MESSENGER RNA VACCINE, A GENETIC TOOL TO IMPROVE LIVESTOCK PRODUCTION- A REVIEW

[1]*Oluwole, O.O., [2] Oladejo, O.A., [1] Bolaji, A.A., [1]Oladele-Bukola, M.O., [1]Makanjuola, B.A., [1]Boladuro, B.A., [1] Olalere M.O. and [1]Olorungbohunmi, T.O.

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University, Nigeria.

[2]Bowen University, Iwo, Osun State

*Corresponding author: oluwoleolufunke@gmail.com; +234(0)8037422069

ABSTRACT

This review explores the current understanding of mRNA vaccines, the technologies involved in their development and it examines future directions and challenges. As emerging technologies and new diseases pose threats to animal health and human food security, research into innovative methods for improving animal health is continuously evolving. One such technology under extensive study is the use of messenger RNA (mRNA) vaccines in livestock production. The potential displacement of traditional livestock vaccines by mRNA vaccines remains uncertain. Factors such as manufacturing costs, the requirement for cold storage to prevent degradation, and varying efficacy of mRNA vaccine types need resolution before widespread adoption can occur. mRNA vaccines function by introducing a small piece of genetic material known as messenger RNA (mRNA) into the body. This mRNA carries instructions to produce a protein similar to a part of the virus or bacteria the vaccine targets. The immune system recognizes this protein as foreign, triggers an immune response, and creates a memory of the invader, aiding the body in combating future infections. Traditional vaccines have long safeguarded food animals against numerous diseases. Restricting the use of mRNA vaccines at this stage would forego a new approach to protecting animals from pathogens that existing vaccines may not adequately address such as African swine fever virus in pigs etc. The public needs to be educated to prevent fear stemming from disinformation or misinformation, which can lead to widespread misunderstanding and confusion. Messenger RNA vaccines have the potential to address gaps in animal disease prevention, but further advancements are needed before a vaccine for food animals can be developed.

Keywords: ***Advance technology, Animals, Awareness, Livestock, mRNA, Vaccine,***

INTRODUCTION

The first three sustainable development goals (SDG) of United nations (UNs) were on poverty, hunger and good health with increase number of global human population within the range of 8.7 to 10.7 billion (Mahony *et al*., 2024) at the end of the 21st century are pointing towards solving the problem by increase in production of edible protein (Adam, 2021). A world without vaccines for livestock would jeopardize food security, causing more livestock deaths, reducing supply, and increasing grocery prices. Vaccines impacts animal health and welfare, farmers' economic stability, community economic health, and global food supply (McMorris, 2023). Vaccines are essential and increasingly critical for producing sustainable food while ensuring animal welfare.

A vaccine is a biological preparation made to provide protection against man and animal diseases by stimulating immune responses (Centers for Disease Control and Prevention (CDC), 2021). It represents the most cost-effective pharmaceutical interventions for disease prevention (Amanna and Silfka, 2018). Various approaches have been implemented to streamline the timeline and reduce costs, focusing primarily on the selection of optimal antigens, carriers, and adjuvants (Health for animals (HFA), 2021). Vaccines are recognized as pharmaceutical products offering exceptional cost-effectiveness in disease prevention. However, their development and production are resource-intensive processes that typically span years (Domazet-Lošo, 2022). The role of safe and effective vaccines in improving human and animal health by reducing disease is numerous. In livestock, such vaccines also help in avoiding the use of antimicrobial treatments, crucial in combating antimicrobial resistance. The mRNA vaccines have potential for controlling infectious diseases in animals (Le *et al*, 2022), which are vital for global food

production due to their efficient conversion of low-quality forage into high-quality protein (Mahony *et al.,* 2024). The challenges and opportunities of optimizing mRNA technology for ruminant diseases are explored, with the goal of contributing to sustainable development goals (SDGs) (Mahony *et al.,* 2024). There are various types of vaccines, including those containing living or killed organisms, purified antigens, and newer technologies like DNA and RNA vaccines. Live vaccines often elicit stronger immune responses but may pose risks (Mahony et *al*., 2024). In contrast, killed vaccines are safer but typically require adjuvants for effectiveness. Subunit vaccines use purified antigens and avoid non-essential components, enhancing safety and efficiency. Genetic techniques enable the development of gene-deleted and virus-vectored vaccines, which offer targeted immune responses without causing any disease. These advancements mark significant progress in vaccine technology, catering to diverse infectious agents and ensuring safer, more effective immunization strategies and mRNA vaccine is one of these advancements, since the technology is a promising potential in human health, it could also be valuable in animal health (Carlson, 2023). There is need for both farmers and consumers to be educated on this new advancement of animal vaccination for sustainable livestock production and health.

Historically, animals have played dual roles as recipients and origins of vaccines. The first animal vaccine, developed through laboratory testing, was for chicken cholera in 1879 by Louis Pasteur (The Antibody initiative, 2020). Subsequently, Pasteur created an anthrax vaccine for sheep and cattle in 1881, followed by a rabies vaccine in 1884 (Antibody initiative, 2020). Monkeys and rabbits were utilized to cultivate and attenuate the rabies virus (Meeusen *et al.,* 2007). Starting in 1881, dried spinal cord material from infected rabbits was administered to dogs to immunize them against rabies (WHO, 2020). The virus was weakened through the drying of infected nerve tissue (Long, 2020). In 1885, this vaccine was successfully administered to Joseph Meister, a 9-year-old boy infected with rabies, who became the first person to survive the disease (Long, 2020). This achievement was hailed as a breakthrough by the French National Academy of Medicine and garnered worldwide attention, prompting scientists to collaborate and advance Pasteur's pioneering work (WHO, 2020; Long 2020).

