BIOSCIENCES REPORTS

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ISSN: (Print)
ISSN: (Online)

THE IMPACT OF IMMUNIZATION IN PREVENTING AND REGULATING AVIAN INFLUENZA IN POULTRY

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Abstract

Avian influenza (AI), caused by influenza A viruses, remains a significant challenge to both the poultry industry and global public health. The rapid transmission and potential for mutation of the virus complicate efforts to control outbreaks. Immunization has become one of the most effective methods for managing AI in poultry, helping to reduce the frequency of outbreaks and the severity of infections. This review examines the role of immunization in preventing and regulating avian influenza, focusing on vaccine types, effectiveness, and the challenges associated with their implementation.Inactivated vaccines have played a pivotal role in AI control, offering considerable protection against circulating strains in poultry populations. These vaccines trigger robust immune responses, reduce viral load, prevent severe disease, and limit viral transmission among birds. However, challenges persist, including the emergence of new viral strains, incomplete cross-protection between vaccines and circulating strains, and variability in vaccine efficacy across poultry breeds. Successful vaccination programs require a strong infrastructure for proper administration, surveillance, and monitoring. Recent innovations, such as recombinant technology and adjuvants, are expanding the range of protection, addressing some of these challenges. Despite these advancements, obstacles like high costs, regulatory issues, and concerns about vaccine safety and efficacy continue to hinder widespread adoption. The success of immunization strategies depends on an integrated approach that combines vaccination with biosecurity measures and continuous surveillance to detect emerging strains.In conclusion, effective management of AI outbreaks requires coordinated strategies that integrate vaccination, biosecurity, surveillance, and international cooperation. Ongoing research into improved vaccine formulations and management practices will be essential in reducing AI's impact on the poultry industry and minimizing risks to human health.

Keywords: "Avian Influenza", "Poultry", "Immunization, Protein", "Virus Cycle"

Article History

Received:
January 05, 2024

Revised:
February 08, 2024

Accepted:
March 03, 2024

Available Online:
June 30, 2024

INTRODUCTION

Approximately sixteen million tonnes of poultry products were traded in 2022, making it a major dietary supply for many people. During the next ten years, it is projected that more than half of the growth in meat output will come from poultry. With productivity rising by just 0.6 percent in 2022 over 2021—the weakest rate of expansion on record—the meat from poultry market is slowing down (FAO, 2022). The manufacturing of eggs, which reached over 86 million metric tonnes in 2020, is another vital nutritional item provided by the chicken business. By the year 2029, it is projected that the world's egg output would have increased by thirteen percent, with China and India accounting for almost half of this growth. However, serious illnesses brought on by microbes like Salmonella and germs like avian influenza virus (AIV), Newcastle disorder virus (NDV), and Marek's syndrome virus (MDV) endanger farmed chickens and the egg output that goes along with it (Charkhkar et al., 2024; Scharff, 2020). Since viral outbreaks often lead to reduced growth in weight, decreased production of eggs, and increased risk of pandemic events, infections in chicken flocks are a major cause of financial losses in the worldwide broiler business. The substantial epidemics of highly virulent avian influenza virus (HPAIV) that occurred in various important poultry-producing areas, particularly South and North America and the European Union, are partly to blame for the drop in the trade in poultry meat seen in 2022.

According to Abdul-Cader et al. (2018), vaccination is mostly used to treat avian illnesses, with an emphasis on reducing disease related death and disability as opposed to preventing an infection itself. In chicken farms, antimicrobial treatment for bacterial illnesses is commonly used as a proactive remedy as well as a prophylactic measure. Determining the precise pathogenic agent, knowing how much to give, and being aware of potential drug interactions are all necessary for successful treatment. Due to a shortage of antimicrobial treatments for poultry, accurate diagnosis is essential for both preventing the growth of resistant bacteria to antibiotics and effectively treating diseases. When taken as a whole, these factors make chicken vaccination a safer and more economical method of preventing disease in flocks of birds (Bodman-Harris, Rollier, & Iqbal, 2024).

