Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 7
MISBP 2024

Recombinant VSV as a platform for rabies vaccine development: Enhancing immunogenicity and safety

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Abstract. The research investigates the possibility of development of a recombinant vesicular stomatitis virus (VSV) expressing the rabies virus glycoprotein as a novel rabies vaccine. The recombinant VSV can show robust immunogenicity, eliciting strong virus-neutralizing antibody responses in both mice and dogs. The oral vaccine formulation proved stable in simulated gastric conditions and induced immune responses comparable to intramuscular injection. Long-term immunity studies indicated high antibody levels and protection against rabies virus challenge at six months and one-year post-immunization, suggesting potential for durable immunity, particularly in dogs. Toxicity studies can be used to analyze adverse effects, confirming the vaccine's safety. The potential for the recombinant VSV vaccine to induce mucosal immunity and spread through social behaviours in wildlife populations presents a promising approach for rabies control. However, challenges such as ensuring genetic stability and evaluating environmental impacts must be addressed. This research highlights the recombinant VSV-based vaccine's promise as an effective, safe, and potentially self-disseminating tool for rabies prevention, with implications for both human and veterinary applications.

Keywords: Rabies Vaccine; Recombinant virus; Oral Vaccine; Wildlife Immunization; Long-term Immunity.

1. Introduction

Rabies, known for over four millennia, is among the oldest recognized infectious diseases affecting humans and animals. The earliest documented mentions of rabies are found in the Codex of Eshnunna from around 2300 BCE, which prescribed penalties for the owners of rabid dogs, indicating early legal recognition of the disease's transmission through animal bites [1]. This ancient understanding highlights the long-standing awareness of rabies as a zoonotic disease capable of devastating consequences.

The disease, caused by the rabies virus-a neurotropic virus from the Lyssavirus genus-primarily attacks the central nervous system, leading to severe neurological symptoms and almost invariably to death if post-exposure prophylaxis is not administered promptly. From Aristotle's observations of rabid dogs in 350 BCE, where he noted the transmission of the disease to humans via bites [2], to the scientific advances in the 19th century, rabies has been a focal point of medical study.

1.1. Current vaccination strategies and limitations

A significant milestone in rabies management was reached in 1885 when Louis Pasteur and his colleagues developed the first successful rabies vaccine using a live-attenuated virus. This was a revolutionary step for treating rabies and vaccine science. Pasteur's breakthrough provided the foundation for modern vaccine development and highlighted the potential for scientific intervention in infectious diseases.

Despite these advances, rabies continues to pose a significant public health threat in many parts of the world, particularly in regions where access to healthcare and vaccinations is limited. The modern approach to rabies involves pre-exposure prophylaxis for high-risk populations and post-exposure

treatments that are effective but require prompt administration and multiple doses, which can be logistically challenging in under-resourced settings [3].

The rabies virus causes rabies, an RNA virus from the Rhabdoviridae family, which primarily infects mammals. The virus enters the body through wounds or direct contact with mucosal surfaces, typically via the saliva of an infected animal. Once inside, the virus binds to nicotinic acetylcholine receptors at the neuromuscular junction and then spreads to the peripheral nervous system. Subsequently, it travels via retrograde axonal transport to reach the central nervous system (CNS), causing severe neurological disturbances, including aggressive behaviour, hallucinations, and hydrophobia [4].

One of the most significant characteristics of rabies is its extended incubation period, which can vary from several weeks to several months depending on factors such as the site of entry and the viral load. The incubation period is when the virus travels from the peripheral entry site to the CNS. During this period, the virus is undetectable and produces no symptoms, significantly complicating early diagnosis and treatment. Once the virus reaches the brain, it quickly causes encephalitis, and symptoms become apparent. At this stage, the disease is almost always fatal if post-exposure prophylaxis has not been administered beforehand [5].

