Review Article

Low Pathogenicity Avian Influenza H9N2 Virus in Africa

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Abstract | Avian influenza virus (AIV) of the subtype H9N2 virus has been detected worldwide in wild and domestic birds as well as in few occasions in mammals. The virus was recorded in several African countries, with endemic status in Egypt and frequently reported in different other countries like Morocco, Tunisia, Uganda, Benin and Togo since 2016. Egypt and Morocco have applied a mass vaccination programme for commercial poultry to control the infection. The isolated viruses from poultry in Africa have been classified as G1 'Western' Viruses and related to the circulating viruses in the Middle East. In addition to Y439 lineage recorded in South Africa farmed ostriches. The infection with H9N2 is frequently displaying moderate-to-high morbidity and mortality in the field especially when comes associated with bacterial or viral infections or with many other factors such as poor biosecurity, mal-nutrition and managemental problems. LPAIVs H9N2 in chickens tend to show more respiratory tropism with some strains also show gastrointestinal tropism. The ideal mechanism of transmission among birds varies by host species and virus strain. The respiratory and contact transmission are likely the primary routes of transmission and may partly arise initially as an adaptation to poultry which clearly has implication for zoonotic transmission. Inactivated vaccines are the most common vaccines used to control H9N2 viruses, however they have a wide antigenic variability in different lineages.

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Introduction

Avian influenza virus (AIV) is a type of influenza A virus in the Orthomyxoviridae family. It contains eight negative-sense, single-strand RNA segments encoding 10 core proteins and other accessory proteins. Influenza A viruses are generally typed by their combinations of hemagglutinin (HA) and neuraminidase (NA) genes, lead to multiple different subtypes. AIVs are also classified into high pathogenicity avian influenza viruses (HPAIVs) and low pathogenicity avian influenza viruses (LPAIVs) according to their intravenous pathogenicity index (IVPI) in chickens.

Avian influenza virus (AIV) of the subtype H9N2 was isolated for the first time from turkey flocks in Wisconsin, United States in 1966 (Homme and Easterday, 1970). Since then, the virus has been detected worldwide in wild and domestic birds as

well as in few occasions in mammals (Kawaoka *et al.*, 1988). H9N2 AIVs can be classified into 2 major lineages: Eurasian and American. American lineage of H9N2 AIVS are mainly recorded in wild birds, while Eurasian lineage of H9N2 AIVs are subgrouped into several sublineages: The G1 lineage (A/quail/Hong Kong/G1/1997), the Y280 lineage (A/duck/Hong Kong/Y280/1997), and the Y439 lineage (A/duck/Hong Kong/Y439/1997) (Guo *et al.*, 2000). The G1 and Y280 lineages have zoonotic potential with many cases have been reported in Asian countries and Egypt (Zhang *et al.*, 2012; Pu *et al.*, 2014; Carnaccini and Perez, 2020).

History and phylogeography of H9N2 virus in poultry

Avian influenza of subtype H9N2 has been recorded in several African countries, Egypt has an endemic status in poultry and the virus has been frequently reported in Libya and Tunisia (Alexander, 2007; Monne *et al.*, 2013; Tombari *et al.*, 2011; Kammon *et al.*, 2015). Morocco, Burkina Faso, Ghana Algeria and Uganda have been isolated the virus since 2016 for the first time (El-Houadfi *et al.*, 2016; Zecchin *et al.*, 2017; Rubrum *et al.*, 2018; Awuni *et al.*, 2019). From those countries, Morocco has applied a mass vaccination programme for commercial poultry (El-Houadfi *et al.*, 2016). The isolated viruses from poultry in Africa have been classified as G1 'Western' Viruses and related to the circulating viruses in the Middle East in Israel, Jordan, Lebanon, Saudi Arabia and the United Arab Emirates.