A chicken illness known as avian influenza (AI) is brought on by infections with kind A influenza viruses, most especially the viruses that cause avian influenza (AIV). The 8 segments of gene that make up all avian influenza viruses (AIV) can be divided into distinct subtypes based on the glycoproteins on the outside of them. These types include the 9 neuraminidase (NA) variants (N1–9) and the sixteen a protein called (HA) subtypes (H1–16), which are stored by gene segments 4 and 6, accordingly As a result, every AIV will have a classification that encompasses hemagglutinin (HA) and neuraminidase (NA) variants, including H5N1, H9N2, and so on. Based on their capacity to cause illness and death in hens (Gallus gallus domesticus) via an intravenous pathogenicity test, AIVs are divided into two main pathotypes: low pathogenesis (LP) and high pathogenesis (HP).Low virulent avian influenza viruses (LPAIV) can be of any H1–16 a subtype, but all naturally found highly infectious avian influenza viruses (HPAIV) are either H5 or H7 subtypes. At one stage of its existence, the HPAIV is the result of an alteration in either the H5 or H7 LPAIV. For more than 20 years, the extremely virulent avian influenza virus (HPAIV) A/goose/Guangdong/1/1996 (Gs/GD) branch of H5 genotype AIVs has been widely distributed. LIV

causes a localised disease in the digestive and breathing mechanisms of poultry, resulting in a variety of medical presentations ranging from moderate to severe respiratory problems to preclinical disease (Swayne & Sims, 2021).

The highly infectious avian influenza (HPAI) A (H5N1) clade 2.3.4.4b viruses has been spreading over the globe since the year 2021, causing significant mortality and morbidity in domesticated birds as well as affecting the chain of custody for poultry goods and the safety of consumer food. The function of vaccination in controlling avian influenza in chicken had to be reevaluated in light of this circumstance. The World Organization for Animal Health (WOAH) recognized the constraints and unsustainable nature of relying solely on mass slaughter and conventional biosecurity protocols as control options throughout its 90th Annual Meeting on May 25, 2023. About sixty-four million industrial ducks will be the target of a trial immunisation program launched by France on the first of October in 2023.Up until now, vaccination has been a very controversial method of preventing HPAI in chickens. The main reason for this, which is in line with current methodical requirements for based on research veterinary care, is concerns that vaccination may impede investigations by masking HPAI-related death (Swayne & Spackman, 2013; Swayne, Spackman, & Pantin-Jackwood, 2014), leading to unnoticed transmission (Peyre, Fusheng, Desvaux, & Roger, 2009) and widely alteration (Tian et al., 2015), potentially causing human spread and a new worldwide epidemic wave. However, challenging tests with small sample sizes provide the majority of the information on the efficacy of immunization in poultry at home.