A significant aspect of animal vaccination can be seen through the history of smallpox. The vaccine initially administered to humans was derived from animals. Smallpox, known for its severe rash and high mortality rate of 30% among those infected (Smallpox, 2020), saw Edward Jenner's groundbreaking experiment in 1796. Jenner demonstrated that individuals previously infected with cowpox were protected from smallpox, marking the beginning of efforts to eradicate the disease (Steward and Delvin, 2006). Under the auspices of the World Health Organization (WHO), extensive vaccination efforts led to at least 80% coverage in every country (Smallpox, 2020). This was followed by case identification and ring vaccination strategies, culminating in the eradication of smallpox in 1980, a historic milestone as the first disease eradicated through vaccination (Smallpox, 2020).

There are different types of vaccines, some may contain either living or killed organisms or purified antigens from these organisms. These antigens were processed by the host immune systems and present them to either T or B cells. The T cells are a vital subset of white blood cells in the immune system and are crucial for the adaptive immune response. They are characterized by the presence of a T-cell receptor (TCR) on their cell surface, setting them apart from other types of lymphocytes (Pascolo *et al.*, 2004; Marthinon *et al.,* 1993). B cells belong to a subset of white blood cells known as lymphocytes and they play a key role in the humoral immunity branch of the adaptive immune system while Live virus vaccines, on the other hand, infect host cells and grow briefly.

Live virus vaccines, on the other hand, infect host cells and grow briefly. Non- living vaccines containing killed organisms or purified antigens might be less immunogenic than live vaccines and they resemble the living organisms as closely as possible but Chemical inactivation cause minimal change to their antigens. These Compound chemicals used include formaldehyde, ethylene oxide, ethyleneimine, acetyl ethyleneimine, and beta-propiolactone (Tizard, 2023). These inactivate them and prevents them

from growing and spreading within the host, making them less likely to optimally stimulate the immune system while killed organisms and purified antigens are often less expensive and potentially safer (e.g., no risk of reversion to virulence). The infected cells process the viral antigens, triggering a response dominated by cytotoxic T cells (a type 1 response) and commonly stimulating antibody-dominated responses (a type 2 response). This type of response may not provide optimal protection against some organisms. Consequently, vaccines with killed organisms or purified antigens may require multiple doses and the use of adjuvants to maximize their effectiveness (Tizard, 2023).

mRNA Vaccines

Vaccines work by using components of the pathogen, which can either be inactive (killed) or weakened (live attenuated), to train the immune system to recognize and defend against the pathogen effectively. Scientists have developed a novel vaccine type employing messenger RNA (mRNA), a molecule essential for protein synthesis, rather than fragments of actual pathogens. Biotechnology analysts predict that mRNA will become the leading vaccine technology within the next 15 years (African Development bank group (ADBG), 2022). Unlike traditional vaccines, mRNA vaccines do not integrate into the nucleus or affect DNA. Instead, they function by introducing mRNA fragments that encode specific viral proteins, typically derived from surface proteins of the virus. This allows cells to generate these proteins, prompting an immune response where antibodies are produced to combat the perceived foreign protein (Maruggi *et al*., 2019). These antibodies remain in the body to swiftly recognize and neutralize the virus upon future exposure, thus preventing severe illness. mRNA vaccines function by introducing a small piece of genetic material known as messenger RNA (mRNA) into the body (Figure 01) (Rackley, 2024, Riggs, 2023). This mRNA carries instructions to produce a protein similar to a part of the virus or bacteria the vaccine targets.

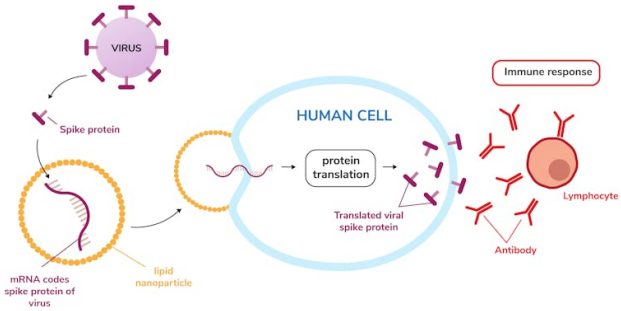

Figure 01: Messenger RNA vaccines get the recipient's body to produce a viral protein that then stimulates the desired immune response. Trinset/iStock via Getty Images Plus
Source: https://theconversation.com/what-is-mrna-the-messenger-molecule-thats-been-in-every-living-cell-for-billions-of-years-is-the-key-ingredient-in-some-covid-19-vaccines-158511

