABV-G is critical for optimizing vaccine antigen design.

4.1. Structural characteristics of RABV-G

RABV-G shares a common trimeric architecture and pH-sensing mechanism with other *Lyssavirus* glycoproteins.^{78,79} The full-length RABV-G consists of approximately 524 amino acids, comprising a 19-amino acid N-terminal signal peptide (SP), an extracellular domain (ectodomain), a transmembrane domain (TM), and a CT. The SP directs the nascent glycoprotein polypeptide into the lumen of the endoplasmic reticulum (ER). After cleavage of the SP, the glycoprotein undergoes further folding and post-translational modification within the ER and Golgi apparatus,^{83,84} and is subsequently transported to the plasma membrane, where it assembles into spikes on the budding virion surface.

Based on high-resolution structural studies,^{6,8,71,80,85} the RABV-G ectodomain can be categorized into five distinct subdomains: Central domain (CD, domain I), pleckstrin homology domain (PHD, domain II), fusion domain (FD, domain III), transition hinge domain (THD, domain IV), and ecto-C-terminal domain (ECD, domain V) (Figure 2).

In the pre-fusion state, the CD is located at the center of the trimeric axis as the inner core of RABV-G, where it stabilizes the trimeric structure. The THD comprises two separate linker regions that connect the PHD. During the fusion process, the THD refolds and repositions beneath the CD. The PHD sits on top of the FD and mediates receptor binding through residues 190–203. The FD is located adjacent to the PHD and contains two hydrophobic fusion loops that can interact with the host membrane. The ECD has a flexible structure, and its conformation has not yet been fully characterized. In the pre-fusion state, the ECD forms a compact conformation and loosely associates with the FD, CD, TM, and membrane. In the post-fusion conformation, the ECD extends to elevate the CD, and the FD detaches from the membrane-proximal position and subsequently inserts its fusion loops into the host membrane.

4.2. Antigenic epitopes of RABV-G

Multiple antigenic sites (I, II, III, and IV) have been defined on the RABV-G (Figure 2), among which antigenic sites II and III are the most significant.⁸⁶ Antigenic site II is primarily located in the CD of RABV-G, encompassing two discontinuous regions (residues 34–42 and 198–200), which are linked by a disulfide bond.^{82,87} Site III is situated

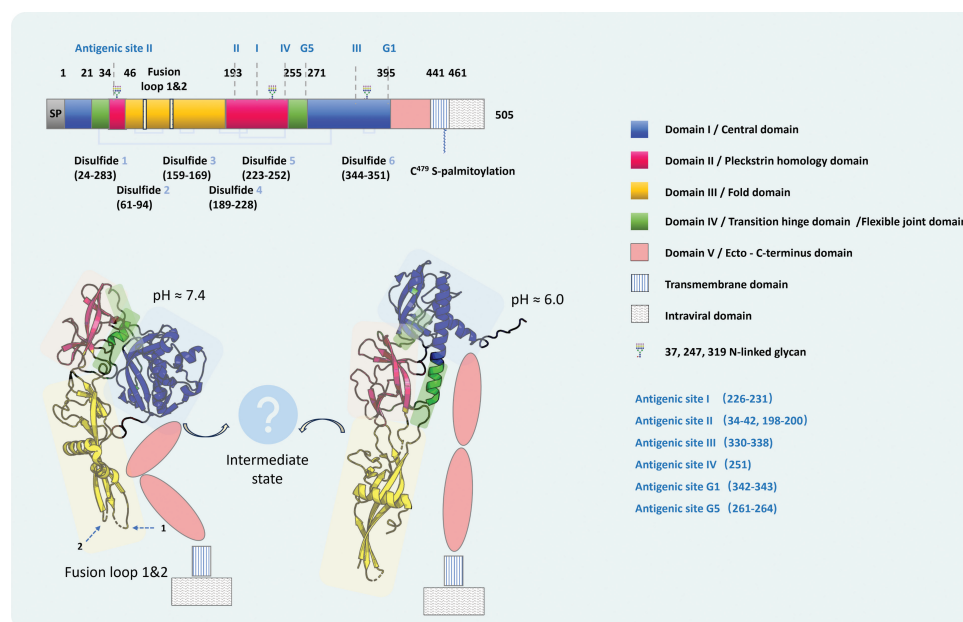


Figure 2. Structural organization and pH-dependent conformational dynamics of rabies virus glycoprotein (RABV-G). Antigenic sites I, II, III, IV, G1, and G5, with their residue ranges, are mapped onto the structure. Six disulfide bonds and N-linked glycosylation sites are indicated in the diagram. pH-dependent conformational transitions of the RABV-G ectodomain illustrate the structural rearrangement. At physiological pH, the glycoprotein adopts a prefusion conformation with compactly arranged domains. Upon acidification, a conformational rearrangement, likely involving an intermediate state, results in an extended post-fusion conformation conducive to membrane fusion. RABV-G structures (PDB accession codes: Low pH structure, 6LGW; high pH structure: 6LGX) were generated with PyMOL (<https://pymol.org/>). Image created by the authors using PyMOL (for molecular visualization) and assembled using Microsoft PowerPoint.

in the PHD at the top of the trimer.⁸⁸ This site includes residues 330–338, with critical residues at positions 330, 333, 336, and 338. Structural analyses reveal that the 17C7 antibody (Rabishield) binds parallel to the membrane surface on the flank of the CD, engaging residues, such as Asn336 and Arg346^{89,90}; whereas the 1112-1 antibody binds almost perpendicularly to the membrane at the PHD, interacting with residues Asn194, Arg199, Gln244, and Thr245 (Figure 3).⁷¹

Several viral strains exhibit amino acid polymorphisms at site II, with known variants reducing the binding and neutralization efficacy of site II-targeting antibodies, such as 17C7,^{89,91} suggesting that reliance on a single site II antibody may be insufficient to neutralize all RABV variants. Recently, broadly neutralizing antibodies targeting conserved regions of the RABV-G have been identified. For example, the human monoclonal antibody RVC20 recognizes a highly conserved epitope on domain III of RABV-G and efficiently neutralizes 100% of tested RABV strains, as well as multiple bat-derived rabies-related viruses. The neutralization mechanism of RVC20 involves “locking” the RABV-G in the pre-fusion conformation, thereby preventing low-pH-induced conformational changes and subsequent membrane fusion.