Nigerian poultry and agricultural workers showed high seropositivity against H9N2 without virus isolation (Oluwayelu *et al.*, 2016; Okoye *et al.*, 2013). Although surveillance for HPAIVs is ongoing in Nigeria, serological investigations revealed presence of H9N2 antibodies in poultry without virus isolation. The presence of H9N2 virus in poultry across different regions of Africa suggests that other regions or countries may have infection. However, active surveillance is lacking or not found, and not being reported due to LPAIs such as H9N2 infections not being diseases that are notifiable to the World Organisation for Animal Health (OIE).

Phylogeographical analysis of the H9 subtype of AIV separated viruses into two basic lineages: American and Eurasian and subdivided into four clades based on HA gene segments (Hu *et al.*, 2017; Carnaccini and Perez, 2020). The H9.1 clade is derived from the A/turkey/Wisconsin/1/1966 virus found in bird populations in North America. The H9.2 clade of the American lineage is related to A/quail/Arkansas/29209-1/93 strain. While the Eurasian lineage is composed of two primary subgroups: Y439-H9.3 (A/duck/HongKong/Y439/1997) and G1-H9.4 (A/Quail/Hong Kong/G1/1997). The Y439 lineage has spread in wild bird species across Asia, Africa, and Europe. The G1 lineage has been frequently reported in commercial poultry and live poultry markets in the Middle East region and Africa (Peacock *et al.*, 2019). The H9.4 clade has been divided into two subclades: H9.4.1 and H9.4.2. Since 2013, the H9.4.2 subclade of the H9N2 AI virus has been discovered in China (Gu et al., 2017).

In this review, the evolutionary history of H9N2 viruses from Africa was inferred by using the maximum likelihood method and Tamura-Nei model (Tamura and Nei, 1993) (Figure 1). The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 69 nucleotide sequences available in NCBI GenBank database for Africa. Codon positions included were 1st+2nd+3rd+Noncodingusing Mega11 (Tamura *et al.*, 2021).

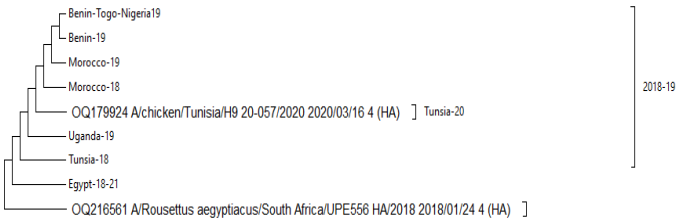

Figure 1: *Phylogenetic tree for the hemagglutinin gene of H9N2 influenza viruses in African countries. The tree was generated using maximum likelihood method. Evolutionary analyses were conducted in MEGA11.*

North Africa

LPAI H9N2 is currently enzootic in countries of North Africa. H9N2 viruses is circulating in North Africa causing economic losses even in the vaccinated flocks. Genetic analysis showed markers of adaptation of mammals, including humans (Moghoofei *et al.*, 2018; Kaboudi, 2019).

