Vaccines, Existing and Prospective Measures against Avian Influenza – A review

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Abstract

Avian influenza (AIV) is a respiratory disease in bird species that causes frequent outbreaks in poultry farms worldwide, leading to economic loss, loss in food security, a threat to the longevity of wild bird populations, and a threat to public health. This review aims to sum up and discuss existing and prospective measures against highly pathogenic avian influenza outbreaks and the developments and possibilities to use vaccination as a supporting strategy. Traditional methods to control AIV outbreaks consist of biosecurity, education, diagnostics and surveillance and elimination of infected poultry. As this system has been found insufficient to curb AIV outbreaks without great pushbacks for the economy and food-security, vaccination is considered an important possible second-tier component. Though having some drawbacks, inactivated vaccines are the most commonly used vaccines against AIV due to their safety to use and ability to induce decent humoral immune responses. Recent research on inactivated vaccines found it possible to administer inactivated vaccines via inhalation as a means of passive immunization, making it possible to circumvent some of the drawbacks to inactivated vaccines. Several studies opt for using virus-like particles against AIV outbreaks. These vaccines are seemingly cheap to produce, suitable for mass vaccination via oral intake and have high immunogenicity. While recent research seems promising, this review finds that additional developments need to be made to existing vaccine concepts in order for vaccine implementation to be feasible and effective in real-world settings. Efficient means of mass administration should further be researched and translated to real-world settings, as well as the practicality of vaccine development and transport of vaccines for locally emerging strains. Moreover, for vaccination strategies to be able to take off, countries should consider lifting their current embargos regarding the import of vaccinated poultry and poultry products. In their turn, the used vaccines should be able to adhere to the DIVA-principle (Differentiation Infected from Vaccinated Animals) to ensure safe trade of products from immunized birds.

Key words: Avian influenza – HPAI – Influenza Risk Assessment Tool – Avian influenza infection in humans - Inactivated vaccines - Virus-like particles – Plant-based vaccines – DIVA principle

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Introduction

Avian influenza is a respiratory disease in bird species caused by influenza A viruses (Perez *et al.*, 2019). For the past decade, there have been concerns about the rising emergence of avian influenza. Researchers contribute this increase in avian influenza virus (AIV) outbreaks to a combination of factors, including the global increase of commercial poultry under conditions that do not prevent contact with wild birds and carriers of AIV. Increased monitoring of avian influenza in recent years is also seen as an important contributing factor to the increase in AIV numbers, as we have become better at detecting AIV (Perez *et al.*, 2019). According to the European Centre for Disease Prevention and Control, 2,653 highly pathogenic avian influenza (HPAI) virus detections were reported in 33 EU/EEA countries and the UK between December 9th 2021 and March 15th of 2022 alone. In more recent months, distress has been rising due to ongoing avian influenza outbreaks in both Europe and the USA. The USA reported many infections throughout 36 states, with 40 million poultry being infected with H5N1 AIV (HPAI strain) this year, and 1635 infected wild birds (CDC, 2022). The Netherlands knows an ongoing outbreak as well, as there are generally bi-weekly reports of AIV in poultry (Animal Rights, 2022; Rijksoverheid, 2022), resulting in the culling of nearly 3 million birds in the first six months of 2022 (Animal Rights, 2022).

The prevalence of AIV is considered a threat for several reasons. Firstly, AIV infection of one or a few birds in poultry farms often leads to the culling of all of the birds in the farm, causing dangers to food security and economic losses (Zadorsky, 2022). Consequent restrictions in trade also lead to economic losses and dangers to food security (Moore and Morgan, 2006). Secondly, the pathogenicity of AIV poses a danger to wildlife bird populations (Zadorsky, 2022). Thirdly, there are concerns about public health with regards to AIV outbreaks (Li et al., 2019). Though rare, humans can get infected with zoonotic strains of avian influenza, usually through intensive contact with AIV-infected birds (Li et al., 2019). Human infection with AIV often presents with severe symptoms and possibly death (WHO, 2018). For example, the H5N1 AIV strain has an estimated case fatality rate between 14% and 60% (Li et al., 2008; WHO, 2018; World Health Organization Western Pacific Region, 2022). Globally, from January 2003 to June 2022, there have been 864 cases of human infection with H5N1 AIV reported from 18 countries. Of these 864 cases, 456 were fatal, with the most recent case of AIV infection in humans being reported in the USA in April of 2022. Another serious public health concern about AIV outbreaks is the possibility of pandemic and/or epidemic situations. Mutations in an avian influenza virus could cause the virus to become capable of human-to-human transmission (Li et al, 2019). In the past century, there have been several pandemics caused by avian influenza viruses, with the Spanish Flu being the most infamous example of these pandemics (Berche, 2022; CDC, 2019a).

Traditionally, elimination of infected poultry (stamping out) was a popular method to control AIV outbreaks, though this method has enormous costs for food security, economy and animal wellbeing (Swayne, 2012). More recently, more attention is given to biosecurity, diagnostics and surveillance as well to control AIV outbreaks to prevent infection, secure the production sector and food security, as well as to limit the risk of human infection (Swayne, 2012; Perez *et al.,* 2019). Even so, when taking all these components into account, AIV outbreaks can still have devastating effects and are sometimes incontrollable (Swayne, 2012). Therefore, methods to reduce host susceptibility, e.g. vaccination are also being experimented with, and methods to optimize vaccination (Swayne, 2012; Nurzijah et al., 2022). Vaccines can be implemented in three ways: as a preventive tool when risk of poultry infection is high, as an emergency response after outbreak initiation, or as a routine measure when infection is enzootic within the country or area (Swayne, 2012). Still, the use of vaccines faces some challenges. Current vaccines often fail to induce a proper immune response against the rapidly evolving AIV strains, administration of vaccines by injection is impractical for poultry, there are trade restrictions against vaccinated poultry, and the costs of AIV vaccines discourages their use (Perez et al., 2019; Wageningen Bioveterinary Research centre, 2021; Swayne, 2012; Nurzijah et al., 2022).