In addition to sites II and III, RABV-G harbors antigenic sites I, IV, V, and other letter-designated epitopes, such as “a” or antigenic site G1 located at residues 342–343. Compared to sites II and III, antibodies elicited against these other epitopes generally exhibit lower breadth and protective efficacy.⁸⁶ Moreover, RABV-G contains several

linear epitopes that are independent of higher-order structure, such as G5, which is exclusively recognized by monoclonal antibody 6-15C4. This epitope induces only low levels of neutralizing antibodies but can still confer protection against live-virus challenge.^{92,93}

4.3. Post-translational modifications of RABV-G

Newly synthesized RABV-G is translocated into the ER via its SP, processed through the secretory pathway, and undergoes glycosylation and trimerization in the Golgi apparatus.⁸³ It is then transported to the host cell membrane for incorporation into budding virions.⁹⁴

4.3.1. Disulfidation

Upon entry into the ER, the glycoprotein polypeptide begins to fold and forms disulfide bonds with the assistance of protein disulfide isomerase.^{95,96} This folding process is further facilitated by molecular chaperones, such as calnexin and binding Ig protein.⁹⁷ Disulfide bonds are critical for the structural stability of glycoproteins, facilitating the formation of their functional three-dimensional conformation.

4.3.2. Glycosylation

Subsequent glycosylation modifications depend on the stable conformation. Within the lumen of the ER, glycoproteins undergo N-linked glycosylation catalyzed by glycosyltransferases, which transfer glycans to the asparagine residues of the glycoprotein. N-linked glycosylation is essential for the folding, stability, and

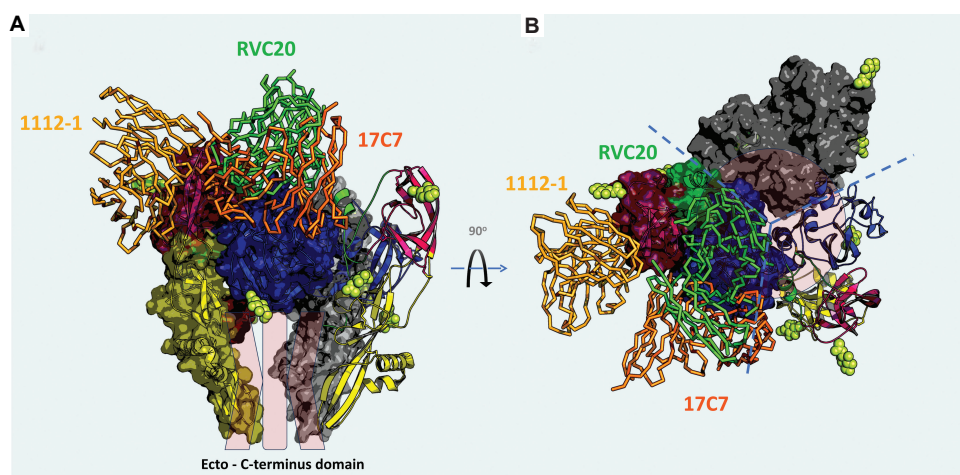


Figure 3. Binding of neutralizing antibodies to the prefusion ectodomain of rabies virus glycoprotein. RVA122, 1112-1, and 17C7 Fab fragments bind quaternary epitopes on the pre-fusion ectodomain. (A) Side view and (B) top view of the molecular model of rabies virus glycoprotein. Two protomers are displayed as surfaces, and the third protomer is shown as a cartoon. Color-coded domain organization of rabies virus glycoprotein: central domain (blue), pleckstrin homology domain (red), fold domain (yellow), and transition hinge domain (green). RVA122 is shown as a green ribbon (PDB: 7U9G), while 1112-1 and 17C7 Fab complex are shown as yellow-orange and salmon ribbons, respectively (PDB: 8A1E). The ecto-C-terminus domain structure (shown in pink) remains unresolved. Image created by the authors using PyMOL.

antigenicity of glycoproteins.^{87,98} For example, the asparagine residue at position 319 (Asn319) on the RABV-G is highly conserved across all RABV strains. Glycosylation at this site is essential for the proper folding of nascent RABV-G and its subsequent transport to the cell surface.^{99,100} The molecular chaperone calnexin recognizes partially processed glycans and binds to them, assisting in the folding of RABV-G to achieve the conformational stability required for its function.

4.3.3. Palmitoylation

Beyond glycosylation, the mature RABV-G contains a conserved cysteine residue (Cys461) located on the cytoplasmic side near the transmembrane region, which undergoes covalent palmitoylation (a type of lipid modification). Palmitoylation is believed to contribute to the stabilization of the trimeric RABV-G spikes on the membrane and may promote interactions between the CT of the RABV-G and the matrix protein, thereby enhancing viral assembly and budding efficiency.¹⁰¹ After glycosylation is completed, RABV-G is transported via vesicular trafficking to the Golgi apparatus, where it is prepared for subsequent trimer assembly.