Egypt recorded a genetic report of AIV H9N2 in 2006

from live poultry markets without virus isolation, while from February 2009 to April 2012, antibodies against H9 viruses were widespread in poultry in Egypt (Afifi *et al.*, 2013). Virus isolation of H9N2 was recorded in Egypt from December 2010 and it was different from the first introduction recorded in 2006. Since then, AIV H9N2 infected a wide range of birds in Egypt including chickens, quails, ducks, turkeys and pigeons in commercial and backyard sectors (Kandeil *et al.*, 2014; Arafa *et al.*, 2012a, b; Abdel-Moneim *et al.*, 2012; Monne *et al.*, 2013; El-Zoghby *et al.*, 2012; Awad *et al.*, 2013; Li *et al.*, 2020). Most of infected chickens and turkeys exhibited respiratory distress and/or decrease in egg production, but some quails and broilers flocks showed no overt symptoms (Afifi *et al.*, 2013; Arafa *et al.*, 2012b; Monne *et al.*, 2013; El-Zoghby *et al.*, 2012). The majority of outbreaks are reported during the winter months but outbreaks are observed year-round particularly in the Nile Delta (Arafa *et al.*, 2012b; Abdelwhab and Abdel-Moneim, 2015). Co-infections with other viruses (like NDV, IBV, IBD) or mycoplasma (MG, MS) are common. Interestingly, out of 86 broiler flocks 42% were co-infected with H9N2 and IBV and in 41% a mixed triple infections with IBV-H5-H9-NDV were observed in 2012–2014 (Hassan *et al.*, 2016; Naguib *et al.*, 2017). There is evidence of reassortment between the co-circulating HPAI-H5N8 and LPAI-H9N2 viruses has been reported in 2018 leading to a new virus HPAI-H5N2 (Hagag *et al.*, 2019; Hassan *et al.*, 2020). And experimentally through coinfection of embryonated eggs with HPAI-H5N1 and LPAI-H9N2 (Naguib *et al.*, 2017). Inactivated vaccines using local and non-local field strains of H9N2 are frequently used in Egypt and the emergence of antigenic drift variants has been reported. It is worth mentioning that the Egyptian viruses reacted poorly against serum samples from the ME including vaccination derived sera (Kandeil *et al.*, 2014; Adel *et al.*, 2017; Kandeil *et al.*, 2017). In 2015, three children with a history of exposure to poultry were found positive for AIV H9N2 RNA (Abdelwhab and Abdel-Moneim, 2015) and up to 7·5% seroprevalence in exposed humans was reported (Gomaa *et al.*, 2015). Human infections showed transient influenza like illness but subsided without sequelae.

H9N2 viruses was first recorded in Libya in commercial poultry in 2005–2006 (Alexander, 2007). Then further recorded in 2013 from layers, broilers chicken flocks and peacock (Kammon *et al.*, 2015). The infection spread rapidly all over the country and the flocks were concurrently co-infected with NDV. Birds in the affected flocks showed respiratory signs and high mortality (Kammon *et al.*, 2015). There are no reports of application of H9N2 vaccines in the field.

H9N2 viruses have emerged in Tunisia in 2009 causing several outbreaks in poultry flocks (Tombari *et al.*, 2011). AIV H9N2 virus was also isolated from wild birds (Tombari, 2016). Moreover, a nationwide serosurvey of 800 flocks in 2010–2011 indicated widespread AIV infection including H9N2. A total of 223 flocks had anti-NP antibodies (28·7%) particularly in the coastal areas during the autumn and winter. The infection was mainly reported in layer and breeders. Low biosecurity measures and contact to wild birds were claimed to be the source of infection (Tombari, 2013). In 2012, H9N2 was isolated from a broiler flock (Aouini et al., 2016). Antivirals zanamivir and amantadine decreased virus replication in experimentally inoculated chickens (Umar, 2016).

There are no data available on the prevalence of H9N2 in Algerian poultry and without H9 antibodies were detected from broilers, turkeys or layers flocks in 2012-2013. It was also no vaccination against AIV is implemented in Algeria (Sid et al., 2015).

The first record of H9N2 in Morocco was in 2016 from broilers and breeders (El-Houadfi *et al.*, 2016). Widespread of virus to several locations in the country was observed in layer and breeder chickens. The symptoms were mainly inducing respiratory signs and decrease in feed consumption, drop in egg production with mortalities from 2 to 15%. Similar signs were also reported in turkey flocks with mortality rates about 10%. Emergency vaccination was implemented for poultry in all production sectors. The control of the virus was announced the by April 2016 (El-Houadfi *et al.*, 2016).

West and central Africa

Since 2017, H9N2 viruses have been detected in several Sub-Saharan African countries like Ghana, Nigeria, Burkina Faso, Uganda, Kenya, Benin, Togo and Senegal, where a human case was recently reported (Jallow *et al.*, 2020; Fusade-Boyer *et al.*, 2021).

H9N2 subtype virus was identified in West Africa, where highly pathogenic H5 strains of the A/goose/

Guangdong/1/1996 lineage (Gs/GD) have been widely distributed since 2015. The H9N2 has a concern because of animal health implications and its negative effect on local economies as well as possible emergence of reassortant viruses with zoonotic risk.