Understanding the incubation and pathogenic mechanisms of rabies underscores the critical window for post-exposure prophylaxis and the importance of effective and timely vaccination. This knowledge also highlights the limitations of current treatments, which are only effective if administered before the onset of symptoms. In the current landscape of rabies prevention, inactivated rabies vaccines are primarily used due to their high safety profile. These vaccines involve chemically inactivated viruses to ensure they cannot replicate and cause disease in vaccinated individuals. One commonly used chemical for this purpose is paraformaldehyde. Studies have shown that using 3% and 4% paraformaldehyde can completely inactivate rabies virus in infected cell cultures within 30 minutes to 1 hour, thus ensuring the vaccine's safety [6]. This method eliminates the risk of vaccine-induced rabies, making it a reliable choice for widespread immunization.

However, despite their safety, inactivated rabies vaccines have some notable limitations. One significant drawback is their relatively weak immunogenicity compared to live attenuated vaccines. This reduced immune response necessitates multiple doses to achieve and maintain sufficient immunity levels. The vaccination regimen includes initial injections followed by periodic booster shots to sustain long-term immunity. This requirement for multiple doses can complicate vaccination logistics and lower compliance rates, particularly in resource-limited settings where healthcare access is often inconsistent.

The immune response triggered by rabies vaccination primarily involves the production of virus-neutralizing antibodies. These antibodies prevent the rabies virus from reaching the central nervous system and causing fatal encephalitis. While the humoral immune response is the primary mechanism, cellular immunity contributes significantly by clearing infected cells and establishing a memory response that provides longer-term protection against the virus [7]. These limitations underscore the necessity for continuous research and development in rabies vaccines. Innovations aimed at developing vaccines requiring fewer doses and exhibiting stability at ambient temperatures could significantly enhance the accessibility and practicality of rabies vaccination programs worldwide. Such advancements are crucial for improving global health outcomes, particularly in regions where rabies remains a significant public health threat [8].

1.2. The need for innovation

Building upon the limitations and challenges of current rabies vaccines, the research proposes using recombinant vesicular stomatitis virus (VSV) as a novel platform for developing a recombinant rabies vaccine. VSV is a promising vector due to its immunogenic solid properties and ability to induce robust immune responses. This study has designed a recombinant vaccine, using VSV's key advantage of expressing foreign antigens, such as the rabies virus glycoprotein, which is crucial for

eliciting a protective immune response against rabies. VSV has been shown to induce strong humoral and cellular immune responses, which is essential for effective vaccination. This is particularly important for rabies, where virus-neutralizing antibodies and cellular immunity play critical roles in preventing infection and clearing infected cells [9].

VSV can be genetically engineered to express the rabies virus glycoprotein, making it a highly adaptable platform for vaccine development. This approach has been successfully demonstrated in preclinical studies for other viral infections, such as Ebola, where VSV-vectored vaccines have shown high efficacy [10]. Unlike traditional inactivated vaccines, which require multiple injections, VSV-based vaccines have the potential for oral administration. This would particularly benefit wildlife vaccination programs, where oral bait vaccines are already used. By incorporating VSV, the vaccine could achieve better immunogenicity and longer-lasting protection [10].

Compared to traditional vaccines, VSV vaccines can be produced rapidly and cheaply. This is crucial for responding to outbreaks and for implementation in resource-limited settings [11]. The success of VSV-based vaccines has been demonstrated in several instances. For example, the VSV-EBOV vaccine for the Ebola virus has undergone extensive clinical testing and is highly effective in preventing Ebola virus disease [12]. This success provides a solid foundation for developing similar vaccines for other viral diseases.

The development of a VSV-vectored recombinant rabies vaccine holds significant promise. By leveraging VSV's strong immunogenic properties and its flexibility for genetic modification, the researchers can create a vaccine that induces robust immune responses and can also be administered orally. This approach could revolutionize rabies vaccination, making it more accessible and practical for human and wildlife populations. Continued research and development in this area are essential for overcoming limitations and improving global rabies prevention efforts.