5. Development of rabies vaccines

5.1. Early rabies vaccine based on tissue culture

The rabies virus was first cultivated in animal neural tissues and developed into an attenuated vaccine. In 1879, Victor Galtier began investigating RABV infection using domestic rabbits as hosts.^{102,103} In 1882, Louis Pasteur isolated a RABV strain from the brain of an infected cow and serially passaged it in the brains of rabbits. By the 50th passage, the virus had acquired a shortened incubation period of 7 days and attenuated virulence, becoming what he termed the “fixed virus.”^{103,104} The spinal cords of infected rabbits, when air-dried at room temperature for approximately 15 days, exhibited complete attenuation and could protect over 50 dogs from intracerebral RABV infection challenge.¹⁰⁵ In 1885, Pasteur administered 13 doses of the desiccated spinal cord preparation to a 9-year-old boy, Joseph Meister, who had been severely bitten by a rabid dog. The treatment successfully prevented the onset of rabies in the child.¹⁰⁵⁻¹⁰⁷ Pasteur’s method of post-exposure prophylaxis gained worldwide acceptance and spurred further improvements in vaccine production.

Although inactivated neural tissue vaccines provided effective protection, their production—such as in the case of the Semple vaccine—involved the use of 5–10% brain tissue suspensions, which contained substantial amounts of CNS material.^{108,109} These vaccines required multiple injections over consecutive days, and repeated exposure

to myelin components in the brain and spinal cord often triggered allergic encephalomyelitis, potentially leading to serious complications, including paralysis, generalized neurological impairment, and even death.¹⁰⁹⁻¹¹¹ In 1955, Fuenzalida and Palacios¹¹² sought to reduce adverse neurological effects by using brains from 3- to 5-day-old suckling mice, based on the observation that myelin content increases with the maturity of the animal. They developed a vaccine using 1% suckling mouse brain suspensions, inactivated either by ultraviolet irradiation or by 1:1,000 phenol.^{112,113} However, subsequent studies revealed that myelin was already present in the brains of 9-day-old suckling mice, and these preparations could still induce allergic encephalomyelitis in guinea pigs.¹¹⁴

Between 1964 and 1969, 40 cases of neurological complications following suckling mouse brain vaccination were reported across eight countries, with an incidence of approximately 1 in 8,000.¹¹⁵ In response, manufacturing processes were further refined: Brains were sourced from mice no older than 1 day, and suspensions were centrifuged at 17,000 × g to reduce myelin content. These improvements enabled the vaccine’s broad adoption in Latin America for a period of time.¹¹⁵

5.2. Embryo vaccines

The development of embryonated egg vaccines originated from the need to replace neural tissue vaccines, which contained highly sensitizing myelin components. In 1931, Goodpasture¹¹⁶ first utilized fertilized chicken eggs as a viral culture medium, laying the foundation for adapting RABV to embryonated eggs. In 1940, the Flury RABV strain was inoculated into 1-day-old chicks, leading to the establishment of a chicken embryo-adapted strain. After 40–50 passages in chicken embryos, the virus was designated in 1948 as the Flury low egg passage (LEP) strain, which exhibited significantly attenuated virulence while retaining immunogenicity. Further serial passaging exceeding 180 passages generated the Flury high egg passage strain, which completely lost neurotoxicity and became a safer candidate for a live attenuated vaccine. However, clinical trials revealed insufficient immunogenicity, and it was ultimately not approved for human use.¹¹⁷

In 1956, Culbertson *et al.*¹¹⁸ developed the duck embryo vaccine by inoculating fixed RABV into the yolk sac of 7-day-old duck embryos, culturing for 12–14 days, preparing a 40% suspension, and inactivating it with β -propiolactone. This vaccine was widely used for human immunization in the United States until the 1980s. Compared to neural tissue vaccines, the duck embryo vaccine substantially reduced the risk of CNS complications, but its immunogenicity was relatively weak,

requiring multiple doses to achieve adequate protection. With the successful development of human diploid cell vaccines (HDCVs), the use of embryonated egg vaccines was eventually discontinued.

5.3. Cell culture vaccines

In 1958, Kissling¹¹⁹ inoculated fixed and wild-type RABV strains into primary hamster kidney (PHK) cells, successfully achieving 15 serial passages of the fixed strain and four passages of the wild strain in a non-neural tissue system for the 1st time, laying the foundation for cell substrate vaccine development. Fenje¹²⁰ serially passaged the Street Alabama Dufferin strain in PHK cells, obtaining high-titer viral suspensions that, after formalin inactivation and immunization of rabbits, induced robust antibody responses and conferred complete protection against natural infection by fox salivary gland virus challenge. In 1963, Kissling and Reese¹²¹ inoculated the adapted challenge virus standard (CVS) strain into PHK cells, successfully producing the first experimental cell culture rabies vaccine (PHKCV). In 1968, PHKCV produced from the CL 60 strain (derived from the Street Alabama Dufferin strain) was approved for use in Canada.¹⁰⁴

Since the late 1950s, WI-38 human diploid cells, developed by Leonard Hayflick, have been shown to support RABV replication without latent viral contamination. The supernatant of the Pitman-Moore L503 3M strain cultured in human embryonic fibroblast MRC-5 cells was concentrated 10- to 20-fold by ultrafiltration or ultracentrifugation and inactivated with β -propiolactone. Animal and human trials demonstrated superior immunogenicity¹²² and fewer adverse effects¹²³—such as local inflammation, lymphadenopathy, headache, and fever—compared to duck embryo and suckling mouse brain vaccines. The vaccine was approved for pre- and post-exposure prophylaxis in Europe in 1976 and in the United States in 1980,¹²⁴ and MRC-5 cell-based vaccines have since been widely adopted.