The H9N2 subtype virus identified in Burkina Faso in 2017 and it seems to be introduced from Morocco (Zecchin *et al*., 2017). Then between November 2017 and February 2018 it was identified as GI-19 lineage in Ghana (Awuni *et al*., 2019).

The H9N2 viruses from Benin and Togo were clustered with previously isolated in Western Africa, however, viruses from Uganda were genetically distant and clustered with viruses from the Middle East. Antigenic drifting viruses were suggested in Benin that showed decreased cross-reactivity with those from Togo and Uganda. The viruses exhibited mammalian adaptation markers similar to those of the human strain A/Senegal/0243/2019 (H9N2) (Jallow *et al*., 2020; Fusade-Boyer *et al*., 2021). The H9N2 Nigerian strains were also belonging to the G1 lineage, which has zoonotic potential, and are clustered with H9N2 viruses identified in Africa between 2016 and 2020. There are two distinct clusters of H9N2 viruses in Nigeria, suggesting different introductions into the country (Sulaiman *et al*., 2021).

In Kenya, previous AIV surveillance detected AIV in 0.8% of chickens sampled in live bird markets (LBMs), but no virus was successfully isolated and the detected sample was not further subtyped (Kariithi *et al*., 2020).

South Africa

South Africa isolated H9N2 viruses from farmed ostriches and they were similar to wild bird viruses of the Y439 lineage, so these viruses are representing dead-end spill over actions from wild migratory birds from 2017-2018 (Abolnik *et al*., 2006; Wang *et al*., 2018; Peacock *et al*., 2019).

H9N2 virus pathogenesis

H9N2 viruses are of low pathogenicity in experimental infections when tested by IVPI (Guo *et al*., 2000; Alexander, 2003; Iqbal *et al*., 2009), however, they frequently display moderate-to-high morbidity and

Table 1: *African countries with laboratory confirmed H9N2 infections.*

Country	Years of poultry isolates	Lineages	Species	Status	Recorded human cases/ Serology
North Africa					
Egypt	2006, 2011–2021	G1-W	Chicken, Quail, Duck, Turkey, Pigeon, Bat, pig	Endemic	4 cases /and serology
Libya	2006 & 2013	G1-W	Chicken	Sporadic	No
Tunisia	2010–2012, 2014-2020	G1-W	Chicken Turkey, Wild birds	Potentially endemic	No
Morocco	2016-2019	G1-W	Chicken	Potentially endemic	No
Algeria	2017	G1-W	Chicken	Sporadic	No
Central Africa					
Kenyia	2017	G1-W	Chicken	Sporadic	No
West Africa					
Nigeria	2013 & 2019	n/a	Chicken, Wild birds	Potentially endemic	Serology only
Burkina Faso	2017	G1-W	Chicken	Sporadic	No
Ghana	2017–2018	G1-W	Chicken	Sporadic	No
Uganda	2017-2019	G1-W	Chicken, Wild birds	Potentially endemic	No
Benin	2017-2020	G1-W	Chicken, Duck	Potentially endemic	No
Senegal	2017-2019	G1-W	Chicken	Potentially endemic	1 case
Togo	2018-19	G1-W	Chicken	Sporadic	No
South Africa					
South Africa	1995, 2008–2009 2018-2019	Y439 G1-W	Ostrich, Chicken, Wild birds	H9N2-free	No

Table 2: Available HA gene sequence data on 2 public databases for African H9N2 viruses.

