Review Article

Epidemiological Situation and Spread of Avian Influenza H9N2 in Poultry in North Africa

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Abstract | Avian influenza (AI), which is recognized as one of the most contagious respiratory diseases in poultry worldwide, is caused by avian influenza A virus (LPAIV). Wild birds are considered the primary natural reservoirs of low pathogenicity avian influenza (LPAI) viruses. This review focusses on the epidemiological situation and the spread of LPAI viruses of the H9N2 subtype in poultry in North African countries including Morocco, Egypt, Algeria, Tunisia, and Libya. Although H9N2 virus infections do not typically cause severe disease in poultry, they can result in significant economic losses to the poultry industry. Furthermore, the H9N2 subtype has zoonotic importance as human H9N2 virus infections have been identified, particularly among poultry workers. The potential for reassortment (exchanging genome segments with other influenza A virus subtypes) that could produce a virus with pandemic potential also poses a significant threat to human health. Phylogenetic analysis has shown that most of the H9N2 viruses circulating in North Africa belong to the G1-like lineage. This review highlights the need for continued surveillance, accompanied by sequencing and phylogenetic analysis of the viral genome to monitor for emerging viruses of concern and to inform updating vaccines to match the emerging field strains.

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Introduction

Avian influenza (AI) is one of the most contagious respiratory diseases in poultry, and wild birds are considered the primary natural reservoirs. Avian influenza A virus (AIV) belongs to the *Alphainfluenzavirus* genus, in the *Orthomyxoviridae* family (Walker *et al*., 2022). Influenza A viruses are classified in subtypes according to the surface glycoproteins haemagglutinin (HA) and neuraminidase (NA). These have traditionally been determined serologically using haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests, but more recently have been determined by sequencing the HA and NA genes. There are sixteen HA subtypes (H1–16) and nine NA subtypes (N1–

N9) found in wild birds, while H17–H18 and N10–N11 were uniquely identified in bats (Tong *et al.*, 2013). Based on their pathogenicity, AIVs are divided into two categories, highly pathogenic AIV (HPAI) and low pathogenicity AI (LPAI) (Liu *et al.*, 2015).

The LPAIV H9N2 subtype was detected for the first time in 1966 in Wisconsin (USA) from turkeys (A/turkey/Wisconsin/1/1966 (H9N2)) (Homme and Easterday, 1970), while its first detection in humans was reported in 1998 in Hong Kong (Peiris *et al.*, 1999). From December 2015 to February 2024, 94 human cases H9N2 infection have been reported to WHO of which 92 were reported from China and two from Cambodia (WHO, 2024).

Although traditionally, AIV surveillance focussed on virus isolation and serosurveillance, molecular identification by reverse transcription and PCR is now the main approach. Often, initial screening is done to detect sequence in the matrix (M) protein gene that is conserved across all IAV subtypes, following which primers specific for HA and NA can be used to determine the subtype. Complete genome sequencing allows for the characterization of the virus and provides valuable information about its origin, transmission, and evolution (James *et al.*, 2022). This information plays a crucial role in understanding its properties and developing effective vaccines and control measures. In addition, genomic data can help track the spread of the virus and identify any mutations that may affect its pathogenicity or resistance to treatments. Due to the segmented nature of their genome, influenza viruses can generate new viruses through reassortment. Reassortment can lead to antigenic shift when it involves the HA and NA gene segments, but H9N2 viruses can transfer their internal genes to other influenza virus subtypes. Some of these reassortants identified in China, such as H5N1, H7N9, H10N8, and H5N6, have been identified as capable of infecting humans and causing severe illness (Pu *et al.*, 2017). Serosurveillance using the haemagglutination inhibition (HI) test can shed light on the circulating subtype.

Sequence analysis of the HA1 portion of the HA (containing the variable antigenic regions) of H9N2 viruses revealed the presence of two distinct phylogenetic lineages: American and Eurasian. The latter lineage is further subdivided into three sub-lineages identified as the (i) G1 sub-lineage, represented by A/quail/Hong Kong/G1/97; (ii) the Y280-like sub-lineage represented by A/chicken/Beijing/1/ 94, A/duck/Hong Kong/Y280/97, and A/chicken/Hong Kong /G9/97, and (iii) the Korean or Y439-like sub-lineage (represented by A/chicken/Korea/38349-p96323/96 and A/duck/Hong Kong/Y439/1997) (Dong *et al.*, 2011; Guan *et al.*, 1999; Guo *et al.*, 1992).