In the late 1970s, the primary chicken embryo cell vaccine derived from LEP Flury virus underwent extensive animal and human trials, showing immunogenicity comparable to HDCV with good tolerability.¹²⁵ In 1984, this vaccine was marketed as Rabipur® in Germany and has since been widely used in over 60 countries worldwide.¹⁰⁶

During the 1980s, African green monkey kidney cells (Vero cells) were found to efficiently propagate RABV, leading to the development of the purified Vero cell rabies vaccine. Clinical trials involving 106 patients bitten by rabid animals showed that intramuscular injections administered on days 0, 3, 7, 14, 28, and 91, combined with human rabies Ig at 20 IU/kg for 47 severely exposed

patients, resulted in 100% survival at 1 year with negligible side effects. Rabies-neutralizing antibodies appeared by day 14 and persisted for 1 year.¹²⁶ Approved since 1985 in Europe and multiple countries worldwide,¹²⁷ purified Vero cell rabies vaccine is used for both prophylactic and post-exposure immunization, offering immunogenicity comparable to HDCV, with higher production efficiency and lower cost,¹²⁸ and remains widely in use today.

Recombinant viruses expressing the RABV-G, such as vaccinia virus and baculovirus vectors, have been constructed and shown to protect mice from live-virus RABV challenge, confirming the protective effect of RABV-G. However, due to safety concerns regarding viral vectors, no RABV vector vaccines are currently licensed for human use.^{129–131}

6. Progress of mRNA vaccines

Traditional human rabies inactivated vaccines have undergone over a century of research. Various viral strains with different characteristics and cell substrates have been identified, leading to the widespread application of rabies vaccines and a significant reduction in rabies incidence.^{132,133} Unlike the traditional inactivated vaccines, which involve live virus culturing and inactivation procedures, mRNA vaccine manufacturing follows a different process to design and synthesize mRNA.

The journey of mRNA vaccine development has been long and cumulative (Figure 4).^{134–137} The existence of mRNA, an unstable informational intermediate between DNA and protein synthesis, was first demonstrated in 1961 by Brenner *et al.*¹³⁵ through experiments involving bacteriophage infection of bacteria. Lockard and Lingrel¹³⁸ achieved the first *in vitro* mRNA-directed protein synthesis in 1969. A significant hurdle, the rapid degradation of mRNA, was overcome in 1984 when Krieg and Melton¹³⁹ successfully transcribed mRNA *in vitro* using SP6 RNA polymerase. In 1989, Malone *et al.*¹⁴⁰ first demonstrated that mRNA could be delivered into eukaryotic cells via liposomes and successfully expressed proteins intracellularly, following their initial work in 1987 involving cationic lipid-encapsulated mRNA. Martinon *et al.*¹⁴¹ later demonstrated the feasibility of inducing specific cellular immune responses *in vivo* with mRNA vaccines, showing that liposome-encapsulated mRNA encoding influenza virus nucleoprotein induced anti-influenza cytotoxic T lymphocytes in immunized mice.

However, early *in vitro*-synthesized mRNA was readily recognized as a foreign substance *in vivo*, triggering the innate immune system, leading to inflammatory reactions and rapid mRNA degradation,^{142,143} which limited its application in vaccines and therapeutics.

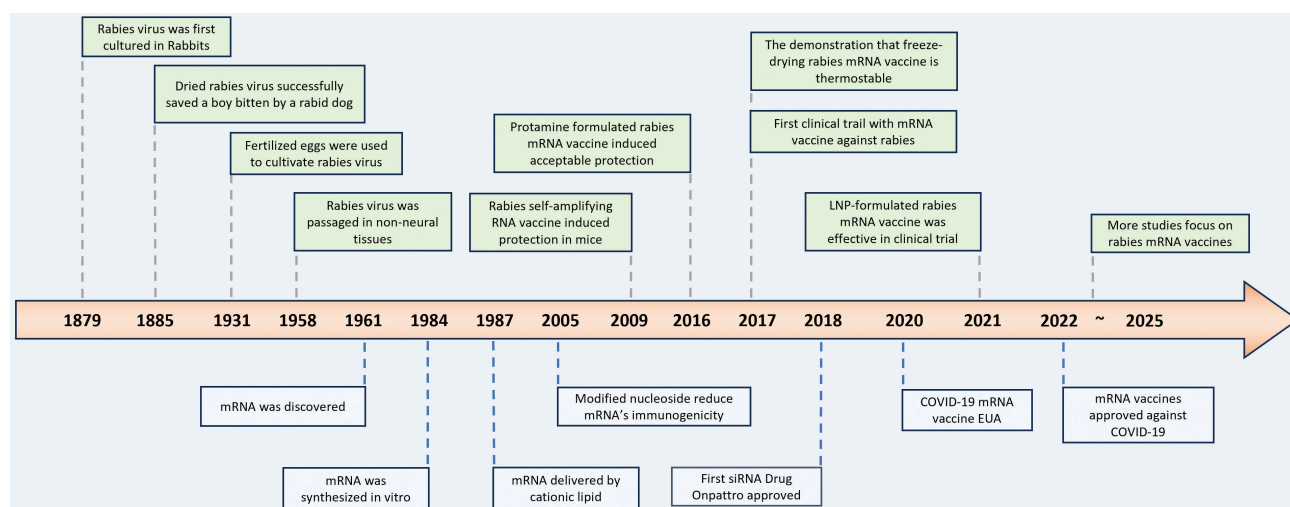


Figure 4. Timeline of key milestones in rabies research and mRNA vaccine development. Green boxes indicate major events related to the rabies virus and rabies vaccines, while blue boxes represent key advances in mRNA vaccine development. Image created by the authors using PowerPoint. Abbreviations: EUA: Emergency Use Authorization; LNP: Lipid nanoparticle; siRNA: Small interfering RNA.