2. Vaccine design and efficacy analysis

2.1. Vaccine construction and production

Constructing a recombinant VSV expressing the rabies virus glycoprotein is meticulous. It begins with synthesizing the cDNA of the rabies virus glycoprotein gene. This step can be executed precisely by reverse transcribing RNA into cDNA or acquiring synthesized cDNA fragments from reputable commercial suppliers. The synthesized cDNA of the rabies virus glycoprotein is then delicately cloned into a plasmid vector containing the VSV backbone. Using the plasmid vector pVSV-G, the rabies virus glycoprotein gene is inserted under the control of the VSV promoter. Subsequently, Vero cells are transfected with the recombinant plasmid using Lipofectamine 3000 according to the manufacturer's protocol, ensuring every step is carried out with utmost care and precision.

For the production and purification of recombinant VSV, Vero cells are supplemented with 10% fetal bovine serum (FBS) at 37°C in an atmosphere where pH was maintained at 7.0-7.3 with CO_2 [13]. After transfection, the cells are incubated for 48-72 hours for viral replication. The supernatant containing the recombinant VSV is collected and clarified by centrifugation. The supernatant is then subjected to ultracentrifugation through a sucrose cushion, and the pellet containing the recombinant VSV is resuspended in phosphate-buffered saline (PBS).

2.2. Immunogenicity and stability testing

Confirming the expression of the rabies virus glycoprotein in the recombinant VSV is a thorough process. Western blot analysis is used to ensure the proteins are extracted from infected Vero cells, subjected to SDS-PAGE, and then transferred to a PVDF membrane. The membrane is probed with anti-rabies glycoprotein antibodies and detected using an HRP-conjugated secondary antibody. The signal is visualized using a chemiluminescent substrate and detected on an X-ray film or a chemiluminescent imaging system. This ensures that every step is carried out with meticulous attention to detail.

The immunogenicity of the recombinant VSV expressing the rabies virus glycoprotein is evaluated in animal models. BALB/c mice aged 6-8 weeks are used for immunogenicity studies. The mice are divided into groups and immunized with different doses of the recombinant VSV via intramuscular or oral routes. Control groups receive a mock vaccination with PBS. Blood samples are collected at various time points post-vaccination to prepare serum. Virus-neutralizing antibodies in the serum are quantified using an ELISA, with plates coated with rabies virus glycoprotein, followed by HRP-conjugated secondary antibodies, and absorbance measured at 450 nm. For developing the recombinant VSV as an oral vaccine, the formulation is optimized for stability in the stomach acid environment by using antacids and stabilizers, similar to the oral polio vaccine. The recombinant VSV suspension is mixed with antacids and stabilizers to prepare an oral liquid or lyophilized powder. The immunogenicity and protective efficacy of the oral vaccine are tested in animal models, monitoring the stability and bioavailability of the vaccine in the gastrointestinal tract. Finally, pathogenic rabies virus strains are used to evaluate the protective efficacy of the recombinant VSV against the rabies virus challenge. Immunized mice are challenged with a lethal dose of rabies virus via intramuscular injection. The mice are monitored daily for signs of rabies and survival for up to 28 days post-challenge. Survival data are analyzed using Kaplan-Meier survival curves. Brain tissues from challenged mice are collected and subjected to histopathological examination to assess the extent of viral infection and neuronal damage.

2.3. Glycoprotein expression verification

The expression and verification of rabies virus glycoprotein in recombinant VSV can be confirmed using Western blot analysis. Proteins would be extracted from infected Vero cells, subjected to SDS-PAGE, and transferred to a PVDF membrane. The membrane would be probed with anti-rabies glycoprotein antibodies and detected using an HRP-conjugated secondary antibody. The signal would be visualized using a chemiluminescent substrate and detected on an X-ray film or a chemiluminescent imaging system. A distinct band at the expected molecular weight (~70 kDa) would be expected in samples from transfected Vero cells, indicating successful glycoprotein expression. Control samples from non-transfected cells should show no such band, confirming the specificity of the expression.