The immune system recognizes this protein as foreign, triggers an immune response, and creates a memory of the invader, aiding the body in combating future infections (Jain *et al.*, 2021; Medilineplus, 2022). Rackley (2024) also explained that mRNA vaccines carry instructions to produce a specific pathogen protein that the immune system will recognize and neutralize, with the body manufacturing this protein instead of a laboratory. This mimics a viral infection without causing disease, as the protein is only a small, non-disease-causing part of the pathogen. The immune system mounts a strong response to these foreign proteins. Since no live viruses are involved, the development process is much quicker than traditional vaccines, which require time to deactivate, weaken, or kill a virus. Scientists have researched mRNA vaccines for over three decades with the aim of preventing diseases like Cytomegalovirus (CMV), Influenza (flu), Rabies, and Zika virus (Yangzhuo *et al.,* 2022). Nolan (2023) reported that the use of mRNA vaccines in livestock has become a significant issue. It has been revealed that these vaccines have been used in the pork and poultry industries for several years, with trials

currently underway in the beef industry. Sharif (2024), made statement that it is a common vaccine like other vaccine used before for improving animal health. He stated that according to the Livestock Research Innovation Corporation, vaccines have been used on animals since 1879, and Canada currently has an approved mRNA vaccine for swine. Rackley (2024) reported that currently, there are two types of mRNA vaccines: one that closely resembles the natural mRNA found in the body, and another that is self-amplifying (saRNA), enabling the production of higher levels of proteins. SaRNA provides the instructions for the body to produce more proteins but does not include modified nucleotides (Rackley, 2024). In the United States, all vaccnes, including mRNA vaccines, must receive authorization or approval from the Food and Driug Administration (FDA) before use. Currently, mRNA vaccines authorized for COVID-19 utilize mRNA to instruct cells to produce copies of the coronavirus's spike protein. Ongoing research explores the potential of mRNA technology for creating vaccines against various other diseases for both human and animals. (Medlineplus, 2020).).

University of Queensland researchers emphasize the potential of mRNA vaccine technology in enhancing livestock production to meet global food needs. Mahony (2023) underscores the importance for the livestock industry to capitalize on the significant investment in mRNA vaccination spurred by the COVID-19 pandemic. He highlights that infectious diseases currently hinder the production of edible protein, impacting both its quality and quantity. The potential of mRNA vaccines lies in their ability to adapt swiftly to new disease variants, unlike conventional vaccines which require separate formulations for each pathogen. This flexibility could revolutionize livestock production by mitigating substantial economic losses due to diseases like Bovine respiratory disease (BRD), African swine fever (ASF), Bird flu etc. A report estimates that such losses amount to $US 982 million daily for BRD, impacting productivity and global food supply, while mRNA vaccines for livestock are not yet approved, their development holds promise pending rigorous safety assessments. Investment and collaboration among governments, industry bodies, and veterinary health sectors are crucial for advancing this technology to protect ruminants and other livestock effectively (Mahony, 2024) (https://www.uq.edu.au/news/article/2024/03/can-mrna-vaccines-help-boost-livestock-production).
Traditional vaccines have been successful against many animal pathogens, but diseases like porcine reproductive and respiratory syndrome, hoof-and-mouth disease, H5N1 influenza, and African swine fever still pose threats due to ineffective vaccines. mRNA vaccines could address these gaps, as advancements allow for rapid isolation of disease-specific genetic sequences (Rackley, 2024).

In Australia, mRNA vaccines for animals are currently under research and development globally and hold significant promise. The COVID-19 pandemic has demonstrated that mRNA vaccines can be produced quickly, are easily adaptable to new virus strains, and can differentiate between natural infection and vaccination through diagnostic testing. With appropriate regulatory measures to ensure their safety, these vaccines could eventually be safe and effective for use in livestock (Australia government, 2023). The Australian Government, along with the New South Wales and Queensland governments and Meat & Livestock Australia, is investing $4.95 million in a research project focused on developing mRNA vaccines for livestock (Australia government, 2023).

mRNA In Africa Continent

According to Hwanda *et al.* (2022), Africa is the second most populous continent, bears 25% of the global disease burden but imports 99% of its vaccines and 95% of its medicines (Saied *et al.*, 2022). Africa consumes nearly 25% of globally produced vaccines, a figure expected to rise with a projected 2.5% yearly population growth (Saied *et al.*, 2022). The African Development Bank is funding the Africa Centres for Disease Control and Prevention, pledging $3 billion over the next decade to enhance vaccine manufacturing and meet the African Union's goal of producing 60% of needed vaccines by 2040 (Saied, 2022b).

mRNA vaccines hold significant potential for Africa, offering a way to combat various bacterial and viral infections beyond COVID-19 (Saied, 2022a). This is the same for animal or livestock health where the mRNA can be a great weapon for combating animal diseases if accepted. This technology could

enable the development of vaccines for diseases like ASF, providing African nations with effective tools to fight these animal diseases without vaccine yet. Rwanda has become a trailblazer by hosting the first mRNA vaccine manufacturing facility in Africa. This is as a result of collaborative efforts between Rwanda, Ghana, Senegal, the African Union, WHO, and the European union (EU). These three countries (Senegal, South Africa, and Kenya) will also establish their own mRNA vaccine manufacturing facilities on the continent (Saied, 2023). Challenges in building vaccine production capacities in Africa include a lack of trained personnel, specialized equipment, cold chain infrastructure, power outages, and public health infrastructure. Their pharmaceutical sectors are still in their early stages, facing challenges such as a limited scientific workforce and difficulties in importing reagents and equipment (Bowman, 2024). He added shortage of skills and experience, long lead times for equipment and raw material supplies, and no access to existing procedures and analytics to expedite development. Investments made in ultra-cold chains for COVID-19 vaccines can be repurposed for future mRNA vaccine storage. Stabilizing mRNA vaccines through lyophilization (freeze-drying) is proposed as a strategy to manage these challenges, as it would eliminate the need for a cold chain for some vaccines. The commencement of large-scale mRNA vaccine manufacturing in Africa is crucial for creating a sustainable healthcare system. It also provides an opportunity to enhance vaccine training in African countries, as expertise is often gained within manufacturing facilities. Rwanda becoming the first African country to host an autonomous mRNA manufacturing facility.

