

Scientific and Technical Challenges in Developing mRNA Vaccines for Veterinary Applications

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Abstract Messenger RNA (mRNA) vaccines are a revolutionary technology in the field of human medicine and are currently being considered to be utilised in veterinary work. Their main strengths include the ability to induce humoral as well as cellular immunity, their short production cycles and their relatively acceptable safety profiles. Molecular instability has been identified to be one of the most significant obstacles to the translation of these advantages into veterinary practise, which is not unique to veterinary medicine, but is also a serious concern with human mRNA vaccines development and storage. The possible constraints related to the veterinary use of mRNA vaccines include issues with the delivery system, potential species-related differences in immunogenicity, scalability of production, use of and dependence on cold-chain logistics. Though these considerations have not been currently determined to be significant barriers in the field of veterinary medicine, the active work done in the field of (LNP) formulations, thermostable preparations, and self-amplifying mRNA platforms in the period of 2020-2025 helps to have them mitigated. to enhance the attempts to resolve these problems mRNA vaccines have shown protection against rabies, porcine coronaviruses and feline infectious peritonitis virus, but poultry models have shown good immune responses to Marek's Disease and avian influenza. The above case studies indicate that the veterinary mRNA vaccines have the potential to transform the field by offering customizable, fast deployable and cost effective alternatives to traditional vaccines. The future research directions include to eliminate the use of a cold-chain to improve the stability of RNA distribution.

Keywords: mRNA vaccine, ruminants, veterinary medicine, lipid nanoparticles

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Introduction The COVID-19 pandemic demonstrated that mRNA vaccines could be developed and deployed faster than ever before with high efficacy and altered the global vaccine strategy and underscored the flexibility of nucleic acid-based systems [1,2]. This breakthrough has triggered great interest in applying the mRNA technologies to veterinary medicine where infectious diseases persistently threaten the productivity of livestock, health of companion animals, food security and eventually, human health due to the zoonotic spillovers [3]. Veterinary vaccines are viewed in the One Health framework as the means of protecting health not only of the animals, but also of the population and animal production systems sustainability. Historically, traditional veterinary vaccines, both live attenuated and inactivated, have proven to be effective but are generally less demanding in terms of cold-chain requirements compared to modern mRNA-based formulations. Nevertheless,

their relatively lengthy production cycles and biosafety concerns may limit their capacity to respond rapidly during emergent outbreaks of novel pathogens[4,5]. Also, the fact that they cannot be easily adapted to newly emerging variants of the pathogen. In comparison, mRNA vaccines have different technological advantages: they can be designed directly based on the sequences of the pathogen genome, produced in a cell-free condition on a large scale, and engineered to produce both a strong humoral and cellular immune response [6,7]. These attributes render mRNA vaccines especially attractive to veterinary medicine where a wide range of hosts and fast-evolving pathogens require flexibility and accuracy. Recent advances have provided strong preclinical evidence supporting the veterinary application of mRNA vaccines across multiple animal species. For instance, mRNA-based formulations have demonstrated protective efficacy against rabies in several animal

models [8,22,23], as well as against porcine coronaviruses, including porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV), in piglets [10,11]. Likewise, promising results have been reported for experimental vaccines targeting feline infectious peritonitis virus (FIPV) in laboratory models [6,18]. In poultry, mRNA vaccine candidates have induced measurable immunogenic responses against diseases such as Marek's disease and avian influenza under both laboratory and field-relevant conditions [14,15,17]. Furthermore, aquaculture represents an emerging frontier for mRNA vaccine technology, with lipid nanoparticle (LNP)-based formulations currently being explored for the protection of economically significant species, including Atlantic salmon [16]. Collectively, these findings underscore the broad potential and adaptability of mRNA vaccine platforms in diverse veterinary contexts. demonstrate the versatility of the platform, but also its possible ability to make veterinary vaccinology a more responsive, customizable and globally relevant discipline. This development is being contributed even more by technological refinements. LNP innovations, thermostable systems and self-amplifying mRNA constructs are enhancing the efficiency of antigen delivery and longevity of immune responses, and may lower dosage and production costs [7-9,19]. State-of-the-art technologies, including exploiting chiral self-assembling peptides as delivery vectors, widen the portfolio to improve the stability of vaccines and specific cellular internalisation [18]. However, there are still substantial obstacles to scale on the way to the mRNA vaccines entering the veterinary practise. These involve species-specific optimization of delivery systems, long-term safety demonstrated in a wide range of hosts, economic feasibility of use in large-scale livestock applications, and regulatory systems that tend to be slow to adapt to scientific advances [12,20,21].