Country	Year	Host	No.	Database
South Africa	1995	Avian	2	IVD
	2008	Avian	1	IVD
	2018	Bat	1	IVD
	2009	Ostrich	1	GISAID
Egypt	2004	Avian	1	IVD
	2011	Avian	17	IVD
	2012	Avian	52	IVD
	2013	Avian	28	IVD
	2014	Avian	41	IVD
	2015	Avian /1 Swine	80	IVD
	2016	Avian	38	IVD
	2017	Avian/1 Bat	26	IVD
	2018	Avian	42	IVD
	2019	Avian	94	IVD
	2020	Avian	108	IVD
	2021	Avian	22	IVD
	2011-2021	Avian	441	GISAID
Libya	2006	Avian	1	IVD
	2013	Avian	3	IVD
	2006	Avian	1	GISAID
Tunisia	2010	Avian	4	IVD
	2011	Avian	3	IVD
	2012	Avian	2	IVD
	2013	Avian	1	IVD
	2014	Avian	3	IVD
	2015	Avian	2	IVD
	2016	Avian	1	IVD
	2018	Avian/ 1 Env	8	IVD
	2020	Avian	2	IVD
	2010-2018	Avian/ 1 Env	15	GISAID
Morocco	2016	Avian	17	IVD
	2017	Avian	3	IVD
	2018	Avian	3	IVD
	2019	Avian	4	IVD
	2016-2018	Avian	22	GISAID
Burkina Faso	2017	Avian	1	IVD
	2017	Avian	1	GISAID
Uganda	2017	Avian	59	IVD
	2018	Avian	76	IVD
	2019	Avian	92	IVD
	2017-2019	Avian	191	GISAID
Algeria	2017	Avian	37	IVD
	2017	Avian	37	GISAID
Senegal	2017	Avian	3	IVD
	2019	Human	1	GISAID
Ghana	2017	Avian	3	IVD
	2018	Avian	4	IVD
	2017-2018	Avian	7	GISAID
Benin	2018	Avian	2	IVD
	2019	Avian	31	IVD
	2020	Avian	2	IVD
	2018-2020	Avian	35	GISAID
Togo	2019	Avian	3	IVD
	2019	Avian	3	GISAID
Nigeria	2019	Avian	19	IVD
	2019	Avian	19	GISAID
Kenya	2017	Avian	5	IVD
	2017	Avian	5	GISAID

GISAID: Global Initiative on sharing all influenza data; IVD: Influenza virus database.

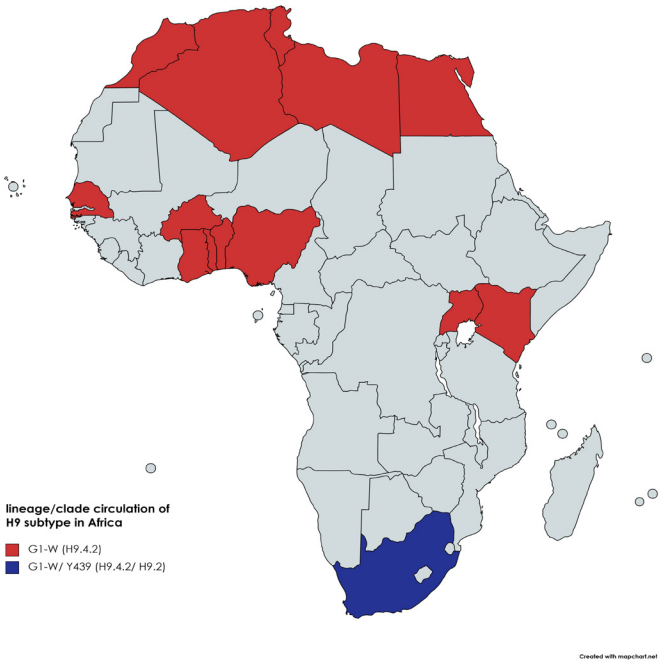

Figure 2: *Africa map representing spread of H9N2 lineages in different countries. The map was created by mapchart.net.*

mortality in the field. There are many reports of mortality rates more commonly associated with HPAIV outbreaks (Banet-Noach *et al.*, 2007; Awuni *et al.*, 2019). They also reported with co-infection with bacterial or viral pathogens, and many other factors such as poor biosecurity, mal-nutrition and housing problems (Kishida *et al.*, 2004; Seifi *et al.*, 2010; Wang *et al.*, 2016). H9N2 viruses are considered as a source of gene doner to produce a highly pathogenic or zoonotic viruses, therefore it is important to control H9N2 viruses to prevent the emergence of new zoonotic viruses (Liu *et al.*, 2014).