Advantages of mRNA Vaccines

mRNA in cells carries instructions to produce proteins necessary for various functions. Vaccines use mRNA to encode proteins from pathogens, training immune cells to identify and combat these proteins. This creates immunological memory, enabling a rapid and robust immune response when the actual pathogen enters the body as shown in Figure 1. Sharif (2023) noted that mRNA vaccines for livestock are easier and quicker to produce and can be updated for new pathogen variants more efficiently, thus potentially saving more livestock. Folta (2024) highlights the advantages of mRNA vaccines, describing them as cleaner and easier to use than other type of vaccines. They demonstrate consistent effectiveness in inducing immune responses (Yangzhuog, 2020). They are efficient, safe, and can be produced in large quantities quickly and with minimal effort, even without prior knowledge of biological pharmacological agents (Saied, 2022b). mRNA vaccines can stimulate both humoral and cellular immunity (Pardi *et al.*, 2020). Additionally, RNA directly interacts with pattern recognition receptors (PRRs), eliciting a strong innate immune response without the need for additional adjuvants (Painter *et al*., 2021; Gutiérrez-Bautista et *al* 2022). mRNA vaccines are safe, avoiding many risks associated with conventional vaccines, such as reversion to virulence or insertional mutagenesis (Domatez-Loso e*t al.,* 2022; Hajissa and Musa, 2021). Additionally, as a minimal genetic unit, mRNA does not trigger antivector immunity, making repeated administration of mRNA vaccines both safe and straightforward (Xu *et al.*, 2020; Sadarangani *et al*., 2021).

According to Loomis *et al*. (2018) mRNA vaccine is a rapid and inexpensive vaccine to manufacture, because the synthesis of mRNA vaccines mainly uses in-vitro transcription (IVT), which bypasses the time-consuming steps of in-vivo protein expression (i.e., cloning and cell culture) (Chen, *et al.*, 2022; Noor *et al.*, 2022). mRNA is a manageable transient modulator of physiologic processes (Yangzhuo *et al.,* 2022). Acabonac farms (2023) reported that mRNA vaccines offer quick development, allowing for faster responses to emerging diseases. Their technology is flexible and easily adaptable to various pathogens, making them versatile for fighting multiple diseases. Additionally, mRNA vaccines do not use live viruses or bacteria, enhancing their safety by reducing the risk of causing disease in vaccinated animals. mRNA vaccines do not use or manipulate live viruses, eliminating the risk of causing the disease they target. According to Verhoeven (2024), the absence of virus cultivation shortens the manufacturing process to months or even days, enabling a rapid response to disease outbreaks (Rackley, 2024). Due to their long-lasting responses, ease of production, ability to encode complex protein designs, and safety, mRNA vaccines are also an excellent platform for HIV vaccine development (Saied, 2022).

Limitations to mRNA Vaccines

Challenges of mRNA vaccines include the instability and rapid degradation of mRNA without proper storage, complicating distribution. Efficient delivery systems to target cells are essential and may need further research for livestock. Additionally, the long-term effects on animal health are not fully understood due to the novelty of mRNA technology (Acabonac farms (2023). The main challenge has been the rapid degradation of natural mRNA and its destruction by enzymes called RNases (Rackley, 2024). To be effective, as shown in Figure 1, mRNA vaccines must be packaged to survive long enough to instruct cells to produce sufficient protein for an effective immune response as seen in human viral disease (COVID-\19) as an example (Riggs, 2023). Vahoeven (2024) reported that while the basic technology for mRNA vaccines has existed for decades, newer methods prevent immediate rejection. Mixing modified and unmodified nucleotides allows the mRNA to evade detection and train the immune system. Additionally, packaging the mRNA in a fatty outer shell helps it avoid cellular sensors. Despite this stabilization, mRNA vaccines are eventually broken down by normal cellular processes. In ability of SaRNA to include modified nucleotides with other proteins instucted to produce (Rackley, 2024) but identifying the protective antigens of some diseases, remains a critical knowledge gap (Mahony 2024).

Misconception About mRNA Vaccines

Despite the promising benefits of mRNA vaccines demonstrated by their effectiveness against COVID-19, concerns and misinformation regarding their safety circulated concurrently. These misunderstandings have extended to fears about potential exposure to vaccine components through agricultural animals, such as meat or milk (Vahoeven, 2023).

There is a misunderstanding that administering these vaccines to animals poses a health risk to people who consume their products. However, these vaccines have proven effective in reducing diseases on farms, and it is highly unlikely for them to transfer into the food supply. Nolan (2023) reported that mentioned that mRNA technology is seen as genetic manipulation rather than a traditional vaccine while the synthetic spike protein it uses can persist and spread throughout the body. Pathogens adapt quickly, necessitating multiple boosters, as seen with COVID-19 shots in human. European studies, cited by Peter McCullough, suggest mRNA remains in the body, can transfer to humans through meat, and survives digestion. The long-term effects on animal or human health remain unknown.