A groundbreaking discovery in 2005 by Karikó *et al.*,¹⁴⁴ showing that replacing uridine with pseudouridine and other modified nucleosides in mRNA significantly reduced its immunogenicity while enhancing stability and protein expression efficiency, laid the foundation for mRNA vaccine development. This paved the way for the Food and Drug Administration approval of Onpatro, the world's first small-interfering RNA drug, in 2018.¹⁴⁵ The ultimate breakthrough came in 2020, with the successful large-scale application of mRNA vaccines for COVID-19 prevention, achieving over 90% clinical and real-world efficacy.¹⁴⁶ This success subsequently spurred widespread research into mRNA vaccines for other infectious diseases, including rabies.

6.1. Generation of mRNA vaccine

A typical mRNA molecule comprises a 5' cap structure, 5' and 3' untranslated regions, an open reading frame, and a poly A tail. The preparation of mRNA vaccines begins with the construction of plasmids encoding the designed antigen gene. The plasmids are linearized by restriction endonucleases and used as templates for *in vitro* transcription. RNA polymerases utilize linear DNA templates to generate raw mRNA, and mRNA capping enzymes add the 5' cap. After purification, the mRNA is dissolved in an aqueous buffer, whereas the lipid components, such as lipid nanoparticles (LNPs), are prepared in an ethanol (organic) phase.¹⁴⁷ Typically, liposomes contain positively charged cationic or ionizable lipids,¹⁴⁸ while the phosphate groups in mRNA carry negative charges. Upon mixing, mRNA and liposomes spontaneously assemble into nanoparticles through

electrostatic interactions.¹⁴⁹⁻¹⁵¹ The mixture is then buffer-exchanged (e.g., by ultrafiltration) into the final formulation buffer and concentrated to the desired strength. The mRNA vaccine production process does not involve live virus or cell culture and allows flexible antigen design to adapt rapidly to evolving epidemic strains. From vaccine design to preliminary animal experiments, the entire process can be completed within a few months.¹⁵²

6.2. mRNA vaccine types

At present, approved and underdeveloped mRNA vaccines can be categorized into three types based on the structure and function of mRNA.

- (i) Non-replicating linear mRNA vaccines consist solely of mRNA sequences encoding the target antigen¹⁵³
- (ii) Circular RNA vaccines consist of RNA molecules with covalently closed circular structures, typically formed through chemical, enzymatic ligation, or splicing events of pre-cursor mRNAs¹⁵⁴⁻¹⁵⁷
- (iii) Self-amplifying RNA vaccines retain, in addition to the antigen-encoding sequence, replicase genes derived from viruses, such as alphaviruses,¹⁵⁸ allowing intracellular self-replication of RNA and substantially amplified antigen expression.^{149,159} This mechanism enables durable immune responses at extremely low doses.¹⁶⁰⁻¹⁶²

7. Development of a rabies mRNA vaccine

In 2009, Saxena *et al.*²⁴ developed Sin-Rab-G, the first self-replicating RNA vaccine encoding RABV-G (CVS strain), utilizing a Sindbis virus RNA replicon. Immunization of mice with 10 µg of Sin-Rab-G was

compared to immunization with a rabies DNA vaccine and a commercial cell culture-derived vaccine (Rabipur®). The self-replicating rabies RNA vaccine provided protective immunity, although it was generally less effective than the inactivated vaccine.

7.1. CureVac trial

In 2016, CureVac developed CV7201, a non-modified mRNA vaccine encoding the glycoprotein of the Pasteur strain, based on their RNActive platform and delivered with cationic peptide protamine.²⁵ This vaccine demonstrated significant protective effects in both mouse and pig models. Moreover, neutralizing antibodies remained stable for up to 1 year. The vaccine also activated both cluster of differentiation (CD)4⁺ and CD8⁺ T cells, with the CD4⁺ T-cell response being significantly superior to that induced by traditional inactivated vaccines, and complete protection was achieved in challenge experiments.

In 2017, CureVac published stability data for a lyophilized rabies mRNA vaccine.²⁶ In contrast to traditional inactivated vaccines (e.g., HDC and Rabipur®), the mRNA vaccine showed no significant decline in protection after high-temperature storage, whereas traditional vaccines exhibited a significant reduction in protection after 4 weeks at 60°C. In 2017, clinical data from 101 participants evaluating the safety and immunogenicity of the first mRNA vaccine, CV7201, were published.¹⁶³ The results indicated that CV7201 caused local injection site reactions in 95% of all participants and systemic reactions in 78% of participants, including 10 severe Grade 3 events. Crucially, the immunogenicity data revealed that neither intradermal nor intramuscular administration via traditional needle syringe, using three doses at 80 µg/dose, 160 µg/dose, or 320 µg/dose, resulted in subjects reaching the WHO protection standard (neutralizing antibodies ≥0.5 IU/mL).

As the first clinical data for a prophylactic mRNA vaccine and compared to other prophylactic mRNA vaccines reported later (e.g., CV7202, mRNA-1273, BNT162b1), CV7201 required relatively higher doses yet still did not demonstrate sufficient efficacy. Given the almost 100% fatality rate of rabies once symptoms appear, further progress on CV7201 has not been reported.

To investigate the impact of delivery systems on vaccine efficacy, an initial study compared the delivery efficiency of luciferase-encoding mRNA into mice via a cationic nanoemulsion (CNE) or LNP. *In vivo* imaging confirmed that LNP significantly enhanced delivery efficiency in mice.¹⁶⁴ Compared to the inactivated vaccine Rabipur®, CV7202 showed a competitive advantage in non-human primates: A single 10 µg dose of the rabies vaccine yielded

antibody titers (4.9 IU/mL) exceeding the full dose of Rabipur® (1.8 IU/mL), and a 100 µg booster dose resulted in titers (842 IU/mL) more than 20-fold higher than Rabipur®.