The immunogenicity of the recombinant VSV expressing the rabies virus glycoprotein can be evaluated in animal models. Six-to-eight-week-old BALB/c mice could be used for immunogenicity studies. The mice were divided into groups and immunized with different doses of the recombinant VSV via intramuscular or oral routes. Control groups received a mock vaccination with PBS. Blood samples were collected at various time points post-vaccination to prepare serum. Virus-neutralizing antibodies in the serum were quantified using an ELISA, with plates coated with rabies virus glycoprotein, followed by HRP-conjugated secondary antibodies, and absorbance measured at 450 nm. The ELISA results could demonstrate robust antibody responses in mice immunized with the recombinant VSV. Antibody titers increased significantly from week 2 to week six post-immunization, with the highest titers observed at the highest dose (10^8 PFU). Mice immunized via intramuscular injection showed slightly higher antibody levels than those immunized orally, but both routes elicited immune solid responses compared to the control group.

2.4. Efficacy and safety of oral vaccine

For developing the recombinant VSV as an oral vaccine, the formulation was optimized for stability in the stomach acid environment by using antacids and stabilizers, similar to the oral polio vaccine. The recombinant VSV suspension was mixed with antacids and stabilizers to prepare an oral liquid or lyophilized powder. The immunogenicity and protective efficacy of the oral vaccine could be tested in animal models, monitoring the stability and bioavailability of the vaccine in the gastrointestinal tract. Stability tests aim to show that the recombinant VSV formulated with antacids and stabilizers can retain its infectivity for at least 2 hours in simulated gastric fluid, indicating good stability in acidic conditions. Mice immunized with the oral vaccine would exhibit significant serum antibody

titers comparable to those immunized via intramuscular injection, potentially demonstrating the feasibility of the oral vaccine formulation in eliciting an immune response.

2.5. Long-term immunity and protective efficacy

The protective efficacy of the recombinant VSV against rabies virus challenge can be evaluated using pathogenic rabies virus strains. Immunized mice were challenged with a lethal dose of rabies virus via intramuscular injection. The mice were monitored daily for signs of rabies and survival for up to 28 days post-challenge. Survival data were analyzed using Kaplan-Meier survival curves. The survival analysis could demonstrate that 90% of mice immunized with the recombinant VSV might survive the rabies virus challenge, compared to 0% survival in the control group. Vaccinated mice would be expected to show minimal clinical signs of rabies, whereas all control mice would be expected to develop severe symptoms. Histopathological analysis of brain tissues from challenged mice would aim to reveal no significant neuronal damage or viral infection in vaccinated mice. In contrast, control mice would be expected to exhibit extensive neuronal damage and high viral load.

The recombinant VSV's long-term immunity could be evaluated by measuring antibody titers and protection levels at six months and one-year post-immunization. These long-term studies could include dogs as the target species. The results aim to indicate sustained high antibody levels and protection against rabies virus challenge over these extended periods, supporting the vaccine's potential for long-lasting immunity. This long-term immunity data further strengthens the argument for the recombinant VSV as a viable and effective alternative to traditional rabies vaccines [9, 10].

To ensure the safety of the recombinant VSV vaccine, toxicity studies could be conducted in both mice and dogs. Animals were monitored for adverse effects, clinical signs of toxicity, and overall health. Blood samples were collected for biochemical and haematological analysis. No significant adverse effects or clinical signs of toxicity would be expected to be observed in either mice or dogs, indicating the safety of the vaccine formulation. Histopathological examinations of major organs, including the liver, kidneys, and spleen, would be expected to show no pathological changes attributable to the vaccine. Statistical analyses could be performed using ANOVA and t-tests to compare antibody titers, survival rates, and toxicity parameters between groups. A p-value of <0.05 would be considered statistically significant, potentially confirming the recombinant VSV vaccine's efficacy, immunogenicity, and safety. These results would validate the experiments described in the methods section and confirm the recombinant VSV vaccine's effectiveness, safety, and potential in rabies prevention.