There is a belief that mRNA vaccines altered genes of an animal when vaccinated which is not true but contains genetic code which do not alter the recipient's mRNA (Folta, 2024). The mRNA acts as an intermediary between the gene and the products it encodes, similar to a construction worker using a blueprint to build a house while the mRNA uses DNA information to build part of a cell`s structure without changing the DNA itself (Folta, 2024).

There is believe that there is transfer of mRNA through meat consumption or the vaccine is in the food (Carlson, 2023). The vaccine is not present in the food, it's a vaccine. After vaccination there is a need for withdrawal period of 7 days before entry into the food chain. The vaccine will modify the genes of the organisms when vaccinated. This is untrue according to Carlson (2023).

Applications in Animal Vaccination

The mRNA is essential in livestock such as pigs, chicken and cattle), Companion animals (dogs, cats), Aquaculture (fish and shellfish) and Wildlife conservation. For livestock, diseases include foot-and-mouth disease, swine fever, and avian influenza. For example, mRNA vaccine for avian influenza in chickens (Naidoo, 2019) African wine fever in pigs (Gong *et al*., 2024). For companion animals, diseases include rabies, distemper, and parvovirus. E.g mRNA vaccine for rabies in dogs and cats (Xia *et al*., 2019). Diseases include salmon anemia and white spot syndrome (Wang *et al*., 2020), while for wild life, diseases include rabies and Ebola.

Way Forward

Recently, researchers are developing mRNA vaccines for pork production to combat porcine reproductive and respiratory syndrome (PRRS), a viral disease causing approximately $664 million in economic losses annually in the U.S. (Montaner-Tarbes, 2019). Incorporating mRNA technology provides an additional tool that could be useful in fighting diseases like African swine fever (ASF), avian influenza, and other illnesses affecting food animals. Advocates for mRNA vaccine technology highlight its potential to combat diseases such as Foot and Mouth Disease, African Swine Fever, Porcine Reproductive and Respiratory Syndrome, and Highly Pathogenic Avian Influenza. Research on mRNA vaccines for livestock has been ongoing for over a decade and requires government approval before use especially for cattle. Some countries like Tennessee lawmakers proposed bans and labeling requirements for meat from animals treated with mRNA vaccines in 2023. Like existing vaccines, mRNA vaccine components are broke9n down by immune cells, leaving no trace in animal tissues, and withdrawal periods before slaughter would be mandatory (Policy development, 2023). to enhance vaccine training in African countries, as expertise is often gained within manufacturing facilities.

Research and development needed to overcome challenges in mRNA vaccines for animal vaccination are;

1. Regulatory frameworks: There should be regulatory considerations for mRNA Vaccines (Talagrand-Reboul *et al.*, 2020; Videc *et al*., 2020).
2. Large-scale production and distribution: There should be scalable production, manufacturing, challenges and solutions of mRNA Vaccines (Guzman, 2020).
3. mRNA Vaccine Manufacturing, Challenges and Solutions (Kim *et al.,* 2020). Public acceptance and education: There should be public perceptions, education and awareness of mRNA vaccines (Savinovia *et al*., 2020)
4. mRNA Vaccine Education and Awareness (Dudley *et al* 2024; Bell *et al*. 2020)
5. Continuous monitoring and evaluation of safety and efficacy animal mRNA vaccines: There should be safety monitoring and efficacy (Jamous *et al* 2023; Li *et al* 2024).
6. Overcoming technical challenges: mRNA Vaccine Delivery Systems, Stability and Storage (Bouazzaoui and Abdellatif, 2024).

CONCLUSION

Messenger RNA (mRNA) vaccines are rapid and cost-effective to produce because they use in-vitro transcription (IVT), bypassing the lengthy steps of in-vivo protein expression. Their quick development allows for faster responses to emerging diseases and easy adaptation to different pathogens, making them versatile for combating various diseases. Since mRNA vaccines do not use live viruses or bacteria, they are safer, eliminating the risk of causing disease in vaccinated animals. This technology is especially beneficial for livestock, as it allows for quicker production and updates to address new pathogen variants. mRNA vaccines have the potential to fight diseases like Foot and Mouth Disease, African Swine Fever, Porcine Reproductive and Respiratory Syndrome, and highly Pathogenic Avian Influenza, if accepted by the public and if all challenges can be tackled in details to produce effective vaccines

REFERENCES

Acabonac Farms (2023). mRNA Vaccines for Livestock: Benefits, Risks, and Ongoing Debates. https://www.acabonacfarms.com/blogs/in-the-field/mrna-vaccines-for- livestock-benefits-risks-and ongoing-debates

Adam, D. (2021). How far will global population rise? Researchers can't agree. *Nature*, *597*, 462–465.

Amanna, I.J., Slifka, M.K. (2018). "Successful Vaccines". In Lars Hangartner; Dennis R. Burton (eds.). *Vaccination Strategies Against Highly Variable Pathogens*. *Current Topics in Microbiology and Immunology*, vol. 428. pp. 1–30. doi:10.1007/82_2018_102. ISBN 978-3-030-58003-2. PMC 6777997. PMID 30046984.