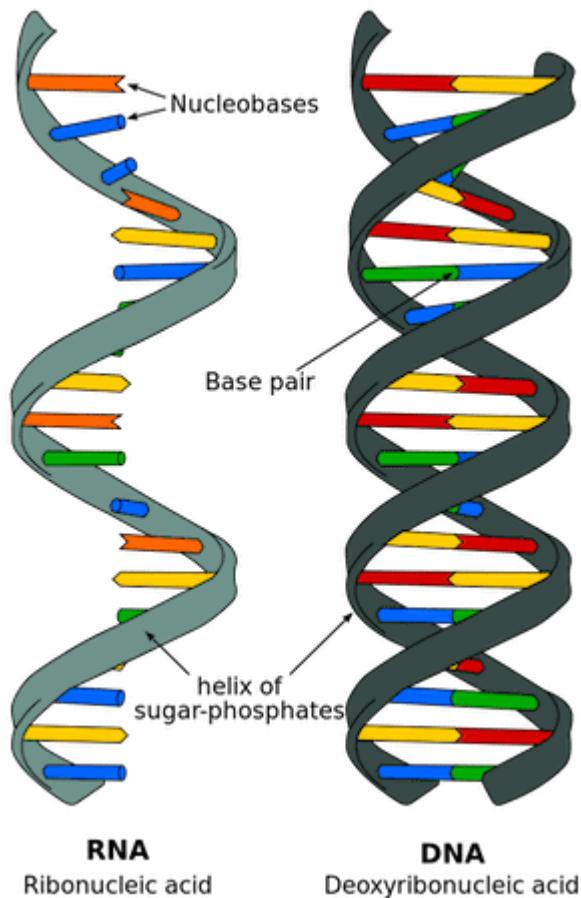
. Principles of mRNA Vaccines

The mRNA vaccines are essentially constructed on the concept of in vitro transcription (IVT) where a DNA template is transcribed into messenger RNA molecules that encode the antigen of interest [5,9]. This synthetic mRNA is then translated into the antigenic protein in the cytoplasm of the host cell and is then processed and delivered via the major histocompatibility complex (MHC) pathways triggering both humoral and cellular arms of the adaptive immune response once it is transported into the cytoplasm [5,6]. This bidirectional induction of immunity has been cited as one of the benefits of mRNA vaccines over the protein-based or inactivated platforms [6,7].

In order to maximise antigen expression, and circumvent the natural instability of RNA, numerous structural alterations have been implemented into mRNA vaccine