Having established that avian influenza outbreaks are a serious issue, researchers and authorities have been searching for effective solutions. This review will discuss and evaluate existing and prospective strategies to tackle highly pathogenic avian influenza outbreaks in poultry that are focused on decreasing host susceptibility. Firstly, the viral properties of AIV will be analysed. Transmission of AIV to humans will be discussed as well. Then, current solutions to AIV outbreaks will be referred to, with a focus on vaccination possibilities. Ultimately, this review aims to answer: What (vaccine) measures are effective to tackle avian influenza outbreaks?

Avian influenza

Structure of avian influenza viruses

Influenza viruses are negative, single stranded RNA viruses that are part of the *Orthomyxoviridae* family in the Influenza A viral species (Wong and Yuen, 2006). The eight RNA segments of the influenza A virus genome encode 11 viral proteins, including RNA polymerase proteins (PB1, PB2, PA, PB1- F2) and glycoproteins neuraminidase (NA) and hemagglutinin (HA) (Wong and Yuen, 2006). Hemagglutinin and neuraminidase sit evenly distributed over the surface of the virion and are the major antigenic determinants of influenza A viruses. These two proteins make up the framework for influenza subtype classifications (Wong and Yuen, 2006; Sogaolu, 2005). The HA protein is essential for host-cell adhesion, and does so by binding to sialic receptors at the hosts' cell surface. The binding affinity of HA to the sialic acid residues partially contributes to the host specificity of the various influenza A virus subtypes. Hemagglutinin is also the central viral target of protective humoral immunity by neutralizing antibody (Wong and Yuen, 2006). Neuraminidase (NA) is important in releasing virus copies from infected cells (Young, 2016). The NA-protein facilitates the spread of virions in the host by cleaving the glycosidic linkages to sialic acid residues on host cells and the surface of viral particles (Wong and Yuen, 2006). As of 2022, there exist 18 hemagglutinin types (H1 to H18) and 11 neuraminidase types (N1 to N11) (Chen, Pu-xuan and Yu-xin, 2021).

The surface proteins of viruses such as influenza are subjected to antigenic drift, making them a tricky target for adaptive immune responses. The virus constantly evolves because the viral polymerase lacks proof-reading ability, leading to amino acid changes on the viral surface proteins caused by replication errors (Perez et al., 2019). As a consequence, surface proteins change their antigenic features in such a way that preceding antibody responses against the virus are no longer effective (Perez et al., 2019). Especially antigenic drift in HA is common and impactful for the viruses' ability to survive. Antigenic drift is also observed in NA, but NA's mutation rate is slower than the one of HA, most reasonably because antibody responses against NA are not as influential to stop virus infection and spread (Perez et al., 2019). As the genome of influenza viruses are segmented, gene reassortment is possible, leading to more viral diversity (Wong and Yuen, 2006; Perez et al., 2019). When two strains infect the same cell simultaneously, it is possible that a fitter, reassorted strain emerges from the cell, further complicating an adaptive immune response and increasing the survival rates of influenza viruses (Perez et al., 2019).

Relevant strains

Generally, strains of avian influenza are categorized in HPAI (highly pathogenic Avian Influenza) and LPAI (lowly pathogenic avian influenza). LPAI strains rarely result in illness for poultry, although they can be potential progenitors for HPAI strains (Sogaolu, 2005). In HPAI strains, there is an insert of additional basic amino acids at the proteolytic cleavage site of HA, allowing proteases in many organs to cleave the protein, causing viral particles to spread widely in the body (Perez et al., 2019; Young, 2016). This causes a portion of HPAI strains to be extremely deadly in some poultry species, notably in chickens, which leads to high mortality in flocks within a day or two upon infection (Young, 2016). Such deadly HPAI viruses with inserts at the proteolytic cleavage site have been restricted to the avian influenza H5 and H7 subtypes to this date (Young, 2016). Though, the majority of influenza viruses circulating in avian species lack this insert and therefore do not cause significant disease in birds, including most H5 and H7 viruses (Young, 2016). To this date, only H5 and H7 subtypes have been linked to HPAI outbreaks (Perez et al., 2019). Most recently, the highly pathogenic subtypes H5N1 and H7N9 are known to have caused two major AIV zoonosis outbreaks, causing human casualties and damages to the poultry industry (Chen, Pu-xuan and Yu-xin, 2021; Li et al., 2019). Hence, these subtypes are often described in literature and surveyed in studies and are often the targeted strains for vaccines.

Human infection with avian influenza

In general, the chance of a human getting infected with AIV (zoonosis) is relatively low (Li et al., 2019; Chen, Pu-xuan and Yu-xin, 2021). Human infections with AIV's can take place when virus gets into a person's eyes, nose or mouth, or is inhaled, usually through droplets or dust in the air or when a person touches something with virus on it and then makes contact with their eyes, nose or mouth (CDC, 2017). The most commonly reported means of infection are either through unprotected contact with infected birds or with unprotected contact with surfaces contaminated with avian influenza viruses (CDC, 2017).