African Development Bank Group (2022). Six African countries to receive initial transfer of mRNA vaccine technology.https://www.afdb.org/en/news-and-events/six-african-countries-receive-initial-transfer-mrna-vaccine-technology-49460

Australian government (2023). https://www.agriculture.gov.au/about/news/correcting-the-record-on-mrna-vaccines

Bouazzaoui, A. and Abdellatif, A.A. (2024). Vaccine delivery systems and administration routes: Advanced biotechnological techniques to improve the immunization efficacy. *Vaccine: X*, *19*, 100500. https://doi.org/10.1016/j.jvacx.2024.100500

Bowman, V. (2024). Why Africa is facing an uphill battle to make its own vaccines. *The telegramme.* https://www.telegraph.co.uk/global-health/science-and-disease/why- africa-faces-an-uphill-battle-to-make-its-own-vaccines/

Calson, P. (2023). Livestock and mRNA Vaccines: What You Need To Know *Porkbusiness.* https://www.porkbusiness.com/news/education/livestock-and-mrna-vaccines-what-you-need-know

Chen, J., Chen J. and Xu Q. (2022) Current developments and challenges of mRNA vaccines. *Annuual Review Biomedical Engineering*. 24:85-109.

Centers for Disease Control and Prevention (2021). Immunization: The Basics". https://www.cdc.gov/vaccines/vac-gen/imz-basics.ht

Danijel Videc, Odon Planinšek, Dejan Lamešić, (2020). Design of Experiments for Optimization of the Lactose Spherical Crystallization Process, *Journal of Pharmaceutical Sciences,* Volume 109, Issue 9, 2020, Pages 2774-2786,ISSN 0022-3549,https://doi.org/10.1016/j.xphs.2020.04.024. (https://www.sciencedirect.com/science/article/pii/S0022354920303026)

Domazet-Lošo T. mRNA vaccines: why is the biology of retroposition ignored?. *Genes (Basel)*. 2022;13(5):719.

Dudley, M. Z., Bernier, R., Brewer, J., and Salmon, D.A. (2021). Walking the Tightrope: Reevaluating science communication in the era of COVID-19 vaccines. *Vaccine*, *39*(39), 5453-5455. https://doi.org/10.1016/j.vaccine.2021.08.037

Folta Kevin. (2024). University of Florida. Livestock and Mrna Vaccines What You Need Know. *Farm Journal Pork*. https://www.porkbusiness.com/news/education/livestock-and-mrna-vaccines-what- you-need-know

Gong L., Zhang Y., Wang L., Zhao X., Wang L., Qiu X., Yang X., Zhu W., Lv L., Kang Y., Wu Y., Zhang A., Du Y., Wang X., Zhang G., Sun A. and Zhuang G. (2024). Advancing vaccine development: Evaluation of a mannose-modified lipid nanoparticle-based candidate for African swine fever p30 mRNA vaccine eliciting robust immune response in mice. *International Journal of Biological Macromolcules*. Jun;270(Pt 1):132432. doi: 10.1016/j.ijbiomac.2024.132432. Epub 2024 May 17. PMID: 38761609.

Gutiérrez-Bautista, J.F., López-Nevot M., Gómez-Vicente E., (year). Study of humoral and cellular immunity in vaccinated with mRNA-1273. *Apmis*. 2022;130(5):261-269.

Guzmán, C.A. (2020) Next Generation Influenza Vaccines: Looking into the Crystal Ball. *Vaccines (Basel)*. 8(3):464. doi: 10.3390/vaccines8030464. PMID: 32825635; PMCID: PMC7563445.

Hajissa, K., Mussa, A. (2021). Positive aspects of the mRNA platform for SARS-CoV-2 vaccines. *Hum Vaccin Immunother*. 17(8):2445-2447.

Health for animals (HFA) (2020). Global health Association. https://healthforanimals.org/animal-medicines/vaccines/

Hwenda, L., Sidibe, M. and Makanga, M. (2022). The African Medicines Agency: the key to unlocking clinical research in Africa. *Lancet Glob Health.* 2022; 10: e1088-e1089

Jorge, S., Dellagostin, O.A. (2017). "The development of veterinary vaccines: a review of traditional methods and modern biotechnology approaches". *Biotechnology Research and Innovation.* 1(1):6–13. doi:10.1016/j.biori.2017.10.001

Jain, S., Venkataraman, A., Wechsler, M.E, Peppas, N.A. (2021). Messenger RNA-based vaccines: Past, present, and future directions in the context of the COVID-19 pandemic. *Adv Drug Delivery Review*. 2021 Oct 9;179:114000. doi: 10.1016/j.addr.2021.114000. Epub ahead of print. PMID: 34637846; PMCID: PMC8502079.

Jamous, Y.F. and Alhomoud, D.A. (2023). The Safety and Effectiveness of mRNA Vaccines Against SARS-CoV-2. *Cureus*, *15*(9). https://doi.org/10.7759/cureus.45602

Jingjie, W., Jun X., Jingyi Z., Hongxing C., Dan L.L, Jinling C, Lingtian X, Yongju Luo (2020). Effects of dietary Cu and Zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of Nile tilapia (Oreochromis niloticus). *Fish Shellfish Immunology*. 100:198-207. doi:10.1016/j.fsi.2020.03.012.

Kim, J.W., Ko, Y.S., Chae, T.U. and Lee, S.Y. (2020). High-level production of 3-hydroxypropionic acid from glycerol as a sole carbon source using metabolically engineered Escherichia coli. *Biotechnology Bioengineering.* 2020 Jul;117(7):2139-2152. doi: 10.1002/bit.27344. Epub 2020 Apr 8. PMID: 32227471.