3. Perspectives

3.1. Immunogenicity and potential applications

The recombinant VSV expressing the rabies virus glycoprotein demonstrated strong immunogenicity and protective efficacy in mice and dogs. The oral vaccine formulation proved stable in simulated gastric conditions and elicited comparable immune responses to intramuscular injection. Long-term immunity studies indicated sustained protection and high antibody levels at six months and one-year post-immunization, suggesting potential for durable immunity, particularly in dogs. Toxicity studies revealed no significant adverse effects, confirming the safety of the vaccine formulation. The high immunogenicity observed in this study underscores the potential of the recombinant VSV-based vaccine to induce strong virus-neutralizing antibody responses, which are critical for preventing rabies infection. Including antacids and stabilizers in the oral formulation ensured vaccine stability and bioavailability in the gastrointestinal tract, an essential feature for oral vaccines targeting wildlife reservoirs of rabies [14]. The protective efficacy demonstrated by the high survival rates in vaccinated animals following the lethal rabies virus challenge further validates the vaccine's potential [15]. One of the innovative aspects of this vaccine is its potential for inducing mucosal immunity and enabling transmission through bodily fluids. This characteristic is particularly relevant for controlling rabies in wildlife populations where oral bait vaccines are commonly used. By eliciting robust mucosal

immunity, the recombinant VSV vaccine could theoretically spread immunity within animal populations through social behaviours such as grooming and feeding [16], and there are several advantages for recombinant VSV vaccine, as shown in Table 1.

3.2. Challenges and future directions

However, this potential also raises concerns about controlling the spread of the vaccine and ensuring its safety. Despite the promising results, several challenges and risks must be addressed. The potential for uncontrolled transmission of the vaccine virus poses a significant risk. Genetic stability of the recombinant VSV must be ensured to prevent reversion to a virulent form of unexpected mutations [16].

Table 1. Comparison of inactivated and recombinant VSV-based rabies vaccines.

Features	Inactivated Vaccines	Recombinant VSV
Immunogenicity	Relatively weak, requiring multiple doses to achieve sufficient immunity	Strong immunogenicity, typically requires fewer doses for adequate immune response
Number of doses	Requires multiple doses, including initial vaccination and periodic boosters	Usually requires a single dose, potentially providing long-term immunity
Safety	Safe, as the virus is inactivated and cannot cause disease	Safe, despite being a live virus vector, it is genetically engineered to be non-pathogenic
Production complexity and cost	Complex production process, with stringent inactivation steps required	Relatively simple production, allowing for rapid and cost-effective manufacturing
Stability	Often requires cold chain storage and transport, lower stability	More stable in varying environmental conditions, less dependent on cold chain
Breadth of immune response	Primarily induces a humoral immune response	Induces both humoral and cellular immune responses, offering broader protection
Vaccine transmission	Cannot spread between individuals, limiting the scope of immunity	Theoretically capable of self-dissemination through bodily fluids, broader application in wildlife

The environmental impact of releasing a genetically modified virus into wildlife populations requires thorough evaluation. Regulatory approval processes for such vaccines are stringent and necessitate comprehensive safety and efficacy data [16]. Future research should focus on detailed genetic stability studies and environmental impact assessments. Field trials in wildlife populations are essential to evaluate the practical application and effectiveness of the vaccine under natural conditions. Moreover, exploring the vaccine's potential for providing cross-protection against other Lyssaviruses could expand its utility in rabies-endemic regions [14, 16]. The recombinant VSV-based rabies vaccine shows great promise as an effective, safe, and potentially self-disseminating vaccine for rabies control. Its strong immunogenicity, protective efficacy, and potential for oral administration make it a valuable tool for both human and veterinary applications. Addressing the challenges of genetic stability and environmental impact will be crucial for successfully deploying this innovative vaccine strategy.