One of the reasons why rates of zoonosis are low is that avian and human influenza viruses typically have a different sialic acid binding preference (de Graaf and Fouchier, 2014). Even so, human infection with AIV is still possible when a few amino acid changes in the HA protein cause a conversion from avian – to human receptor specificity through mutations (de Graaf and Fouchier, 2014). Hemagglutinin is essential in determining influenza's host range, pathogenicity and infectiousness through its ability to bind to sialic acid receptors on host cells (de Graaf and Fouchier, 2014). The HA-proteins of AIV preferentially bind to sialic acid receptors with an α2,3 linkage on host cells. α2,3-SA receptors are mainly expressed in the gastrointestinal tract of birds, hence the main site of replication for AIV is the gastrointestinal tract in birds (Li et al., 2019). On the other hand, mammalian influenza viruses - including influenza A viruses that repeatedly infect humans- infect the upper respiratory tract (Li et al, 2019). This is because these human influenza A viruses preferentially bind to receptors that terminate with an N-acetyl sialic acid and that are linked to a residue in a galactose molecule by an α2,6 linkage (Li et al, 2019; de Graaf and Fouchier, 2014). These α2,6-SA receptors are expressed abundantly in the upper respiratory tract of humans, which explains why the main site of infection and viral replication is in the upper airways of the human influenza A viruses (Li et al., 2019). The "avian" α2,3-SA receptors are also expressed relatively high in the lower airways of humans, such as the lungs. As such, human infection with AIV viruses are possible when infectious particles reach the lower respiratory tract and bind to α2,3-SA receptors (Li et al., 2019). Another reason why AIV's usually do not infect humans is that AIV's prefer replication at temperatures above 37 °C, which are not found in the upper respiratory airways of humans (Li et al, 2019). A single mutation in an AIV's polymerase gene can enable temperature adaptation, making it possible for the virus to replicate in a more widespread range of hosts (Li et al., 2019). The risk for a human pandemic rises when avian influenza becomes transmittable between humans, which is possible when mutations occur. The H1N1 virus that caused the notorious Spanish Flu pandemic in the early 20th century was determined to have been of avian origins (Berche, 2022; CDC, 2019a). Moreover, there have been three other recorded pandemics in the past 100 years that were caused by an avian influenza virus; the H2N2 Asian pandemic in 1957, the H3N2 Hong Kong pandemic in 1968 and the 2009 H1N1 pandemic (de Graaf and Fouchier, 2014).

Strategies to tackle Avian influenza

According to multiple reviews, strategies to control avian influenza generally consist out of four components: 1) biosecurity 2) education, 3) diagnostics and surveillance of the virus, and 4) elimination of infected poultry (Swayne, 2012; Perez et al., 2019). In 1995, farms started to vaccinate poultry for the first time as a response to an AIV outbreak -a measure that plays into the component of decreasing host susceptibility (Swayne, 2012). The addition of vaccination is beneficial and necessary when the regular four components: 1) are not sufficient to restrict AIV spread 2) may cause an irreversible impact on the poultry industry 3) pose a hazard to food supply (Swayne, 2012; Perez et al., 2019). This section will review the aforementioned four regular components.

Biosecurity

Biosecurity measures are the first line of defence against avian influenza. They are important in terms of securing the food production sector and food security, and also serve to limit the risk of zoonosis (Perez et al., 2019). Live bird markets are a well-known and major source for both HPAI outbreaks and zoonosis events and hence an example of poor biosecurity implementation (Li et al., 2019; Perez et al., 2019).

Biosecurity measures concern movement management of poultry and captive birds and hygiene of poultry handlers and the used equipment and vehicles (Perez et al., 2019). They also concern limiting the contact between wild birds and poultry, as wild birds are often carriers of AIV that could set off an AIV outbreak in poultry farms when coming into contact (Perez et al., 2019). Effective biosecurity prevents secondary spread of avian influenza from affected establishments, high-density poultry areas and high-risk production sectors (Adlhoch et al., 2022). Biosecurity measures should be applied in poultry farms, on live bird markets, as well as during transport (Perez et al., 2019).

Education

Education practices in the field of AIV-management involve teaching and advocating continuous, well-executed personal and respiratory hygiene amongst all stakeholders involved in poultry (Chen, Pu-xuan and Yu-xin, 2021; Adlhoch et al., 2022). Hygiene and prevention habits include washing hands frequently, keeping the environment clean, and processing and cooking food properly (Chen, Pu-xuan and Yu-xin, 2021). Education and awareness ensure that guidelines are respected, which consequently provides that the risk of human infection and AIV outbreaks are constricted. Moreover, this level of awareness confirms that producers and workers know how to identify clinical signs of AIV and report symptoms and deaths to veterinary authorities (Perez et al., 2019). The European Food Safety Authority advises that veterinary authorities advertise for fast reporting of suspected cases, and implement effective measures to reduce the exposure risk for occupationally exposed people such as farmers (Adlhoch et al., 2022).

Rapid diagnostics and surveillance

Rapid detection of - and typing of AIV is essential during an outbreak, as it allows for quick and appropriate measures to control the severity of the outbreak. With an eye on the possibility for a zoonosis outbreak, the European Food Safety Authority recommends a strengthened surveillance in mammals and humans that are exposed to infected birds (Adlhoch et al., 2022). Furthermore, the European Food Safety Authority strongly recommends authorities to hold sero-epidemiological studies in exposed people during HPAI outbreaks to determine transmission incidents and to support risk assessments (Adlhoch et al., 2022). Finally, they urge that authorities generate and share complete viral genome sequences from sick wild birds, poultry and domestic birds as promptly as possible in order to detect novel virus introductions (Adlhoch et al., 2022).