Le, T., Sun, C., Chang, J., Zhang, G., and Yin, X. (2022). MRNA Vaccine Development for Emerging Animal and Zoonotic Diseases. *Viruses*, *14*(2). https://doi.org/10.3390/v14020401

Lele, G., Yuanyuan, Z., Lele, W., Xuyang, Z., Lucai, W., Xiangqi, Q., Xilong Y., Wenhui, Z., Lijie, L., Yunzhe K., Wu, A.Z., Yongkun, D.X., Gaiping, Z., Aijun, S., Guoqing Z. (2024). Advancing vaccine development: Evaluation of a mannose-modified lipid nanoparticle-based candidate for African swine fever p30 mRNA vaccine eliciting robust immune response in mice. *International Journal of Biological Macromolecules.* Volume 270, Part 1, June 2024, 132432 https://doi.org/10.1016/j.ijbiomac.2024.132432

Li, J., Yu, P., Liu, Q. (2024). Safety and efficacy assessment of an mRNA rabies vaccine in dogs, rodents, and cynomolgus macaques. *npj Vaccines* **9**, 130. https://doi.org/10.1038/s41541-024-00925-w

Long, T. (2007). "1885: Rabies Vaccine Saves Boy -- and Pasteur". *Wired*. ISSN 1059-1028.

Loomis, K.H., Lindsay, K.E, Zurla C., et al. In vitro transcribed mRNA vaccines with programmable stimulation of innate immunity. *Bioconjugational Chemistry*. 2018;29(9): 3072-3083

Mahony, T. J., Briody, T. E. and Ommeh, S. C. (2024). ssssssCan the Revolution in mRNA-Based Vaccine Technologies Solve the Intractable Health Issues of Current Ruminant Production Systems? *Vaccines*, *12*(2). https://doi.org/10.3390/vaccines12020152

Martinon, F,, Krishnan S., Lenzen G., Magné R., Gomard E., Guillet, J.G, et al. (1993). "Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA". *European Journal of Immunology*. **23** (7): 1719–22. doi:10.1002/eji.1830230749. PMID 8325342. S2CID 42640967.

Maruggi, G, Zhang, C, Li J, et al. mRNA as a transformative technology for vaccine development to control infectious diseases. *Molecular Therapy.* 2019;27(4):757-772.

McMorris, Mike (2023). We need to talk about mRNA vaccines in the meats we eat. CBC Kitchener-Waterloo. https://www.cbc.ca/news/canada/-kitchener-waterloo/university-of-guelph-ontario-veterinary-college-covid-19-1.6901872

Medilineplus (2022). What are mRNA vaccines and how do they work? https://medlineplus.gov/genetics/understanding/therapy/mrnavaccines/).

Meeusen, E.N., Walker J., Peters A., Pastoret P.P, Jungersen G. (2007). "Current status of veterinary vaccines". *Clinical Microbiology Reviews*. **20** (3): 489–510, table of contents. doi:10.1128/CMR.00005-07. PMC 1932753. PMID 17630337.

Montaner-Tarbes S., Del Portillo HA., Montoya M., Fraile L.. Key Gaps in the Knowledge of the Porcine Respiratory Reproductive Syndrome Virus (PRRSV). Front Vet Sci. 10.3389/fvets.2019.00038.PMID: 30842948; PMCID: PMC6391865.

Murphy, K. (2012). *Janeway's Immunobiology* (8th ed.). New York: Garland Science. ISBN 9780815342434.

Naidoo, L., Zinhle Mzobe, Steven W. Jin, Erasha Rajkoomar, Tarylee Reddy, Mark A. Brockman, Zabrina L. Brumme, Thumbi Ndung'u, Jaclyn K. Mann. 2019.Nef-mediated inhibition of NFAT following TCR stimulation differs between HIV-1 subtypes,*Virology*, 531, Pages 192-202, ISSN 0042-6822, https://doi.org/10.1016/j.virol.2019.02.011.(https://www.sciencedirect.com/science/article/pii/S0042682219300571)

Nolan, M., 2023, What you need to know about mRNA vaccine and our livestock. https://redhillharvest.com/blog/mrna-vaccines-and-our-livestock

Noor, R. mRNA vaccines as an efficient approach for the rapid and robust induction of host immunity against SARS-CoV-2. *SN Comprehensive Clinical Medicine*. 2022;4(1):88.

Painter, M.M., Mathew, D., Goel, R.R, et al. Rapid induction of antigen-specific CD4(+) T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity*. 2021;54(9):2133-2142.

Pardi, N., Hogan, M..J, Weissman, D..(2020). Recent advances in mRNA vaccine technology. *Current Opinion in Immunology*. 65:14-20.