Clinical studies have shown that two low doses (1 µg or 2 µg) of the mRNA–LNP rabies vaccine (CV7202) induced WHO-compliant neutralizing antibody responses in all subjects (100% achieving ≥0.5 IU/mL).¹⁶⁵ The geometric mean titers were lower than, but not significantly different from, those induced by three doses of the inactivated vaccine Rabipur®. Neutralizing antibody levels were highly correlated with RABV-G-specific IgG titers ($r = 0.8319$, $p < 0.0001$), especially after two doses of CV7202. A single 5 µg dose of CV7202 achieved protective neutralizing antibody levels in only 22% of subjects and was deemed unacceptable due to systemic reactions (e.g., fever, chills) in 90% of participants. The low-dose groups (1 µg/2 µg) demonstrated good tolerability, with primary adverse reactions being mild local pain and transient systemic symptoms (e.g., headache, fatigue). Therefore, two doses of 1 µg or 2 µg of LNP-encapsulated CV7202 effectively induced WHO-compliant neutralizing antibody levels while maintaining low reactogenicity. Overall, CV7202, which utilizes LNP encapsulation, shows a clear advantage over CV7201 formulated with cationic peptide protamine in enhancing immunogenicity and reducing the required dose. However, systemic reactions became unacceptable even at a dose of 5 µg, suggesting lower tolerability compared to mRNA-1273, which is well tolerated at 100 µg.^{166,167} This difference may be attributed to the use of unmodified nucleotides in CV7202.

Beyond these findings, mRNA vaccines offer a more robust and balanced immune response compared to the inactivated Rabipur vaccine. Two doses of mRNA vaccine significantly increased RABV-G-specific plasmablasts, bone marrow plasma cells, and memory B cells, and activated a T helper 1 (Th1)-biased CD4⁺ T-cell response, a response not detected with Rabipur®.²³ Nevertheless, the mRNA vaccine exhibited broader cross-neutralization capabilities against lyssaviruses, such as European bat lyssavirus 1 and Duvenhage virus, likely due to its higher antibody titers.

7.2. GSK trial

In 2020, GSK reported on the non-clinical safety of an alphavirus-based self-amplifying RNA vaccine encoding the RABV-G of the Flury-LEP strain, delivered using CNE.²⁷ Following repeat intramuscular injections, the vaccine induced RABV IgG antibodies and elicited controlled local and systemic inflammatory responses. Biodistribution studies showed that the vaccine persisted at the injection

site and in draining lymph nodes for up to 60 days and was not detected in the CNS. Transient elevations of alanine aminotransferase and aspartate aminotransferase, observed in female rats and not accompanied by liver damage, may be related to sex-specific RNA distribution. Overall, the vaccine was well tolerated.

In a subsequent study evaluating the impact of different delivery systems on vaccine safety, it was found that both LNP-delivered (self-amplifying mRNA [SAM]-LNP) and CNE-delivered (SAM-CNE) rabies SAM vaccines induced transient increases in body temperature (more pronounced in females), local injection site inflammation (muscle/subcutaneous lymphocyte infiltration), and swelling of draining lymph node, but no systemic toxicity.²⁸ Overall, both CNE and LNP delivery systems demonstrated favorable safety profiles, supporting the further development of rabies SAM vaccines.

7.3. Vaccine efficacies in recent pre-clinical studies

Driven by the widespread success of COVID-19 mRNA vaccines, extensive research on rabies mRNA vaccines has been conducted in recent years. In 2022, Liverna company reported excellent protective efficacy in mice and dogs with LVRNA001, an LNP-delivered mRNA vaccine encoding the RABV-G of the CTN-1 strain.²⁰ This LNP utilized the ionizable cationic lipid Dlin-MC3-DMA, a key component of the marketed small interfering RNA drug Onpatro.¹⁶⁸ LVRNA001 induced protective levels of neutralizing antibodies in mice, and challenge experiments demonstrated 100% protection after two doses. Furthermore, the vaccine activated a strong Th1-biased cellular immune response, while the Th2 cytokine interleukin (IL)-4 showed no significant differences.¹⁶⁹⁻¹⁷² In dog experiments, post-exposure immunization with two 25 µg doses of the mRNA vaccine achieved 100% survival, compared to only 33.3% in the group receiving the traditional five-dose inactivated vaccine regimen.

Subsequent research found that a single 5 µg immunization with LVRNA001 in mice resulted in negative serum neutralizing antibodies on day 3, but reached protective levels by day 5, with antibody levels significantly higher than those induced by inactivated vaccines.³⁴ LVRNA001 provided 100% cross-protection against seven China I–VII clades of RABVs (e.g., SC16, GD1) in pre-exposure immunization in mice. SYS6008, developed by CSPC Pharmaceutical Group, encoding RABV-G of the CTN-1 strain, showed similar post-exposure protection against the same seven China I–VII clades of RABVs.²⁹ This might be attributed to the 87.6–97.7% homology of the CTN-1 strain in China.¹⁷³ LVRNA001 received clinical approval from China's Center for Drug Evaluation (CDE) in 2023 (CDE application ID: CXSL2300407).

In 2023, Wan *et al.*³³ and Stemirna Therapeutics developed lipopolyplex (LPP)-mRNA-G, an mRNA vaccine encoding the RABV-G of the Flury-LEP strain, delivered via core-shell structured LPP nanoparticles.^{33,174} The unique double-layered structure of the LPP enhanced mRNA stability and enabled targeted expression of RABV-G primarily in muscle tissue and lymph nodes after intramuscular injection in mice.¹⁷⁴⁻¹⁷⁶ A single 1 µg/dose of LPP-mRNA-G induced protective levels of virus-neutralizing antibodies in mice and provided 100% protection against lethal challenge, outperforming the 50% protection achieved by an inactivated vaccine. In dog studies, a single 2 µg injection induced seroconversion in all animals within 3 weeks, with antibody levels maintained for at least over 6 weeks. LPP-mRNA-G induced the Th2-biased humoral response by promoting T follicular helper and germinal center B cell activation, as well as sustained generation of antigen-specific antibody-secreting cells.