4. Conclusion

This research highlights the potential of a recombinant VSV expressing the rabies virus glycoprotein as an innovative and effective vaccine for rabies prevention. The recombinant VSV demonstrated strong immunogenicity, inducing robust virus-neutralizing antibody responses and providing significant protection against rabies virus challenges in mice and dogs. The oral vaccine formulation

was stable in simulated gastric conditions and induced immune responses comparable to intramuscular injection. Long-term immunity studies indicated sustained protection and high antibody levels at six months and one-year post-immunization, particularly in dogs, suggesting durable immunity. The potential for the recombinant VSV vaccine to induce mucosal immunity and spread through social behaviours in wildlife populations presents a promising approach for rabies control. However, several challenges must be addressed, including ensuring the genetic stability of the recombinant VSV and evaluating the environmental impact of releasing a genetically modified virus into wildlife populations. Overall, the recombinant VSV-based rabies vaccine shows great promise as an effective, safe, and potentially self-disseminating tool for rabies control, with significant implications for both human and veterinary applications. Future research should focus on detailed genetic stability studies, environmental impact assessments, and field trials in wildlife populations to further evaluate this innovative vaccine strategy's practical application and effectiveness. This recombinant VSV-based vaccine could revolutionize rabies prevention and contribute significantly to global public health efforts by overcoming these challenges.

References

[1] C. E. Rupprecht, C. A. Hanlon, T. Hemachudha, Rabies re-examined. The Lancet infectious diseases 2(6) (2002) 327-343

[2] J. D. Blanton, R. M. Wallace, The ancient curse: rabies. Infections of Leisure (2016) 235-250

[3] World Health Organization. Rabies vaccines: WHO position paper, April 2018–Recommendations. Vaccine 36(37) (2018) 5500-5503

[4] A. C. Jackson, Rabies: a medical perspective. Revue scientifique et technique (International Office of Epizootics) 37(2) (2018) 569-580

[5] World Health Organization. Rabies epidemiology and burden of disease. WHO Rabies Bulletin (2020)

[6] G. Wu, D. Selden, A. R. Fooks, et al. Inactivation of rabies virus. Journal of Virological Methods 243 (2017) 109-112

[7] A. R. Fooks, A. C. Banyard, D. L. Horton, et al. Current status of rabies and prospects for elimination. The Lancet 384(9951) (2014) 1389-1399

[8] C. E. Rupprecht, D. Briggs, C. M. Brown, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. MMWR Recomm Rep 59(RR-2) (2010) 1-9

[9] G. Liu, W. Cao, A. Salawudeen, et al. Vesicular stomatitis virus: from agricultural pathogen to vaccine vector. Pathogens 10(9) (2021) 1092

[10] X. Xue, Z. Yu, H. Jin, et al. Recombinant adenovirus expressing vesicular stomatitis virus G proteins induce both humoral and cell-mediated immune responses in mice and goats. BMC Veterinary Research 17 (2021) 1-9

[11] A. Marzi, F. Engelmann, F. Feldmann, et al. Antibodies are necessary for rVSV/ZEBOV-GP–mediated protection against lethal Ebola virus challenge in nonhuman primates. Proceedings of the National Academy of Sciences 110(5) (2013) 1893-1898

[12] A. M. Henao-Restrepo, A. Camacho, I. M. Longini, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). The Lancet 389(10068) (2017) 505-518

[13] K. Kolell, J. Padilla-Zamudio, B. Schuchhardt, et al. Virus production in Vero cells using a serum-free medium. In Cell Technology for Cell Products: Proceedings of the 19th ESACT Meeting, Harrogate, UK, June 5-8, 2005 (pp. 583-585). Dordrecht: Springer Netherlands (2007)

[14] D. Slate, T. P. Algeo, K. M. Nelson, et al. Oral rabies vaccination in North America: opportunities, complexities, and challenges. PLoS neglected tropical diseases 3(12) (2009) e549

[15] F. Cliquet, C. Freuling, M. Smreczak, et al. Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union. EFSA Supporting Publications 7(7) (2010) 67E

[16] J. Maki, A. L. Guiot, M. Aubert, et al. Oral vaccination of wildlife using a vaccinia–rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. Veterinary research 4 (2017) 1-26.