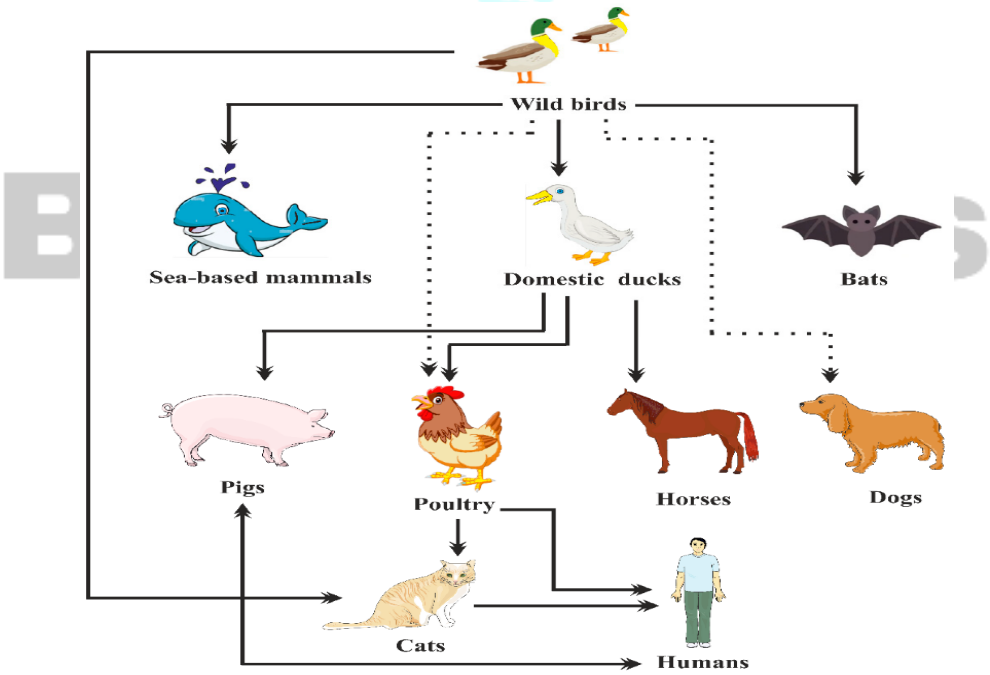


Figure: The onset of flu A virus that comes from wild marine bird reserves. Using groundwater or spores, avian influenza strains can infect household free-range ducks and marine life. Contaminated water, domestic ducks, or direct contact with wild birds can all spread the disease to other species of birds, including poultry. Other household animals susceptible to the influenza virus infections are dogs and cats. People can get the influenza virus from poultry, and cats can also infect people. Lines with

dots indicate distribution that avoids an ordinary duck middleman.

Epidemiology of Avian Influenza Viruses

IVs can cause moderate to severe respiratory tract infections in people, which is an important safety issue. Periodic influenza viruses, such as H1N1, H3N2, and influenza B, cause between three to five million serious infections and 290,000 to 650,000 fatalities globally each year, according to the World Health Organisation (WHO) (Authority et al., 2023). Furthermore, a variety of zoonotic diseases can be caused by avian influenza viruses (AIVs), including H5N1 and H7N9. When infections from animal reservoirs transcend the species barrier, usually as a result of an antigenic change during a reassortment phase between a human influenza virus and an avian influenza virus, pandemics ensue. In addition to causing additional issues and fatalities than regular influenza epidemics, these global epidemics have the potential to claim millions more lives. The global epidemic known as "Spanish Influenza" occurred 440 years ago. About 500 million people, or nearly a third of the world's population size, were infected with the virus during the pandemic of 1918, which led to an estimated fifty to hundred million deaths (Yang et al., 2022). In the year 1979, avian H1N1 infections were found to co-circulate with traditional swine flu viruses after infiltrating a porcine herd in Eurasia. In India, an H1N1 outbreak in 2015, resulted in over 10,000 infections and 774 deaths. Usually, viruses with glycoproteins on the outside like HA and NA—against which the body's immune system is comparatively weak—cause epidemics.

This was true in 1918, when most people appeared to be unaware of the two viruses H1 HA and the N1 NA, and in 1957, when most citizens were immune to the two the H2. While the H3N2 pandemic influenza's N2 element originated from the formerly prevalent H2N2 virus, the H3 HA initially spread to people in the year 1968. The global epidemic variant of the H1N1 virus had antigenically different H1 and N1 surface glycoproteins, while a seasonality version infected individuals in 2009 (Krammer et al., 2018). After spreading among people during the 1968 a worldwide epidemic, the H3N2 virus has since caused other epidemics of influenza.H3N2 infections have come from a variety of origins, including pigs, wild birds, and chickens at home. Several H3N2 infections that emerged in birds have caused severe respiratory illnesses in dogs. An individual influenza outbreak might break out if different H3N2 virus branches were to infect people (Dey et al., 2023).