design. These are codon optimization to match host tRNA abundance, 5' and 3' untranslated region (UTR) modification to improve translation efficiency and the use of modified nucleosides like pseudouridine to evade innate immune sensors and enhance stability [6,9]. Other approaches to further extend half-life and translation efficiency, such as the application of optimised poly(A) tail structure, 5' capping structure, and circular RNA methodology, are actively being pursued [9]. The delivery system is also important as naked mRNA is very vulnerable to enzymatic degradation by ubiquitous RNases. Lipid nanoparticles (LNPs) are the most popular carriers that derive mRNA and protect against degradation, take part in endocytosis into host cells [7]. Targeting LNPs are also amenable to functionalization with targeting ligands (e.g. mannose functional groups) to stimulate delivery of mRNA cargo to antigen-presenting cells and thus increase immunogenicity [19]. Other new delivery methods, including self-assembling peptides, polymer-based carriers, and aerosolized formulations, are also under consideration to deal with species-specific issues in veterinary use [18,20]. Self-amplifying mRNA (saRNA) constructs have also formed another significant breakthrough to develop not only the antigen but also replicase machinery that enables mRNA to amplify itself intracellularly, leading to increased antigen production with reduced doses [14,15,17]. This attribute is quite desirable in veterinary medicine where cost-efficiency and large-scale inoculation to livestock is a significant issue. Moreover, thermostabilization procedures and lyophilized formulations are also being studied to minimise reliance on cold-chain logistics, which continues to be a major impediment to the delivery of vaccines in resource-constrained environments [8,9]. The author provides

Fig. 1. Comparison of RNA and DNA Structures. This character provides an explanation of structural differences between DNA and RNA. RNA is made of a unstranded molecule composed of ribose sugar and a nitrogenous base called uracil which makes it flexible and transient in cellular functions like transcription and protein synthesis. Conversely, DNA is made up of a rigid bi-stranded helix of deoxyribose sugar and the base thymine, thus allowing long term storing and



preserving of the genetic in the cell

Figure 1. The structural differences between the DNA and the RNA. As depicted in the figure, DNA is a stable and double-stranded helix molecule consisting of deoxyribose and thymine whereas RNA is a single strand molecule with ribose and uracil. These variations characterize their biological differences in genetic storage and expression of genes.

Scientific Challenges

mRNA Stability

Among other issues, the basic instability of mRNA vaccines has become one of the most crucial challenges in this specific field of development. Messenger RNA is a fragile molecule, and inherently it is subject to quick degenerative actions by ubiquitous extracellular and intracellular ribonucleases (RNases) [5,7]. Such instability places a harsh limit the on shelf life of mRNA formulations and makes high storage requirements (which are commonly at low temperature) to prevent potential degradation, which is hard to achieve in veterinary field practise, particularly in resource constrained settings (8).

Lipid nanoparticles (LNPs), at the formulation level play not only an active role in pointing to delivery, but also by

providing a shield against degradation by enzymes like RNase [7]. Additional stabilisation of mRNA using novel delivery technologies, which disrupt large molecule assembly, like the mannose-modified LNPs and self-assembling peptide carriers, has been reported to enhance the persistence and immune potency of mRNA [18,19]. Moreover, another option under consideration is the use of lyophilization and other thermostabilization measures to enhance the stability of vaccines under field conditions, eliminating their reliance on cold-chain storage [8].

The biggest issue with veterinary practise is the transfer of laboratory safety to the real world despite this development. Some of these include very high ambient temperatures, long-distance transportation and vocalisation under the conditions of open areas on farms protecting the integrity of vaccines and reducing their effectiveness [8,21]. Additionally, even though certain thermostable reagents have shown potential in test systems e. g. a rabies mRNA vaccine ability to remain at a steady state under cold and side effects [8], their feasibility to the random use on previously untested animal managements remains to be confirmed.

Immunogenicity and Antigen Expression

Preliminary animal trials and micro-trials have demonstrated that mRNA vaccines have the capacity to elicit robust humoral as well as cellular immunity across a number of animal species such as pigs, poultry carnivores [10-12]. These results indicate that the platform can induce both antibody and T-cell mediated immunity that are important in defence against both intracellular and extracellular pathogens.

Another parameter that is important is dose optimization. Upgrades are likely to boost immune responses but on the other hand, higher dosage associated with enhanced immune response also augments possible adverse effects and the cost of production, which is of critical concern in veterinary practises. Differences in pharmacological levels between species, as well as immune stimulation, suggest that dosage schedules that are successful in humans cannot necessarily be directly applied to animals (14).