Pascolo S (2004). "Messenger RNA-based vaccines". *Experimental Opinion on Biological Therapy*. **4** (8): 128594. doi:10.1517/14712598.4.8.1285. PMID 15268662.-S2CID 19350848.Policy development. (2023). mRNA vaccine in livestock. Farm bureau. https://tnfarmbureau.org/wp-content/uploads/2023/07/mRNA-Vaccines-in-Livestock-2023.pdf

Rackley, D. 2024. Using mRNA vaccines in meat animals Part 2: Do these vaccines hold promise or health concerns? https://www.agproud.com/articles/58702-using-mrna-vaccines-in-meat-animals-part-2-do-these-vaccines-hold-promise-or-health-concerns

Ribas-Aparicio Rosa María, Castelán-Vega Juan Arturo, Jiménez- Alberto Alicia, Monterrubio-López Gloria Paulina and Aparicio- Ozores Gerardo (*2017*). The Impact of Bioinformatics on Vaccine Design and Development. *Vaccines,* DOI: 10.5772/intechopen.69273

Riggs, P. 2023. What is mRNA? The messenger molecule that's been in every living cell for billions of years is the key ingredient in some COVID-19 vaccines. *The Conversations*. https://theconversation.com/what-is-mrna-the-messenger-molecule- thats-been-in-every-living-cell-for-billions-of-years-is-the-key-ingredient-in-some covid-19-vaccines-158511

Saied, A.A. (2022a). Africa is going to develop their own health capabilities for future challenges—correspondence. *International Journal Surgery.* 2022: 99106585

Saied, A.A. (2022b). mRNA vaccines and clinical research in Africa—from hope to reality. *International Journal of Surgery.* 2022; **105**106833

Saied, A.A., Metwally, A.A. Dhawan M., Choudhary O. P., Aiash H. (2022). Strengthening vaccines and medicines manufacturing capabilities in Africa: challenges and perspectives. *EMBO Molecular Medicine.* 2022; 14e16287

Saied, A.A. (2023). Building Africa's first mRNA vaccine facility. *The Lancet.* Volume 402, ISSUE 10398, P287-288. DOI: https://doi.org/10.1016/S0140-6736(23)01119-4

Sadarangani, M., Marchant, A., Kollmann, T.R. (2021). Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. *Nature Reviews Immunology*. 2021;21(8):475-484.

Sharif, S. (2023).We need to talk about mRNA vaccines in the meats we eat. CBC Kitchener-Waterloo. https://www.cbc.ca/news/canada/ -kitchener-waterloo/university-of-guelph-ontario-veterinary-college-covid-19-1.6901872

Savinova, T.E.,. Brzhozovskaya, D., Shagin, Y., Mikhaylova, A., Shelenkov, Y., Yanushevich, N., Mayanskiy, A. (2020). Multiple drug-resistant Streptococcus pneumoniae of serotype 15A occurring from serotype 19A by capsular switching, *Vaccine*,Volume 38, Issue 33, 2020, Pages 5114-5118,ISSN 0264-410X, https://doi.org/10.1016/j.vaccine.2020.05.075. (https://www.sciencedirect.com/science/article/pii/S0264410X20307301)

Smallpox (2020). Smallpox vaccination. *Science*. 2009-12-02. https://www.nationalgeographic.com/science/article/Smallpox

Stewart, A.J., Devlin P.M (2006). "The history of the smallpox vaccine". *The Journal of Infection*. **52** (5): 329–34. doi:10.1016/j.jinf.2005.07.021. PMID 16176833.

Talagrand-Reboul E, Westermann B, Raess MA, Schnell G, Cantero P, Barthel C, Ehret-Sabatier L, Jaulhac B, Boulanger N. Proteomic as an Exploratory Approach to Develop Vaccines Against Tick-Borne Diseases Using Lyme Borreliosis as a Test Case. *Vaccines (Basel)*. 2020 Aug 21;8(3):463. doi:10.3390/vaccines8030463. PMID: 32825641; PMCID: PMC7564290.

The Antibody Initiative (2020) -- Veterinary Vaccines and Serums". *National Museum of American History*. Retrieved 2020-05-26.

Tizard Ian Rodney (2023). Types of Vaccines for Animals. *Professional Version*. Can mRNA vaccines help boost livestock production? Queen university https://www.uq.edu.au/news/article/2024/03/can-mrna-vaccines-help-boost-livestock- production

Vahoeven David (2023). mRNA Vaccines Could Prevent Diseases in Farm Animals https://www.scientificamerican.com/article/mrna-vaccines-could-prevent-diseases-in-farm-animals/ https://theconversation.com/vaccines-using-mrna-can-protect-farm-animals-against-diseases-traditional-ones-may-not-and-there-are-safeguards-to-ensure-they-wont-end-up-in-your-food-204774

Wang, J., Xiao, J., Zhang, J., Chen, H., Li, D., Li, L., Cao, J., Xie, L., & Luo, Y. (2020). Effects of dietary Cu and Zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of Nile tilapia (Oreochromis niloticus). *Fish & Shellfish Immunology*, *100*, 198-207. https://doi.org/10.1016/j.fsi.2020.03.012

WHO | Smallpox vaccines (2020). *WHO*. Retrieved 2020-05-26.

Xia, M., Pengwei, H., Xi, J., Ming T. (2019). Immune response and protective efficacy of the S particle presented rotavirus VP8* vaccine in mice. Vaccine. 9;37(30):4103-4110. doi: 10.1016/j.vaccine.2019.05.075.

Xu, S., Yang, K., Li. R., et al. (2020). mRNA vaccine era-mechanisms, drug platform and clinical prospection. *International Journal of Molecular Science*. 2020; 21 (18): 6582.

Yangzhuo, G., Jiangyao, D., Na, Y, Yuxin, Y. and Xing Z. (2022). mRNA vaccines in the prevention and treatment of diseases. *Med Comm.* 3(3): e167.doi: 10.1002/mco2.167

Zimmer, C. (2020). "2 Companies Say Their Vaccines Are 95% Effective.What Does That Mean? You might assume that 95 out of every 100 people vaccinated will be protected from Covid-19. But that's not how the math works". *The New York Times*.