Influenza Virus Life Cycle

As seen in the second figure, the influenza virus's propagation cycle consists of multiple phases and goes like this: (1) The flu attaches itself to the chemical sialic acid receptors; (2) the virus enters the cell that it is attacking; (3) the viral particles fuse and uncoat; (4) vRNPs enter the cytoplasm, where they are tracked by the replication and transcription of the viral RNA genome and afterwards exported from the a nucleus; (5) the viral parts are assembled and budding occurs at the host cell membranes; and (6) fresh viruses are released from the host cells.

The first stage of viral transmission is when hemagglutinin (HA) attaches itself to sialic acid sensors on the outermost layer of the host cell. Glycosidic linkages are what bind sialic acids to HA's carbs. The α (2, 3) connection, which is found in the respiratory tissue of humans, apes, horses, and pigs as well as the upper airway the epithelial and the α (2, 6) linkage, which is found on the outer layer of cells of the human the top breathing system and

the respiratory tract of bats and swin , are both essential for the accuracy of HA (Bao et al., 2022).

After wrapping, the virus is encased within a cell's endosome and reaches the host cell through receptor-mediated endocytosis. When the endosome's acidic pH (pH 5–6) allows the viral and the endosomal membranes to merge, the M2 ion circuit protein is activated, neutralizing the nucleus and causing the merger and removal of viruses. This mechanism permits vRNP to be released from M1, entering the nucleus of the host cell and then into the DNA.

The transcription and expansion follow vRNP arrival in the nucleus. The viral protein molecules that make up the vRNP (NP, PA, PB1, and PB2) identify nuclear localization signals that attach to the molecular DNA import equipment, making it easier for them to enter the nucleus for replication and transcription. Viral Nucleotide-dependent RNA polymerase (RdRp) first transforms negative-sense RNA into positive-sense RNA, which serves as the basis for the generation of viral RNA. This is followed by inner RNA synthesis. By interacting with the major component of RNA Polymerase II (Pol II), the C-terminal portion of the RdRp promotes transcription and the production of adult mRNA. Nuclear pores are then used to export vRNPs from the infectious core (Herold, Becker et al. 2015).

The virus's protein sections, specifically HA, NA, and M2, migrate to the basal border area where viruses bud from polarized epithelial cells, resulting in the formation of viral elements and budding. The production of viral fragments is followed by the expulsion of new viruses from host cells, when neuraminidase cleaves residues of sialic acid from glycoproteins and glycolipids, allowing the release of freshly created viral nanoparticles from the host membrane to neighboring cells.

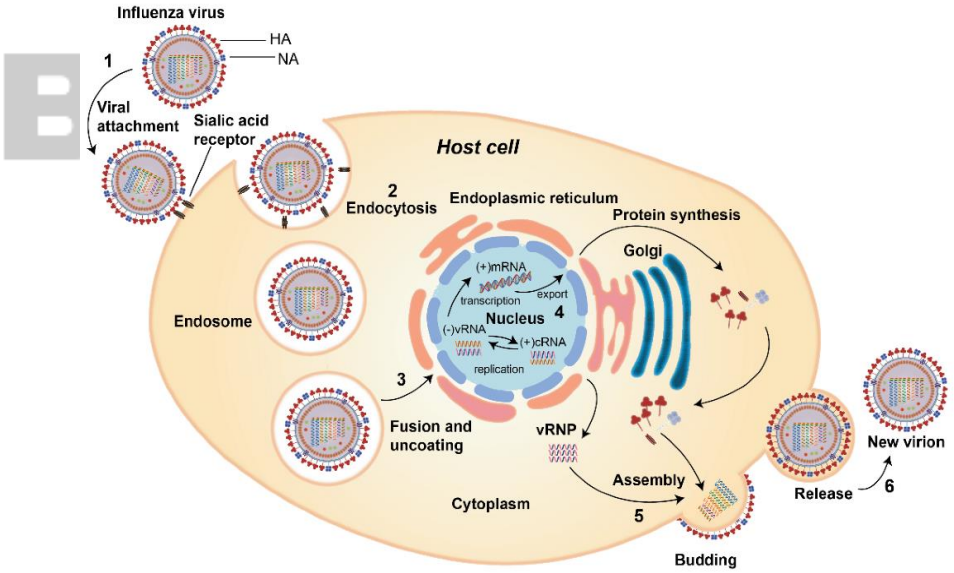


Figure: The existence cycle of pandemic Virus undergoes a series of phases: (1) hemagglutinin (HA) attaches to the sialic acid antibody; (2) endocytosis allows the virus to enter the host cell; (3) combining and removal of the viral particles; (4) viral ribonucleoproteins (vRNPs) enter the nucleus, where they are tracked by replication and transcription of the viral RNA genome and