HPAIV phenotype was not observed in H9N2 virus when HPAIV-like polybasic cleavage site was engineered into H9 virus background. However, when the polybasic H9 HA was combined with the remaining genes from an HPAIV strain the reassortant virus did develop an HPAIV phenotype (Gohrbandt *et al.*, 2011). This implies H9N2 virus internal genes may not be compatible with an HPAIV phenotype in some cases.

H9N2 virus transmission and host tropism in poultry

The main routes of influenza virus transmission are droplet, aerosol, faecal-oral and direct contact (Killingley *et al.*, 2013). Droplet transmission occurred through the upper respiratory tract inhalation of infectious particles (more than 10 nm). While, aerosol droplets are typically less than 5 nm and can reach to the lower respiratory tract (Killingley *et al.*, 2013). Contact transmission occurred through mucous membranes directly, or through an intermediate contact fomite. H9N2 viruses can be transmitted is determined via several viral, host, and environmental aspects, including viral replication and viral titres shedding; distance and frequency between contacts and virus stability in different environmental conditions. In wild aquatic birds such as ducks, the gastrointestinal tropism of AIVs exhibit through the oral-faecal route. LPAIVs H9N2 in chickens tend to show more respiratory tropism and some gastrointestinal tropism (Wang *et al.*, 2016; Killingley *et al.*, 2013; Sorrell *et al.*, 2010). There is good evidence to suggest that many LPAIV strains transmit by the airborne route, the oral-faecal route and the waterborne route (Killingley *et al.*, 2013; Lv *et al.*, 2015). However, the main route of transmission among individuals varies by host species and viral strain.

Direct contact is the important transmission route for H9N2 viruses in chickens, then aerosol and faecal-oral have been also shown to be significant. The respiratory tropism is favored by many strains, however, some other H9N2 strains have been shown to have an extended tropism for the kidneys or oviducts (Bonfante *et al.*, 2018; Perez *et al.*, 2003; Yao *et al.*, 2014). Both in the field and experimentally poultry adapted H9N2 viruses are mostly detected from oral/tracheal rather than cloacal swabs (Peacock *et al.*, 2017). Additionally, inoculation of some H9N2 viruses into the respiratory tract is 40 times more effective than gastrointestinal inoculation at initiating infection (Yao *et al.*, 2014). LBMs have been considered as the major player for transmission of endemic H5N1 and H9N2 viruses as they enhance direct contact transmission among birds. So, respiratory and contact transmission are the primary routes of transmission of H9N2 and has implications for zoonotic transmission.

Vaccination and control

H9N2 causes marked economic damage in many countries including African countries, so vaccination has been adopted at a national level or in a local area to control disease in poultry (El-Houadfi *et al.*, 2016; Kilany *et al.*, 2016). Inactivated vaccines are the most common vaccines used. H9N2 viruses have a wide antigenic variability in different lineages (Peacock *et al.*, 2018). Efficacy trials are generally fewer for H9N2 vaccines than other HPAI H5 vaccines specially against antigenically drifted viruses.

So, vaccination failure has been noticed in many areas applying H9N2 vaccination with high virus shedding and infection to vaccinated birds due to sub-optimal vaccination that may produce antigenic drift and increase zoonotic potential and pathogenicity (Park *et al.*, 2011; Zhang *et al.*, 2008; Peacock *et al.*, 2017; Sealy *et al.*, 2019).

H9N2 vaccination can provide protection for birds by reducing clinical signs and virus shedding. Inactivated whole-virus vaccines may fail to provide sufficient protection when there are differences in antigenicity between the H9N2 vaccine strain and the circulating viruses (Pu *et al.*, 2014; Sun *et al.*, 2012). The H9N2 vaccine needs to be optimized to fit the current status of multiple antigenic groups. The inactivated vaccines induce mainly humoral immunity, which mask virus infection and increase shedding of virus from upper respiratory tract. Besides, field H9N2 viruses can induce efficient chicken-to-chicken aerosol transmission in vaccinated chickens (Zhong *et al.*, 2014) that will increase the difficulty of virus prevention and control. Therefore, it is important to select vaccines that capable to reduce virus shedding to help in controlling H9N2 infections.