Problems

One of the most troublesome scientific issues in the development of mRNA vaccine in veterinary work is the scale of host-specific immune reactions of various animal species. The science utilised to achieve animal vaccination should also take into consideration critical immune, metabolic, and physiological inequalities between mammals, birds and aquatic life, unlike a human immune system, which is highly uniform and thus easily immunised. In fact, comparative research did reveal that inter-species diversity in immune reactivity using homologous vaccine vehicle may be up to 16-fold varies, and the task to develop universal veterinary immunisation

is complex [14-16,21]. E.g. high-immunogenicity self-amplifying mRNA (saRNA) vaccines that were effective in mammals have been found to be ineffective in poultry, probably because of differences in innate immune sensing, and because of differences in the rate of RNA degradation [14,15]. RIG-I and MDA5 antiviral pattern recognition receptors (PRRs) in chicken are also significantly different in this modification of innate activation and antigen expression relative to mammals [14]. Likewise, avian and mammalian lymphoid tissue composition and the abundance of Sub-populations of immune cells differ greatly, making it even more difficult to directly translate mRNA platforms.

Gradually, new solutions actually are closing these gaps in aquaculture. Delivery systems using lipid nanoparticles (LNPs) have demonstrated positive effects in Atlantic salmon, and they are a workable method of safeguarding economically significant species against viral pathogens [16]. Nonetheless, the water stresses also place special limitations, including the dispersal of various vaccines in water, low metabolism of fish, and temperature-related immune system processes, among others, and could directly affect the effect of the vaccines. In addition to birds and fish other issues are complexities of livestock and companion animals. Ruminants, i.e., pose special problems to be seen with the complex gastrointestinal physiology, high mucosal immunity, whereas carnivores, e.g., dogs and cats, can show species-specific innate immune sensitivities that can modulate tolerability and immunogenicity [12,23]. Horses are also under study as recipients of aerosol delivery of mRNA, which avoids certain systemic obstacles even though there is still the need to optimise the delivery to each species [20].

Technical Challenges

Delivery Systems

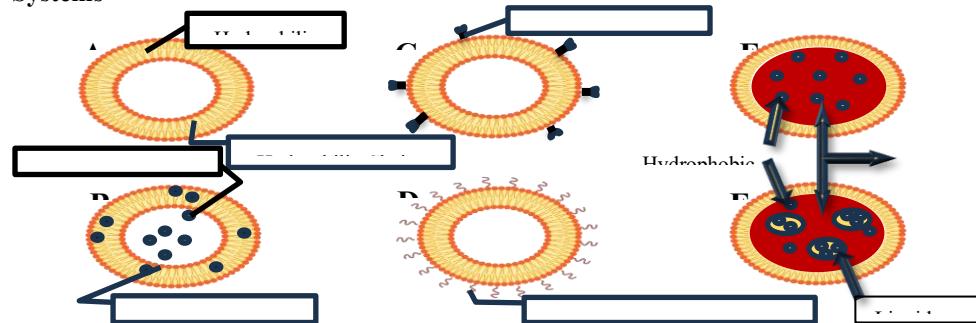


Figure 2: provides a graphic display of the structural forms and composition characteristics of different lipid nanoparticles (LNPs): (A) liposome; (B) drug-loaded liposome; (C) targeted liposome; (D) PEGylated liposome; (E) solid lipid nanoparticle; (F) nanostructured lipid carrier.

Manufacturing and Scalability

Even though a single study cited mRNA platforms as having a strong theoretical benefit in regard to fast and

Another technical challenge in veterinary vaccinology that is still extremely daunting is the efficient transfer of mRNA to host cells. Lipid nanoparticles (LNPs) are the newly available gold standard as they are capable of encapsulating mRNA and protecting it against degradation in addition to facilitating its cytoplasmic release leading to high antigen expression and immunogenicity [7,17]. Nonetheless, their extensive use in veterinary settings has been argued to be hampered by the inconsistency of biodistribution range in various animal species, and is hampered by cost and production issues in high-scale livestock precaution.