Currently, there are rapid diagnostic tests available for AIV that are able to detect the presence of AIV within as little as 30 minutes (Kim et al., 2021). These rapid tests use monoclonal antibodies that target viral proteins and apply either enzyme immunoassay or immune-chromatography (Kim et al., 2021). Such quick diagnostic tests cannot accurately distinguish between HPAI and LPAI, however. Therefore, it is still common to use virus isolation in combination with additional laboratory tests such as RT-PCR to establish more information on the circulating influenza virus and to distinguish between LPAI and HPAI, which can take up to a few days (Charlton 2009; Kim et al., 2021).

The Influenza Risk Assessment Tool (IRAT) is an additional AIV-surveillance tool that allows for more targeted and preventative responses. The IRAT-tool evaluates the potential pandemic risk to avian influenza viruses based on 10 elements (Table 1). IRAT assesses factors in avian influenza viruses that make it possible to shift from being a virus that solely infects birds to becoming a virus that can also infect humans (CDC, 2019; Young, 2018). Stakeholders can then engage in targeted culling of birds infected with the high-risk variant, and researchers will have the chance to make effective vaccines (Young, 2018).

Properties of the Virus	
1. Genomic analysis	*The extent of genetic diversity and/or the presence of known molecular signatures necessary for human infections and disease*
2. Receptor binding	*The host preference of the virus (e.g. human or animal) and preferred tissue and site of infection (e.g. cells, deep lung tissue)*
3. Transmission in animal models	*The measure of the ability of the virus to transmit efficiently in animal models in laboratory studies (e.g. direct contact or airborne etc.)*
4. Antiviral treatment options	*The predicted effectiveness of existing influenza antiviral medications*
Attributes of the Population	
5. Population immunity	*Whether the human population has any existing immunity against the novel influenza virus*
6. Disease severity and pathogenesis	*The severity of illness caused by the influenza virus in question*
7. Antigenic relatedness	*The degree of similarity of the virus in question to seasonal influenza vaccines, pre-pandemic candidate vaccine viruses and stockpiled pre-pandemic vaccines*
Ecology and Epidemiology	
8. Global distribution in animals	*Measure of how widespread the virus in question is in animals, the rate of spread over time, and any management factors that may affect the distribution*
9. Infections in animals	*What animal species become infected by the virus, and the likelihood of human contact with these animals*
10. Human infections	*Evidence and frequency of animal-to-human transmission and under which circumstances these occur*

Table 1: Risk elements used by the Influenza Risk Assessment tool (IRAT), used to assess the potential pandemic risk by influenza A viruses circulating in animals. The 10 risk elements are grouped within three overarching categories. Adapted from CDC (2019b).

Elimination of infected poultry

This part of the strategy is all about effective culling, also referred to as 'stamping out' (Geering and Penrith, n.d.). In short, this involves quarantining suspected infected animals, clean destruction of animals, clean disposal procedures and decontamination of the infected establishment in order to prevent further disease spread (Geering and Penrith, n.d.). This can also involve preventative culling in neighbouring, seemingly uninfected establishments (Peeters et al., 2014). A major downside of stamping out is that it can cause significant damage to food security and the economy (Swayne, 2012).

Vaccination against avian influenza

In many studies, it is emphasized that vaccines are not the sole solution to curb AIV outbreaks (Swayne, 2012; Capua and Marangon, 2004; Perez *et al.*, 2019). Rather, vaccines are considered a valuable supportive tool to other strategies. Vaccines can be implemented in three ways: as a preventive tool when risk of poultry infection is high, as an emergency response after outbreak initiation, or as a routine measure when infection is enzootic within the country or area (Swayne, 2012). One study that researched the use and impact of AIV-vaccination worldwide concluded that vaccination is useful as a second tier component when immediate elimination of poultry is not feasible, to maintain livelihoods and food security and to control clinical disease until a primary strategy can be developed and implemented to achieve eradication of AIV (Swayne, 2012). According to the Wageningen Bioveterinary Research centre (2021), poultry vaccination should follow the following criteria in order to be effective: 1) the vaccine should prevent AIV infection and spread. 2) the vaccine should be suited for mass vaccination, for example through oral administration. 3) the vaccine should live up to the DIVA principle: Differentiating Infected from Vaccinated Animals (DIVA), meaning that the vaccine needs some sort of marker to be able to distinguish infected animals from vaccinated animals during diagnostic surveillances (Wageningen Bioveterinary Research centre, 2021). Additionally, the AI vaccine should ideally be antigenically close to the target virus for the best protection, usable in multiple avian species, and provide single-dose protection (Swayne, 2016). As of this date, there are no licenced AIV vaccines that meet all of the aforementioned ideals (Swayne, 2016).