subsequently exported from the nucleus; (5) viral component assembly and budding at the host cell membrane; (6) new virions are released from the host cell.

Diagnosis of Avian Influenza Virus

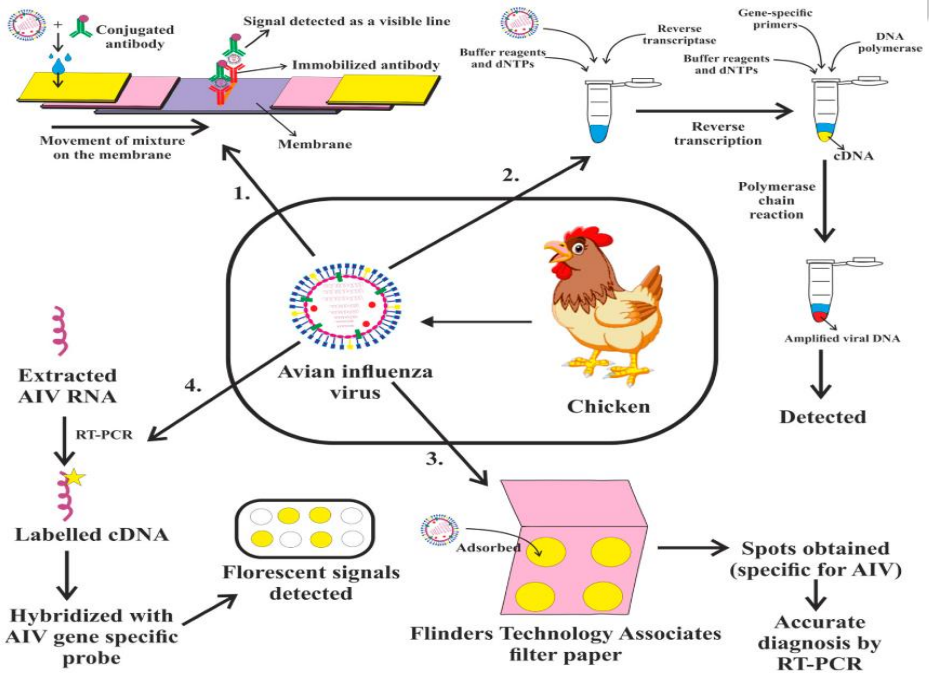
AI treatment involves combining traditional approaches with quickly developing capabilities. A analytical tool's suitability for a given task, scientific ease of use, velocity, detection empathy, and price are some of the variables that may influence the choice. The two types of procedures used to accurately identify the AI virus are those that identify a certain antibody, which indirectly indicates contact, and those that detect the virus directly. Simple identification includes both traditional viral culture techniques and rapid procedures, such the PCR assay or separating viruses in cell culture, that can identify particular viral antigens or nucleic acids.

Serology; Evaluation of Influenza the Use of Serologic Tests to Retrospectively Determine Infections

The Antibody based determination of AI viral contact concludes advancements in traditional agar-gel immunodiffusion and hemagglutination restriction (HAI) strategies as well as effective ELISA-based approaches that offer instant ability to detect and allow the accurate identification of AI subtypes. For the clinical detection and surveillance of IAVs, immunochromatography (IC), an antigen based assay, is an essential or quick Clinical equipment (Sakai-Tagawa et al., 2010).