Inactivated whole-virus vaccines

Inactivated whole-virus vaccines has many advantages including ease of production and its lack of ability to revert to a virulent state. The immunogenicity of the vaccine depends on vaccine candidate strains used and its protective antigenic epitopes as well as the

compatibility between HA and NA (Wang *et al.*, 2019). There are multiple H9N2 antigenic groups were prevalent worldwide that makes antigen-matched vaccine challenging and difficult to predict antigenic variation for the control of H9N2. Antigenicity prediction models based on HA sequences show good potential (Du *et al.*, 2012). Strains with broad-spectrum cross-protection may exist among the naturally prevalent strains (Radvak *et al.*, 2021). An inactivated vaccine modified for the H9 gene was successfully prepared, but its broad-spectrum protection still needs to be further invetigated (Li *et al.*, 2021). A graph-based vaccine design algorithm has been applied and demonstrated its broad protection against H3 subtypes (Bullard *et al.*, 2021). Furthermore, H9/H5N2 recombinant vaccine, that expressed the HA1 of H9N2 and the HA2 of H5N8 viruses, protected chickens against lethal challenge by HPAI H5N8 viruses and significantly reduced virus shedding post infection with both H9N2 and HPAI H5N8 viruses (Kim *et al.*, 2017).

The inactivated whole-virus vaccines are mainly prepared from the formaldehyde inactivation or beta-propiolactone (BPL) or the application of gamma radiation of virus-containing allantoic fluids from infected chicken embryos. A study showed that antibody-mediated immune responses were increased in chickens that received BPL and gamma inactivated whole-virus vaccines compared to formaldehyde prepared vaccines against H9N2 AIV (Astill *et al.*, 2018). Inactivated vaccines mainly induce humoral immunity. Adjuvants could improve the cellular immunity or mucosal immunity induced by inactivated vaccines. CpG oligodeoxynucleotides assist the whole inactivated H9N2 influenza virus in crossing the intestinal epithelial barriers via transepithelial uptake of dendritic cell dendrites (Qin *et al.*, 2015; Yin *et al.*, 2015).

Vector vaccines are developed and licensed for use in the control of avian influenza (Aida *et al.*, 2021). Vector vaccines induce cellular immunity and mucosal immunity to provide extended immunity. There are a variety of vectors have been used for H9 vaccine development, including fowlpox virus (FPV), fowl adenoviruses (FAdVs), Marek's disease virus (MDV) and NDV. In addition to other non-viral vectors, like Lactobacillus and Eimeria acervuline (Shi *et al.*, 2014, 2015; Bo *et al.*, 2019; Li *et al.*, 2020; Zhang *et al.*, 2021a) are also used as vectors to prepare vaccines to control H9N2 avian influenza. However, overcoming maternal antibody interference is a major challenge. Attenuated fowlpox virus (FPV) strains have been used as vaccines against wild-type virus infection. Recombinant fowlpox virus (rFPV-HA) expressing the HA gene of H9N2 AIV- could prevent virus shedding and replication in internal organs in response to H9N2 (Chen *et al.*, 2011). However, the efficacy of recombinant FPV-based vaccines can be affected by preexisting immunity (Swayne *et al.*, 2000). Fowl adenoviruses (FAdVs), with a linear, 26–45 kb, double-stranded DNA molecule, can also be used as virus vectors. By inserting the nucleotide sequence encoding the VP2 protein of IBDV into rFAdV, rFAdV-VP2 was generated (Pan *et al.*, 2021). The protection induced by rFAdV-VP2 vaccination in SPF chickens induced good immune protection, suggesting its potential in the vector vaccine development of H9N2-subtype avian influenza virus. Currently, there is no recombinant FAdVs are being produced as vectored vaccines. Preexisting immunities hamper the application of FAdV vectors in vaccine development.