The different types of vaccines for AIV that currently exist (Table 2) are classified into six groups: 1) inactivated, 2) live-attenuated, 3) subunit, 4) vector-based, 5) DNA, and 6) Virus-like particles (VLP). So far, the most commonly used vaccines against AIV amongst poultry are inactivated vaccines (Nurzijah et al., 2022; Swayne, 2012); between 2002 and 2010, about 95,5% of AIV vaccines used in poultry were inactivated vaccines (Swayne, 2012). The remaining 4,5% of vaccines used in this time period were live recombinant virus vaccines (Swayne, 2012). Recent developments in the field of AIV vaccine research show a trend towards clinical trials with the goal of optimizing inactivated vaccines and subunit vaccines and the invention of new plant-based VLP-vaccines (Scotti and Rybicki, 2013; Landry et al., 2010; Tomar et al., 2018; Shrestha et al., 2021; Nurzijah et al., 2022). AIV vaccines provide protection mainly through systemic humoral immunity against the hemagglutinin protein of a virus subtype. In a humoral immune response, B-cells produce antibodies against the viral protein presented by an antigen-presenting cell. The antibodies provide extracellular protection by preventing viral infection of cells by neutralizing these antigens (Abbas et al., 2018). Cell-mediated immunity contributes to protection against AIV as well (Swayne, 2016). In cell-mediated immunity, phagocytes, cytokines and antigen-specific cytotoxic T-lymphocytes are activated in response to an antigen. These cytotoxic T-lymphocytes destroy AIV-infected cells to hinder the ability of the AIV to survive and replicate (Abbas et al., 2018).

A general distinction in immune responses between non-living and live vaccines must be made. Live vaccines, such as the live-attenuated vaccine, contain attenuated replicating strains of the virus. Administration of a live-attenuated vaccine causes a desired strong immune response, though the major downside of live vaccines is the possibility for the vaccine-administered strain to replicate in an unrestrained manner in an immunocompromised host (Swayne, 2016; Pollard and Bijker, 2021). These live vaccines also have the potential for reversion to virulence and recombination with the circulating field virus (Nuzjirah et al., 2022). These properties pose a significant safety hazard. Hence, non-living vaccines or alternative vaccine options are preferred for poultry vaccination because of their safety (Swayne, 2016). Non-living vaccines (such as inactivated vaccines or subunit vaccines) use either a killed pathogen, purified or recombined proteins of the pathogen, or polysaccharides, thus there is no risk of reversion to virulence, as the pathogen is not alive (Pollard and Bijker, 2021). Drawbacks of non-

living vaccines include that these vaccines require high doses of antigen to provide protection, and that they often require an adjuvant to stimulate an immune reaction (Swayne, 2016).

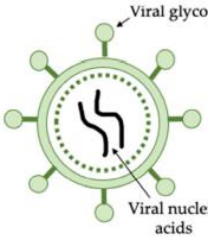
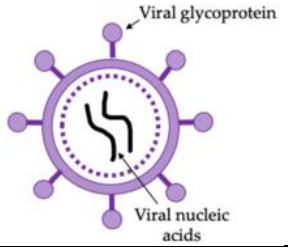
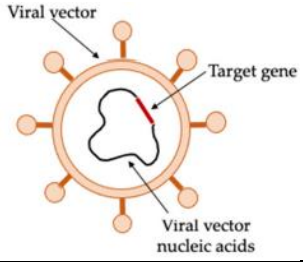
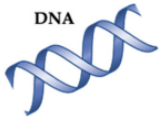
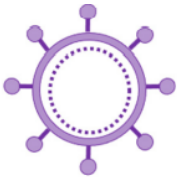
Vaccine type	Advantages	Disadvantages
1. Inactivated vaccines 	- *Good humoral immunity response* - *Possibility for passive immunization* - *Safe, no risk of reactivation of antigen* - *Potential for storage at ambient temperature (20 °C)*	- *Often low immunogenicity* - *Not completely compatible with DIVA principle* - *Requires direct inoculation*
2. Live-attenuated vaccine 	- *Possible to choose most effective antigen* - *High immunogenicity*	- *Safety hazard in immunocompromised birds* - *Possible reversion to virulence* - *Not compatible with DIVA principle*
3. Subunit vaccine 	- *Possibility to choose most effective antigen* - *Safe, no risk of reactivation of antigen*	- *Often low immunogenicity* - *Poor stimulation of cellular immunity* - *Requires direct inoculation*
4. Vector-based vaccine 	- *Stimulation of both humoral and cellular immunity* - *Possible to choose most effective antigen* - *Safe, no risk of reactivation of antigen*	- *Often requires multiple doses* - *Often require cold storage*
5. DNA vaccine 	- *Stimulates cellular immunity* - *Safe, no risk of reactivation of antigen*	- *Usually protects poorly without adjuvant* - *Can have weak humoral immunity* - *Known to be expensive*
6. Virus-like particle vaccine 	- *Stimulates both humoral and cellular immunity* - *Cheap production costs* - *Safe, no risk of reactivation of antigen* - *No need of adjuvant* - *No cold storage required* - *Potential to be compatible with DIVA-principle*	- *Complex production process*

Table 2: Six categories of AIV vaccines and their advantages and disadvantages. Adapted from Nurzijah et al. (2022)

As most research is being done on inactivated vaccines and virus-like particle vaccines in relation to AIV, this review will elaborate further upon inactivated vaccines and virus-like particles (VLP's).

Inactivated vaccines

Inactivated vaccines are a type of non-living vaccines that use a killed virus particle consisting of viral nucleic acids and viral glycoproteins to stimulate an immune reaction (Pollard and Bijker, 2021). The antigen -in this case viral particles of an AIV- will be taken up by an antigen-presenting cell and presented to T-cells, which then stimulates T-helper cells to initiate an immune response against this antigen via the stimulation of B-cells (Pollard and Bijker, 2021). This results in a primarily humoral response of the adaptive immune system, though the addition of an adjuvant to the vaccine can stimulate a cellular response as well (Pollard and Bijker, 2021). Whilst inactivated vaccines are safe to use for poultry and induce a good humoral response, there is a list of disadvantages about them. Firstly, inactivated vaccines induce a humoral response mainly, whilst immune responses against viruses would profit more from a cell-mediated response, or both a cell-mediated and humoral response, as viruses cause intracellular infections (Abbas et al., 2018). The vaccines also need to be administered in multiple doses, which is logistically inefficient, especially when treatment takes place via injection. Another disadvantage is that the rapid antigenic drift of AIV's makes it difficult to develop vaccines against locally emerging strains of AIV. Moreover, inactivated vaccines also make it difficult to uphold the DIVA principle (Nurzijah et al., 2022).