Virus Isolation Test

The idea is similar to that of an ELISA sandwich. Client nose swabs are quickly mixed with colloidal gold, colored latex, antibody to transmissible substances, or a specific enzyme. To get the peptide, the combination is placed on a specimen pad composed of a layer made of nitrocellulose and a protein appropriate to the immobilized viral antigen. When the mixture's antigen–antibody combination crosses the membrane and engages with the immobilized antibody, a line or dot indicates a successful outcome. (Figure 7).



Many techniques for identifying the virus that causes avian influenza. (1) shows how to identify the avian influenza virus (AIV) via immunochromatography; (2) shows how to detect AIV using the RT-PCR methodology; (3) shows how to detect AIV using the filter paper approach from Sheffield Technique Partners; and (4) shows how to detect AIV using DNA microarray technologies.

Rapid Influenza Diagnostic Tests

While additional infections typically feature heterophilic to fibrin cellular infiltration, AI infections might show close edema and lymphocytic infiltration. Turkeys are more likely than poultry to have diarrhea and acinar cell degradation. HPAIV-induced lesions are particularly noticeable in vulnerable bird species. The skin that is exposed and visceral organs have edematous, hemorrhaging, and necrotic lesions (Hunter, 1998). Confirmation testing for the AIV should be started if there is a record of multiple unexpected deaths, frequently within a brief period of time, along with common lesions like cyanosis, edema, and hemorrhage in the head and limbs, petechial hemorrhages in visceral organs, and hemorrhage and necrosis of the mucous membranes. (Songserm et al., 2006).

Types of Vaccines:

The ideal AI vaccine should be affordable, work with various bird species, provide protection after a single dose, use inexpensive mass usage methods, make it easy to identify diseased birds in the immunized group, produce an immune system defense even when the mother has antibodies, be given in ovo or hatcheries at one day of age, and have antibacterial resemblance to field infections. The user must select approved vaccines that provide the greatest number of optimal characteristics relevant to their needs, as no vaccine or vaccine technology currently in use satisfies all eight requirements.

Inactivated Vaccines:

The most common method for producing avian virus vaccines is the deactivated avian influenza virus vaccine, which is created by cultivating the beginning virus in embryonated chicken eggs. The efficacy of this approach is relatively poor, and sufficient antigen production requires a large number of viable eggs. The host immunity recognizes the HA protein's conspicuous and extremely changeable immunodominant spherical head areas, making it a desirable target for vaccine development. Previous studies suggest that HA immunity may be altered by seed viral spread in eggs, giving rise to an inflammatory, disagreement with pandemic isolates and reducing vaccines effectiveness. cultivated lines of mammals may be used for viral replication in order to avoid HA alterations brought on by egg transmission. Moreover, clinical studies have confirmed the safety and effectiveness of a mammal cell based influenza vaccination, which gives equivalent or better immunity protection in the animal models than egg based vaccinations. There are many benefits to using lines of mammals for viral culture, involving the utilization of completely characterized and standardized cell and the ability to facilitate massive cultivation in the event of an impending pandemic. The effectiveness of the manufacturing procedure for cell-based vaccinations depends on the optimization of massive amounts of virus vaccine batches in addition to the capacity of a specific cell to create a virus vaccine. The ability of two immortal cell lines—African green monkey kidney (Vero) cells and Madin-Darby canine kidney (MDCK) cells—to produce influenza virus has currently been

investigated. After being extracted and filtered, the viruses are killed and then mixed with an oil additive. Given its ability to contact with a wide variety of groups that are reactive, such as proteins, RNA, or DNA, causing alkylation and homo- or bifunctional cross- linkage, formaldehyde is regularly used for deactivation to generate complete, inactivated vaccine. However, some disadvantages associated with the use of formaldehyde have been identified: one concerns the harmful effects of remaining formaldehyde in the vaccines, and other concerns the alteration of the antigen epitope as a result of formaldehyde's chemical change (Xu, Zhu, Govinden, & Chenia, 2023).