Marek's disease virus (MDV) which are divided into three species, gallid herpesvirus 2, gallid herpesvirus 3 and meleagrid herpesvirus 1, formally named MDV serotype 1 (MDV-1), MDV serotype 2 (MDV-2) and MDV serotype 3 (MDV-3), respectively.

Turkey herpesvirus (HVT) (of meleagrid herpesvirus 1) has been extensively used as a vaccine against Marek's disease for over the past decades. Attenuated MDV-1 strains and HVT have many advantages that make them appropriate for the development of recombinant vector-based vaccines for poultry diseases. The attenuated CVI988/Rispens MDV-1 strain has been used to express the S1 glycoprotein of IBV (Zhang *et al.*, 2012) and the VP2 of IBDV (Zhou *et al.*, 2010).

Chickens vaccinated with MDV-H9 induced less than 50% protection (Ma *et al.*, 2014). The vector vaccine candidate HVT-H9 could induce humoral and cellular immunity in chickens. In a challenge study, no chicken shed H9N2 virus from the oropharynx and cloaca, and no H9N2 virus was found in the viscera in the vaccination groups when challenged with homologous virus, suggesting that HVT-H9 provides effective protection against H9N2 AIV in chickens (Liu *et al.*, 2019). NDV-based viral vectors

expressing the influenza NA and HA glycoproteins (Nagy *et al*., 2016; Park *et al*., 2006) have been obtained and evaluated as immunogens for chickens. Recombinant NDV expressing H9 HA protects SPF chickens against heterologous avian influenza H9N2 virus challenge (Nagy *et al*., 2016; Liu *et al*., 2018; Xu *et al*., 2019; Zhang *et al*., 2021b).

Conclusions and Recommendations

Avian influenza virus (AIV) of the subtype H9N2 virus has been detected in several African countries with endemic status in Egypt and frequently reported in North Africa (Morocco, Tunisia, Libya, Algeria), West and Central Africa (Nigeria, Burkina Faso, Ghana, Uganda, Benin, Senegal, Togo and Kenyia) since 2016 and South Africa.

The isolated viruses from poultry in Africa have been classified as G1 'Western' Viruses and related to the circulating viruses in the Middle East. In addition to Y439 lineage recorded in South Africa farmed ostriches. The infection with H9N2 is displaying moderate-to-high morbidity and mortality in the field especially when associated with bacterial or viral infections or with other factors related to biosecurity, nutrition and management. LPAIVs H9N2 in chickens showed mainly respiratory tropism with some strains also show gastrointestinal tropism.

The respiratory and contact transmission are the primary routes of transmission and may arise as an adaptation to poultry which clearly has implication for zoonotic transmission.

Inactivated vaccines are the most common vaccines used to control H9N2 viruses, however they have a wide antigenic variability in different lineages. Egypt and Morocco have applied a mass vaccination programme for commercial poultry to control the infection.

Africa now is under threat from zoonotic H9N2 infections. Several H9N2 viruses have mammalian receptor binding properties that could allow efficient transmission to humans with internal gene cassettes that allow efficient replication in humans. There is a clear risk of H9N2 reassortant with the human influenza virus emergence as well as efficient mammalian polymerase reassortant emergence that pose a high zoonotic and pandemic threat.

There is a real need for better understanding of H9 antigenicity based on the molecular determinants and identification of antigenic drifting viruses and their implications on zoonotic potential. Vaccination can protect against current circulating strains in countries applying vaccination like Egypt.

Further surveillance efforts are highly needed particularly in non-officially declared countries for H9N2 as well as in endemic countries applying vaccination. Continual phenotypic characterisation of H9N2 viruses circulating in poultry farms need to assessed over time to examine their properties such as viral pathogenicity, antigenicity and zoonotic potential.

Novelty Statement

This review discuss the findings of the previous studies published and specifically from Africa for H9N2 virus infection and summarizes the updated siltation and highlights on important findings.

Conflict of interest

The author has declared no conflict of interest.

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