DIVA strategies can help to detect specific antibodies which are induced by natural infection rather than vaccination in animals and vice versa, ensuring safe trade of products from immunized animals (Liu et al., 2013). The DIVA principle relies on the use of serological assays against the NA or NS1 proteins. The NA heterologous test is able to detect the neuraminidase (NA) protein subtype of the field virus in birds (Swayne, 2016). Because inactivated vaccines target the HA protein and are only HA-specific, the vaccines contain a HA subtype that is the same as the field virus, along with a random NA subtype. However, this leaves the chance that the vaccine contains a NA subtype that is similar to the field virus, making the serological assay unable to distinguish between infected and vaccinated birds, thus lowering its ability to secure the DIVA principle (Swayne, 2016). The NS1 serological assay relies on the basis that NS1 proteins are only produced by infected cells and are not present in AIV (Swayne, 2016). However, variability in seroconversion and the duration of the antibody response in infected birds make this test less reliable (Avellaneda et al., 2010). Ultimately, the NA and NS1 serological tests only have about 63% sensitivity to detect infection in vaccinated poultry (Swayne, 2016). Consequently, the NA heterologous test would be more suitable for AIV vaccines that only use the HA protein (Swayne, 2016).

There are a number of inactivated AIV vaccines commercially available. These include monovalent inactivated vaccines containing either H5 or H7 strains, bivalent vaccines with both H5 and H7 strains, and both monovalent and bivalent vaccines with homologous or heterologous NA (Nurzijah et al., 2022). As recent as 2018, there have been developments in the creation of an adjuvanted inactivated AIV vaccine dry powder (Tomar et al., 2018). This vaccine is administered via inhalation to induce passive immunization (Tomar et al., 2018). The vaccine was found to be highly protective against morbidity and mortality in HPAI- infected chickens, and also to be able to prevent virus shedding in infected birds, thus providing protection against spread (Tomar et al., 2018). The vaccine is also argued to be suitable for mass-vaccination (Tomar et al., 2018).

VLP vaccines

VLP's (Virus-like particles) are structures that resemble their target virus. However, VLP's are unable to replicate due to a lack of nucleic acid. When administered, VLP's are taken up by dendritic cells and presented to lymphocytes by their MHC-II, initiating an adaptive immune response. VLP's induce both a cellular and humoral adaptive immune response (Pillet et al., 2016). Moreover, the self-adjuvating

effect of VLP's ensure a strong immunogenic response overall. Due to their strong activation of dendritic cells, VLP-vaccines are less susceptible to immune tolerance and thus offer a good protection against infections (Nurzijah et al., 2022).

Plant-based VLP-vaccines are proposed as a cheap option to create VLP-vaccines. When creating plant-based VLP vaccines, a glycoprotein (in the case of influenza, the hemagglutinin surface protein) is selected as a target antigen (D'Aoust et al., 2010). Then, the gene encoding the target antigen protein is integrated into the plant genome of a suitable plant, usually with the help of *Agrobacterium tumefaciens* for gene transformation (Nurzijah et al., 2022). Ultimately, the produced antigen protein is extracted, filtrated and purified from the plant to be used in a VLP-vaccine (Nurzijah et al., 2022). One study also argues that VLP's make good candidates for DIVA, granted that the vaccine is adapted to the epidemiological situation (Liu et al., 2013).

Difficulties of vaccine implementation

Not many countries incorporate the use of vaccines against AIV in their strategy; though more than 113 billion vaccine doses were used between 2002 and 2010 against AIV, 99% of these doses were used in only five countries around the world, with China being the most frequent user (Swayne, 2012). The use of vaccines is still restricted by logistic problems, problems with effectiveness, and faces trade restriction barriers. The import of AIV-vaccinated poultry products is currently not allowed in the European Union (Wageningen Bioveterinary Research, 2021). Besides these trade restrictions, the fact that vaccines are not as commonly used globally could also be accounted for by other factors, such as the relatively high costs of commercial AIV-vaccines. Moreover, inactivated vaccines require cold storage, which makes it logistically impractical to vaccinate poultry in some areas. Also, AIV's undergo mutations quite frequently, making vaccines less effective against locally emerging strains (Nurzijah et al., 2022). Other concerns using vaccines are that the use of live-attenuated and inactivated vaccines do not allow for accessible differentiation between infected and vaccinated animals, which can further complicate diagnosis and control of the AIV outbreak (Nurzijah et al., 2022; Wageningen Bioveterinary Research, 2021). There are also concerns that vaccination will mask clinical signs of AIV. (Perez *et al.*, 2019). Lastly, vaccine delivery via injection is not ideal due to the high number of animals (Wageningen Bioveterinary Research centre, 2021).