New platforms of delivery are thus being actively explored. The literature has identified self-assembling peptides as biocompatible vectors that can lead to an increase in cellular uptake as well as reduction in the use of complicated lipid structures. [18]. On the same note, LNPs are found to have enhanced targeted action to antigen presenting cells like dendritic cells hence magnifying immune reactions at the relatively low doses [19]. Direct aerosolized transfer of naked mRNA to respiratory as the epithelial layer has been successfully and safely done in equine models, which avoids use of any conventional transfection vehicle [20]. Although these innovations have potential to replace more commonly used items, they have not undergone adequate validation in clinical trials other than preclinical and limited scale, and conclusive species specific results in actual veterinary practise does not exist yet. This highlights the importance of a translational research work that helps to achieve the level of innovation in the laboratory and its applicability in the field. **Figure 2** outlines different structural variations and compositional changes of lipid nanoparticles (LNPs) that are used in pharmaceutical and messenger RNA delivery.

customizable scaling; in practise, translating mRNA tools into veterinary settings encounters numerous challenges. The majority of experimental vaccines are still on the

proof-of-concept stage of preclinical testing, with minimal progress to large-scale production pipelines vaccines used to treat veterinary animals [21]. The financial viability producing mRNA animal vaccines is another challenge that has not been overcome, as such a sphere frequently needs to fight with mass vaccination of animals cheaply, believing millions of animals. The existing biomanufacturing facilities are ideally suited to produce high-price human vaccines, as opposed to more affordable veterinary markets. Moreover, scalabilities are complicated by the fact that often the application in veterinary medicine requires formulated and dosed specifically to each host like a ruminant, a poultry or aquaculture species, or pet animals [12,16,21]. Without modular platforms and cost-effective production systems, there is also little likelihood that the mRNA vaccines will be broadly available to be used by routine use by veterinarians in the near future. This challenge might be solved through incorporation of automated IVT technologies, single-use bioreactor technology, and decentralised manufacturing models that would help minimise production-related expenses and enhance flexibility to a wide array of animal health applications.

Cold Chain Requirements

The sensitive characteristics of mRNA demand costly cold-chain logistics with low temperature storage often being the only solution to trace vaccine efficacy. This is a significant barrier to the deployment of veterinary, especially in rural areas or in areas with scarce resources such as in rural areas where cold-chain infrastructure is insufficient [8]. As an example, it is expensive logistically and economically to consider that livestock

vaccines need continuous refrigeration in dispersed farms around the geographical setting.

Solutions that are emerging are on the line of thermostabilization and lyophilized formulations and excipient-based stabilisers wherein ambient or somewhat higher housed temperatures will have no preservative effects regardless of their strength [8,9]. It is noteworthy that rabies mRNA vaccines have thermostable formulations that are resilient to adverse weather conditions in the form of varying temperatures, increasing the potential of extended operation in field conditions among veterinarians [8,22]. Nevertheless, relevance These formulations should be thoroughly tested in the wild among various species, and then only be deemed dependable alternatives to cold-chain dependency.

Veterinary Applications and Case Studies – Ruminants

There has been a fast increase in the application of mRNA vaccines in the veterinary world, which covers a wide range of animal species and viral pathogens. In spite of the fact that ruminants are still a major target due to their role in agriculture, considerable research has been carried out in porcine, avian, companion animal, equine, aquacultural and wildlife species.

To provide a synthesis of both experimental and preclinical discoveries, Table 1 provides a summary of the major mRNA vaccine platforms that have been studied in various animal models and providing a clear outline of their target pathogen and the observed immunological response.

Table 1. Summary of mRNA-vaccine preclinical models which has been tested in veterinary and preclinical models.