Virus-like Particle (VLP) Vaccines;

The development of influenza vaccines has made extensive use of VLP-based vaccinations, which have shown encouraging results in terms of immune defence. Hemagglutinin (HA), neuraminidase (NA), and matrix protein 1 (M1) are the 3 pandemic virus proteins that scientists have shown can be produced in the cells of insects and reassembled into virus-like particles (VLPs) for the H5N1, H3N2, and H9N2 vaccines. A bivalent H5+H7 VLP vaccine was created by (Hu et al., 2021) by producing the HA, NA, and M1 proteins using a baculovirus production method. Both the advertisement inactivated vaccines and the bivalent VLP vaccine produced strong immune responses, as evidenced by the production of antibodies that neutralize the virus, prevented hemagglutination, and concentrated on hemagglutinin.

DNA Vaccines

An antigen-coding gene is integrated into a non-replicative eukaryotic translation plasmid carrier and delivered to the host organism by direct transfer of genes in a traditional DNA vaccination. A plasmid encodes an antigen protein that is expressed by the host's cell and then delivered to immune cells via the major histocompatibility complex, or MHC, pathway. The immune system's reaction generated by DNA vaccinations is largely of the Th1 type, which includes an increased focus on immunity mediated by cells as opposed to immunity mediated by humour. According to a previous study, the DNA vaccination process mimics the virus's cellular pathology. Inside cells the proteasomes synthesize and break down antigenic peptides into smaller amino acids..

mRNA Vaccines

Martinon et al. conducted the groundbreaking study on the efficacy of a conventional mRNA vaccination for flu in 1993. Mice that received a liposomal vaccination containing the influenza nucleoprotein (NP) developed a cytotoxic T-cell response. Later, other studies demonstrated that injecting different mRNA expressing HA, NA, and NP into pigs, ferrets, and chickens elicited detectable immune system reactions. Currently, there are two main forms of mRNA vaccines: self-amplifying mRNA (saRNA), which is produced from an RNA viral vector and has auto-replicative properties, and traditional, non-amplifying mRNA molecules. It is possible to create non-replicating mRNA vaccines by combining various modified nucleosides. The creation of vaccinations targeted especially against influenza viruses has been the subject of numerous investigations. Within four weeks following injection, mice and ferrets given just one 3 µg dosage of an mRNA-LNP-HA vaccination produced neutralising antibody levels greater than 1:120, according to Pardi et al. According to the dosage and method of management, a second dose increased the hemagglutination inhibition antibodies titers to values between 1280 to 20,480. After RNA vaccines

expressing influenza hemagglutinin, nucleotide, and neuraminidase were administered intradermally to mice, ferrets, and pigs, protective immune reactions were seen. activation of T cells was elevated in mice given a subcutaneous injection of the unaltered mRNA-lipid combination encoding the HA of the PR8 H1N1 influenza A virus.A Plasmid DNA, in vitro produced RNA, and virus-like RNA nanoparticles are some of the forms in which saRNA vaccinations can be delivered. The use of saRNA vaccines to fight the influenza virus has been reported in multiple research studies. A study conducted by Fleeton et al. (2001) showed that mice immunised with 10 mg of a saRNA vaccine expressing the HA of the PR8 H1N1 influenza A virus developed an immune defence against a lethal homologous viral challenge. After two intramuscular injections, mice in a different study that used lipid nanoparticles to encapsulate saRNA-encoding HA showed protective coagulation suppression levels (Xu et al., 2023).

CONCLUSIONS

In conclusion, poultry production remains a crucial global food source, with significant contributions from both meat and egg production. However, the industry faces considerable challenges from infectious diseases, particularly avian influenza, which has led to substantial economic losses and concerns regarding public health. While vaccination serves as a key strategy to mitigate disease spread, its implementation remains controversial due to concerns over virus mutation, surveillance hindrance, and potential zoonotic transmission. Advances in diagnostic methods, such as PCR assays and immunochromatographic tests, are crucial for early detection and effective management. Continued research into sustainable control measures and vaccination strategies is vital to ensuring the stability of the poultry industry and safeguarding public health against potential pandemics.

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