Other studies confirm the high efficacy of rabies mRNA vaccines. Bai *et al.*³⁰ demonstrated that a single 3 µg dose of an mRNA vaccine encoding the RABV-G of the Pitman-Moore strain induced neutralizing antibody levels of 20.3 IU/mL in mice, significantly outperforming the 4.07 IU/mL achieved with three doses of inactivated vaccine. Neutralizing antibody levels remained above 10 IU/mL for 25 weeks after a single dose, whereas the inactivated vaccine had dropped to 1.3 IU/mL during the same period. Long *et al.*³² showed that a single 50 µg dose of an mRNA vaccine immunized dogs, achieving 100% seroconversion within 2 days, with a geometric mean titers of 5.19 IU/mL. This further increased to 23.78 IU/mL by day 14, compared to 8.59 IU/mL for the corresponding inactivated vaccine.³² In 2023, Li *et al.*²¹ demonstrated that two intramuscular injections of the rabies mRNA vaccine RV021 protected mice against RABV CVS challenge with a median effective dose as low as 0.032 µg/dose. This study also, for the 1st time, used the National Institutes of Health method to show that a 15 µg/dose mRNA vaccine could achieve 7.5 IU/dose, meeting the Chinese Pharmacopeia's release standard for rabies vaccine potency (>4.0 IU/dose). Unfortunately, no studies on the post-exposure protective effect of this vaccine were conducted.

7.4. Strategies to improve the rabies virus vaccine

7.4.1. Sequence optimization

Wang *et al.*³⁷ used a linear design algorithm to optimize an mRNA vaccine encoding the CTN-1 strain RABV-G, demonstrating significantly superior immune effects compared to inactivated vaccines in rhesus macaques.²² Their optimization strategy integrated minimum free energy and the codon adaptation index to enhance mRNA vaccine stability and translation efficiency. Following a

single 30 µg dose in rhesus macaques, the vaccine induced a peak neutralizing antibody of 385.8 IU/mL, which remained at 94.5 IU/mL after 27 weeks (compared with only 6.5 IU/mL in the inactivated vaccine group at the same time point), demonstrating durable immune memory. The vaccine activated a Th1-biased cellular immune pathway, significantly increasing the proportion of RABV-G-specific interferon- γ^+ CD4 $^+$ and CD8 $^+$ T cells, as well as inducing a three-fold increase in circulating IgG $^+$ memory B cells. The study also observed a transient elevation of IL-6, which may contribute to enhanced long-term antibody production by promoting germinal center reactions.¹⁷⁷⁻¹⁷⁹ Safety assessments showed good tolerability, with all biochemical and hematological indicators fluctuating within normal ranges.

7.4.2. Adjuvant

In 2023, Hongtu *et al.*³¹ co-encapsulated CpG oligodeoxynucleotides and CTN strain RABV-G-encoding mRNA within LNPs as an aqueous phase. CpG oligodeoxynucleotides contain unmethylated CpG motifs that are recognized by Toll-like receptor 9 and induce Th1 immune responses and cytokine production.^{180,181} Compared to LNP+G alone, the LNP+1018+G formulation significantly enhanced both CD4 $^+$ and CD8 $^+$ T cell immune responses and neutralizing antibody levels.³¹ Wan *et al.*³⁸ developed mannose-modified LNPs encapsulating RABV-G circular RNA, which enhanced lymph node targeting, reduced liver accumulation, and prolonged antigen retention. Compared to linear mRNA, the circular RNA vaccine induced more antibody-secreting and long-lived plasma cells, resulting in higher neutralizing antibody titers. Li *et al.*⁴⁰ investigated muscle-targeting LNPs and reported that, compared to traditional liver-targeting LNPs, they generated stronger humoral immunity and exhibited higher post-exposure protection efficacy.

7.4.3. Stabilization mutations

While mRNA vaccines encoding wild-type RABV-G have demonstrated efficacy, antigen structure optimization holds promise for further enhancing immunogenicity. For instance, His270, located within an extended α -helix in the monomeric RABV-G ectodomain crystal structure, has been shown to stabilize the pre-fusion conformation of RABV-G through an H270P mutation.⁷¹ In 2024, Cao *et al.*³⁵ introduced the H270P mutation into a full-length RABV-G encoding mRNA vaccine, designated LNP-mRNA-G-H270P. This construct induced significantly higher neutralizing antibody levels and stronger cellular immune responses, characterized by elevated interferon- γ and IL-2 expression, compared to the wild-type glycoprotein mRNA vaccine.

In 2024, Liu *et al.*³⁶ introduced H261P and H270P mutations into full-length RABV-G to stabilize its pre-fusion conformation. They also appended an MTQ motif to the C-terminus of truncated RABV-G to obtain a soluble trimeric form and constructed virus-like particles (VLPs) by co-expressing RABV-G with matrix protein or with matrix protein and nucleoprotein. These VLPs formed 80–200 nm particles and significantly enhanced early neutralizing antibody responses compared to the wild-type glycoprotein, with the VLP-nucleoprotein group inducing titers 2.2 times higher than the pre-glycoprotein group at day 7. However, full-length glycoprotein ultimately elicited slightly higher antibody levels than VLPs and truncated RABV-G at later time points.

In 2025, Li *et al.*³⁹ reaffirmed that truncated RABV-G provides weaker protection, while the R333Q mutation modestly enhanced immune responses. They also explored a heterologous prime-boost strategy combining inactivated and mRNA vaccines, with an “inactivated RABV prime, mRNA boost” regimen showing strong potential. This effect may be due to the complementary activation of immune pathways: Inactivated vaccines mainly stimulate humoral responses via major histocompatibility complex class II, whereas mRNA vaccines also engage major histocompatibility complex class I pathways to promote T cell activation.