In recent years, several studies have opted for plant-based VLP-vaccines for poultry, vaccines that circumvent most of the problems that inactivated vaccines face (Scotti and Rybicki, 2013; Landry et al., 2010). VLP's have been proven to be more immunogenic than other vaccine types due to their efficient uptake by dendritic cells and macrophages, their self-adjuvating ability and their capacity to induce a cellular immune response (Ninyio et al., 2020). Moreover, production of VLP's can be relatively fast; one study claiming as fast as one month after sequencing of a new strain (Landry et al., 2010). This is because only the HA coding sequence is necessary to start the vaccine production, and the fact that VLP's are relatively simple products (D'Aoust et al., 2010). Production costs of plant-based VLP's are also quite low, allowing VLP's to be used in widespread and particularly in resource-poor settings; one kilograms of plant material would produce enough VLP doses to immunize 30,000 chickens (Nurzijah et al., 2022). The ability to produce VLP's cheaply and locally surmounts some of the logistical disadvantages of inactivated vaccines, such as the need for cold chain storage to stay stable, high production costs, and the inability to develop vaccines against locally emerging strains (Nurzijah et al., 2022). Oral administration of VLP's is possible, though determining and administering the effective dose through oral administration is difficult due to variations in bird size and age (Nurzijah et al., 2022). This could unintentionally lead to immune tolerance or poor immunogenicity when too much or too little is administered (Nurzijah et al., 2022).

Discussion

Every year, there are outbreaks of avian influenza viruses (AIV) amongst birds in poultry farms. This leads to the death of millions of birds, along with huge losses in food security and economy. Recently, particularly AIV subvariants H7N9 and H5N1 have been notorious for causing significant outbreaks of AIV, along with zoonosis outbreaks. A four-component system, consisting of education, biosecurity, rapid diagnostics and surveillance, and elimination is often implemented to mitigate the consequences of an AIV outbreak such as loss of poultry, threats to wild birds and human casualties (Swayne, 2012; Perez et al., 2019). Nonetheless, AIV outbreaks can still have devastating effects and are sometimes uncontrollable (Swayne, 2012). Therefore, optimal methods to reduce host susceptibility, e.g. vaccination are also being experimented with (Swayne, 2012; Nurzijah et al., 2022). According to the Wageningen Bioveterinary Research centre (2021), poultry vaccination should follow the following criteria in order to be effective: 1) the vaccine should prevent AIV infection and spread. 2) The vaccine should be suited for mass vaccination, for example through oral administration. 3) the vaccine should live up to the DIVA principle: Differentiating Infected from Vaccinated Animals (DVIA), meaning that the vaccine needs some sort of marker to be able to distinguish infected animals from vaccinated animals during diagnostic surveillances (Wageningen Bioveterinary Research centre, 2021).

The purpose of this review was to evaluate the existing and prospective strategies against highly pathogenic avian influenza outbreaks (HPAI). All of the reviewed literature agrees that there is not a single solution to curb AIV outbreaks; instead, the combination of multiple components is needed to ensure public health and food security. The traditional four component system is a partially effective system that helps to control AIV outbreaks when all components are used. Considering the bigger picture, the use of vaccines seems promising as an additional tool to contain AIV-outbreaks. They can make the difference by reducing the number of infected poultry, which will reduce the spread and help in eradication of the disease with as little loss of poultry through culling as possible. Moreover, poultry will become less likely to die from an AIV infection. Though, some developments need to be made in order for vaccine implementation to be feasible and effective.

An important aspect to the effectiveness of an AIV vaccine is that it does not just protect the birds from the symptoms, but that the vaccine also prevents infection and limits the spread of AIV. Whilst most vaccine types would be able to overcome difficulties with immunogenicity, the trade-off for achieving this increased immunogenicity tends to bring out more problems. For example, DNA vaccines are low in immunogenicity, though applying three or more vaccinations can provide the protection that is needed. However, nucleic acids are expensive, and administering three or more doses without the possibility for mass vaccination is highly inefficient, both financially and logistically (Swayne, 2016). Live-attenuated vaccines can achieve higher immunogenicity within one dose, but the drawback is the safety hazard (Pollard and Bijker, 2021). Considering the aforementioned examples, it would seem that inactivated vaccines and VLP's would provide a higher immunogenicity without too many known disadvantages. VLP's are known to be highly immunogenic, inducing both a humoral and cellular response, without the need of an adjuvant. In the same line, whilst traditional inactivated vaccines were discouraged because of their initial low immunogenicity, more recent developments in passive immunization with inactivated vaccines seem hopeful in terms of redeeming the applicability of inactivated vaccines in poultry. These dry powder vaccines are argued to be able to protect birds against infection, as well as shedding and spreading viral particles (Tomar *et al.,* 2018). For VLP's, there has been no conclusive study so far to demonstrate whether virus shedding is prevented by vaccination, likely due to the clinical trials of VLP's in poultry still being somewhat recent.
Another aspect concerning the protection that vaccines provide is the rapid antigenic drift that influenza viruses undergo, causing vaccines to become ineffective (Perez et al., 2019). Once a virus evolves, a new vaccine needs to be developed, as no universal vaccine that can protect against any strain exists yet. In this case, it is important that the production process of a vaccine is not very time-

and resource-consuming. The preparation of an inactivated vaccine is time-consuming. Moreover, it requires culturing of the virus in embryonated chicken eggs and protein purification (Tomar et al., 2018), a process that in itself can take up to a few days, and requires having enough readily available embryonated chicken eggs. This causes valuable time-loss, whilst a rapid response is very important when containing AIV outbreaks. On the other hand, whilst VLP's also require a complicated process for production, the production is less time and resource-consuming than inactivated vaccines.