Reference	Vaccine type / technology	Animal model	Target disease	Immune response / outcome
[6] Brostoff et al., 2024	mRNA (FIPV antigen)	Mice (laboratory model)	Feline Infectious Peritonitis Virus (FIPV)	Strong humoral and cellular immune responses
[8] Stitz et al., 2017	Thermostable mRNA	Mice	Rabies	Protective immunity; demonstrated thermostability
[10] Li et al., 2024	Spike-based mRNA	Piglets	Porcine Deltacoronavirus (PDCoV)	Durable and broad protection
[11] Zhao et al., 2024	PEDV-spike mRNA	Piglets	Porcine Epidemic Diarrhoea Virus (PEDV)	Effective protection against viral challenge
[12] Huo et al., 2024	SARS-CoV-2 mRNA	Minks, cats, blue foxes, raccoon dogs	SARS-CoV-2	Safe and immunogenic across carnivore species
[14] Comes et al., 2024	Self-amplifying mRNA (saRNA)	Chickens	Marek's disease	Partial efficacy; species-dependent differences
[15] Snoeck et al., 2023	saRNA reporter mRNA	Broiler chickens	Experimental model (reporter)	Detectable immune responses
[16] Dahl et al., 2024	mRNA-LNP	Atlantic salmon	Aquaculture viral pathogens	Positive protective effects
[17] Fazel et al., 2024	mRNA (MDV antigen)	Chickens	Marek's disease virus (MDV)	Both local and systemic immune responses

[18] Lu et al., 2024	mRNA + self-assembling peptides	Feline models (FIPV, <i>in vitro/in vivo</i>)	FIPV	Improved delivery and immunogenicity
[19] Gong et al., 2024	Mannose-modified LNP mRNA	Mice (ASFV model)	African Swine Fever Virus (ASFV)	Strong antigen-specific immune response
[20] Legere et al., 2021	Aerosolised naked mRNA	Horses	Respiratory delivery (non-specific model)	Safe and efficient pulmonary delivery
[21] Mahony et al., 2024	Theoretical / proof-of-concept mRNA	Cattle (BVDV, extrapolated)	Bovine Viral Diarrhoea Virus (BVDV)	Promising potential but untested in field
[22] Li et al., 2025	Rabies mRNA + muscle-targeted LNP	Mice	Rabies	Strong immune response and protection
[23] Li et al., 2024	Rabies mRNA	Dogs, rodents, cynomolgus macaques	Rabies	Safe and effective across multiple species

The above-presented experimental observations shed some light on the issues and opportunities of ruminant vaccinology, in particular, Bovine Viral Diarrhoea Virus (BVDV), foot-and-mouth disease virus (FMDV), and paste des petits ruminants virus (PPRV).

6. Future Perspectives

The main directions to the research in the future are:

- Production of thermostable mRNA preparations to eliminate cold chain requirements
- High-tech delivery systems designed to accommodate avian, aquatic and ruminant species.
- AI integration to maximise the design of antibodies [9].
- Embrace of the One Health system in order to control the presence of zoonotic pathogens [24].

Close liaisons among academia, industry and regulatory agencies will be critical to translate the efforts achieved in preclinical trials to make field ready veterinary vaccines.

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Conclusion

mRNA vaccination has immense potential in veterinary medicine-based practise because of its potential to be quickly designed, adapted, and elicit robust immune responses. Following scientific difficulties including mRNA stability, immune variability among hosts and technological problems affecting delivery, scalability and logistics however, exist. The innovations in nanotechnology, thermostability and digital design give an opportunity to overcome these challenges. It is possible that with further progress, the vaccine developed on the basis of mRNA by veterinarians will revolutionise the control of diseases in animals and reinforce the strategies of the global One Health.

Conflict of interest

Authors declare no conflict of interest.

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3. Among the critical issues are stability, delivery plans and mass production.

4. Lipid nanoparticles lead to increased protection against degradation and uptake into cells of the mRNA.

Article Highlights

1. mRNA vaccines represent a quite promising medical niche in the field of veterinary medicine.
2. They cause humoral and cellular immunity.



5. Computer-based technology assists in suggesting veterinary mRNA vaccines.