7.4.4. Cytotoxic T lymphocytes

Bai *et al.*⁴¹ identified conserved T cell epitopes across multiple RABV strains to design an mRNA vaccine (RABV-LT) that elicited strong CD4 $^+$ and CD8 $^+$ T-cell responses but did not achieve full protection. They then developed a dual-antigen mRNA vaccine (RABV-G-LT) by combining RABV-G and RABV-LT, which provided 100% protection in mice, outperforming the inactivated vaccine. Although RABV-G-LT induced lower antibody levels than RABV-G alone, it showed better viral clearance, emphasizing the critical role of T cell immunity in protection against rabies.

8. Discussion

In this review, we summarized the present understanding of RABV-G, highlighting its structural characteristics, immunogenic properties, and implications for vaccine design. Despite more than 140 years since the inactivated rabies vaccine was first used to save patients, the use of RABV-G as the key antigen for the development of rabies mRNA vaccines did not achieve significant progress until 2020. As previously mentioned, a self-replicating RNA vaccine encoding the CVS strain glycoprotein was reported as early as 2009. While it induced humoral and cellular immune responses in mice, its protective effect

was inferior to that of inactivated vaccines.²⁴ CureVac's early rabies mRNA vaccine, CV7201, based on their proprietary RActive platform, used cationic peptide protamine to deliver unmodified RNA and demonstrated protective effects in mice and pigs. However, a Phase I clinical trial indicated that intramuscular injection of CV7201 was largely ineffective, even at doses up to 640 µg/dose (approximately 21 times higher than the dose used for the COVID-19 mRNA vaccine BNT162b2),¹⁸² and showed a relatively high incidence of adverse reactions. The subsequent CV7202, which employed LNP delivery of unmodified mRNA, was able to induce neutralizing antibody levels comparable to those induced by inactivated vaccines at a dose as low as 1 µg in its clinical trial, validating the feasibility of mRNA vaccines for rabies prevention.¹⁶⁵

Since 2022, research on rabies mRNA vaccines has been extensively reported, with most vaccine candidates encoding RABV-G. This is primarily attributed to the glycoprotein being the sole spike on the RABV surface and the only glycoprotein capable of inducing neutralizing antibodies. Nevertheless, the RABV-nucleoprotein also possesses four antigenic sites capable of inducing specific antibodies.¹⁸³ Co-encapsulating mRNA encoding both RABV-G and RABV-nucleoprotein has been shown to generate higher antibody titers compared to that induced by RABV-G alone.³⁶ Recent studies also indicate that the RABV-large structural protein contains antigenic epitopes capable of inducing strong CD8⁺ T-cell immune responses, providing modest protection against lethal challenge.⁴¹ Undoubtedly, these recent research results combining virology, structural biology, and immunology will accelerate the development of a modern rabies vaccine in the near future.

RABV-G is critical for viral entry, pathogenesis, and immunity, yet significant knowledge gaps still persist despite extensive research. First, RABV-G undergoes pH-dependent structural shifts during membrane fusion. The exact residues coordinating these transitions and the mechanism by which the pre-fusion conformation is stabilized are not fully explored. Second, compared to attenuated vaccine strains, the genetic and structural factors that confer high pathogenicity to street strains—particularly how they selectively engage receptors for neuroinvasion versus immune evasion—remain unclear. Third, the mechanism by which RABV-G binds to multiple receptors (e.g., neural cell adhesion molecule, p75^{NTR}) is poorly characterized. Finally, key practical challenges include the need to extend the long-term efficacy (>1 year) of present vaccines, establish potential lifelong protection in humans, and improve cross-protection against emerging lyssavirus variants.

Taken together, rabies vaccines are evolving from traditional inactivated platforms toward next-generation mRNA and other more effective strategies, which have the potential to provide enhanced durability and broad protection. However, mRNA vaccines still face several challenges, including strict cold-chain storage requirements, dose-related tolerability issues, and uncertainties regarding long-term protection in humans. Early clinical trials, which reported frequent systemic reactions, underscore that tolerability remains a key barrier to public acceptance.

Future breakthroughs will likely hinge on advances in three key areas: (i) Novel delivery platforms that enhance efficacy and safety; (ii) rational antigen design, including pre-fusion-stabilized RABV-G or soluble trimers; and (iii) the development of cost-effective, thermostable formulations. Success in these areas is essential for ensuring global vaccine accessibility and for realizing the ambitious “Zero by 30” goal of eliminating human rabies deaths.

9. Conclusion

The RABV-G remains the primary target for neutralizing antibodies and the core of modern vaccine design. As summarized in this review, the field is shifting from traditional inactivated vaccines to mRNA platforms, which offer rapid adaptability. However, clinical data suggest that advanced delivery systems alone are not sufficient; success requires precise engineering of the antigen itself.

Future breakthroughs will depend on integrating structural biology with immunology. Specifically, rational design strategies that stabilize RABV-G in its pre-fusion conformation are critical for maximizing immunogenicity. Furthermore, multi-antigen approaches, including the nucleoprotein or large protein, could enhance T-cell responses and durability. Addressing practical challenges, such as thermostability and cost, is also vital for global access. Collectively, these scientific advances will accelerate the development of a highly effective, single-dose vaccine, which is essential to realize the WHO's “Zero by 30” goal of eliminating human rabies.

Acknowledgments

None.

Funding

This work was supported by the Institute of Health and Medicine, Hefei Comprehensive National Science Center Startup funds (grant no.: 2023KYQD011).

Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data used in this work are available from the corresponding author, Xueyao Jin (jinxxy@ihm.ac.cn) upon reasonable request.

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