Another important facet to the use of vaccines is the DIVA-principle (differentiating Infected and vaccinated animals). Applying the DIVA principle will contribute towards detecting and containing AIV cases, and will ultimately ensure safe trade of products from immunized animals. Liu et al. (2013), argue that VLP's show a promising platform for the creation of 'negative DIVA vaccines', VLP's that lack at least one protein that is present in the field virus. However, there has been no further conclusive literature on whether VLP's in poultry sustain the DIVA principle. Thus, so far, the greatest accuracy level that one is able to distinguish vaccinated animals from infected animals sits around 63% with the heterologous NA assay and the NS1 assay. This is the case for both VLP's and inactivated vaccines, as both vaccines rely on the use of both an HA and NA subtype (Liu et al., 2013; Pollard and Bijker, 2021).

It is also worth to consider the more practical aspects of vaccination. It is essential that effective means of mass administration are further researched and translated to real-world settings. It is logistically-wise very costly to have to vaccinate thousands of chickens via injections, especially if multiple doses are required. Vaccination via injection could also pose a public health risk, as intensive contact between those people vaccinating and the possibly infected poultry is needed repetitively. Positively seen, both VLP's and inactivated vaccines demonstrate possibilities for mass administration (Tomar et al., 2018; Nurzijah et al., 2022). Oral administration of VLP's is possible, which provides opportunities for mass administration via e.g. food, though determining and administering the effective dose is complicated (Nurzijah et al., 2022). Less than straight-forward administration protocols could also lead to unsuccessful vaccination, because there remains a responsibility to the employees at poultry farms to administer vaccines (Swayne, 2016).
Storage and transport is also an important prospect. Regular inactivated vaccines require storage at low temperatures to maintain their immunogenicity, though in powder-form, the vaccines can be stored for up to a year at moderate temperature (20 degrees Celsius) (Geeraedts et al., 2010). Similarly, it has been successful to create some VLP's that do not require cold storage (Nurzijah et al., 2022).
Another practical factor is the development of new and effective vaccines with regards to the availability of laboratories in the area. In an ideal case, a suitable vaccine would be developed against any new locally emerging strains. But it is realistic to consider that not every region has a lab and/or qualified personnel to develop a new vaccine. Hence, production of vaccines against locally emerging strains might not even be possible in most cases. The possibility of vaccine production on nation-scale or even continental-scale would already be a great leap in the right direction.

After having discussed all of the prolonging's, practicalities and recent information of vaccination in poultry, one might wonder about the cost-effectiveness of a vaccination strategy. Whilst there is no data available on a global scale, data from China's vaccine response to a H7N9 outbreak might shine some light onto this unknown. A benefit-cost analysis was performed on the H7N9 vaccination program between July 2017 and June 2020, using the period without a vaccination strategy from July 2016 to June 2017 as a baseline for comparison (Tang et al., 2022). In the vaccination program, H5-H7 bivalent inactivated vaccines were used, and the programme was fully financed by the government (Tang et al., 2022). The study concluded that the compulsory routine vaccination program was economically profitable compared to the period where the vaccination program was absent with a benefit-cost ratio of 18.6 (Tang et al., 2022). Whilst the situation in China is definitely not a direct translation of the cost-benefit to other countries or situations, it does give the slight indication that implementing a vaccine strategy could be cost-effective.

Lastly, it is crucial that countries discuss and resolve their current trade limitations to vaccinated poultry. Currently, the European Union does not allow for trade in poultry vaccinated against AIV, and many other countries pose embargos on the import of vaccinated poultry and poultry products as well (Wageningen Bioveterinary Research centre, 2021). If export of vaccinated poultry is not supported, there will be little incentive to invest in poultry vaccination research and – strategies. Nevertheless, considering the positive cost-benefit analysis of the vaccination program in China, in combination with the pressure that nations are facing to tackle the ongoing global AIV outbreaks, there might be an incentive to reconsider the current trade limitations.

In the case of human infection with AIV, the number of infections could be greatly contained by providing bird handlers with proper protective equipment and hygiene protocols. Ultimately, human infection with AIV remains rare, but proper surveillance in humans that could be exposed to infected birds should be maintained (Adlhoch et al., 2022). The IRAT-system (Influenza Risk Assessment tool) could also aid in assessing the danger of a HPAI strain to humans, and can encourage authorities and bird handlers to be extra vigilant. Ultimately, human infection with AIV can best be prevented by controlling AIV outbreaks in poultry itself. Surveillance of avian influenza viruses could also be improved by continued and increased sharing of genomes and information about AIV between countries. This would allow countries to prepare for emergent AIV strains. In the same line, more wide usage of the IRAT-system could help in more (cost)- effective and targeted vaccination and culling, keeping the dangers of a pandemic in check.

To conclude, the combined implementation of the four-component system, along with the use of vaccination is a good strategy to contain avian influenza outbreaks in poultry. Developments in rapid diagnostics and the use of new vaccines could greatly improve the ability to curb AIV outbreaks. Additionally, collaborations on sharing of viral genomes and the IRAT-system should be encouraged between countries for public health protection and more effective eradication for AIV, as a large AIV outbreak is rarely contained by country borders. New vaccines such as plant-based VLP's and dry powder vaccines look promising in the field of (preventative) poultry vaccination for their effectiveness. Although VLP's and inactivated vaccines show significantly more benefits over other vaccine types, there is no 'best' vaccine to be picked. Rather, researchers should be encouraged to further do clinical trials and research. Moreover, there is still a need to translate these vaccines to real-life situations. In the end, it is crucial that nations come to an agreement concerning trade restrictions in vaccinated poultry and poultry products in order for the field of AIV vaccination to make progress.

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