Although LPAIVs cause less severe disease than HPAI viruses, they nonetheless cause economic losses to the worldwide poultry industry (Liu *et al.*, 2015). Recently, in the Middle East, it was reported that the H9N2 subtype caused high morbidity and mortality in poultry; the G1-lineage H9N2 virus was responsible for this outbreak (Lau *et al.*, 2016). The current review focuses on the epidemiological situation of H9N2 LPAIVs in North Africa. The poultry industry is the one of most important activities that contributes to the economic development of North African countries. According to the latest FAO statistics, there are more than 671 million poultry in North African countries (Algeria, Morocco, Tunisia, Egypt, and Libya), around 96% of which are chickens, and 4% Turkeys (Table 1).

Table 1: *Global populations of chickens and turkeys, in North African countries (Morocco, Egypt, Algeria, Tunisia, and Libya) in 2020 (FAO, 2020).*

Country	Total poultry	Percentage	Percentage	
			Chickens	Turkeys
Morocco	218,221,000	32.51	95.18	4.82
Egypt	170,456,000	25.39	2.00	98.00
Algeria	137,374,000	20.46	99.93	0.07
Tunisia	108,720,000	16.19	88.35	11.65
Libya	36,548,000	5.44	100	00
Total	671,319,000	100	96.02	3.98

History and emergence of the H9N2 virus in North Africa

After its initial isolation in North America in 1966 (Homme and Easterday, 1970), H9N2 virus was subsequently detected in domestic ducks in southern China during surveillance conducted from 1976 to 1980 (Shortridge, 1992). Since the late 1990s, H9N2 viruses from southern China have become the dominant strain in Asia (Guan *et al.*, 1999), and have spread to become endemic in Middle Eastern countries (Nili and Asasi, 2002), and North Africa, where successive H9N2 avian influenza waves have occurred since its first detection in 2005 in Libya (Figure 1) (Al-Garib *et al.*, 2007).

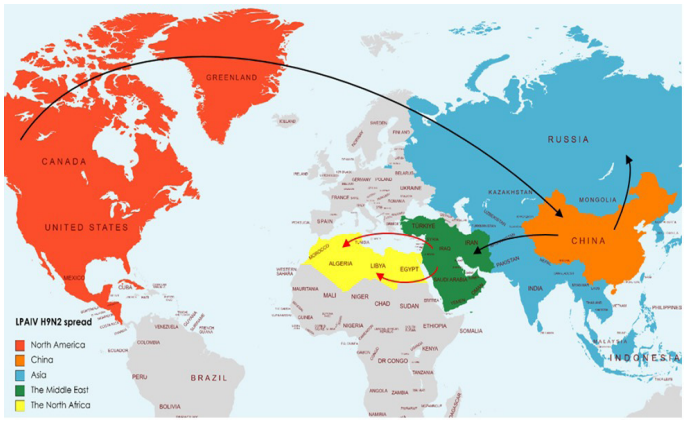

Figure 1: *Map representing the spread of LPAI H9N2 from North America to China, Asia, the Middle East, and finally North Africa (Figure made using: https://www.mapchart.net/africa.html).*

Epidemiological situation of LPAIV H9N2 in poultry in North Africa

Libya

Between March and May 2005, northwest Libya experienced high mortality in chickens aged between 37 and 48 days with a drop in egg production for breeder and layer flocks. The infection was associated with seropositivity and LPAIV H9N2 was confirmed by RT- PCR (Al-Garib *et al.*, 2007; Fares *et al.*, 2010). In 2006, LPAIV H9N2 was detected for the first time in Libya, although the virus could not be isolated (Kammon *et al.*, 2015).

Table 2: *Avian influenza H9N2 viruses detected in North African countries.*

Country	Name	Accession No.	References
Algeria	A/chicken/6BDD/Algeria/2017	MK788274	Barberis *et al.* (2020)
	A/chicken/Algeria/203/2017	MK240260	Jeevan *et al.* (2019)
Egypt	A/chicken/Egypt/11vir4453-276/2010	EPI355114	Kandeil *et al.* (2014)
	A/quail/Egypt/113413v/2011	JN828570	Arafa *et al.* (2012)
	A/chicken/Egypt/D5490B/2012	KF881341	Kandeil *et al.* (2014)
	A/chicken/ Egypt/D7436C/2013	KF881553	
	A/chicken/NLQP194V- AR756/Egypt/2013	EPI557473	Naguib *et al.* (2015)
	A/chicken/Egypt/D10802E/2015	KT216663	Unpublished
Libya	A/avian/Libya/RV35D/2006	JX273538	Slomka *et al.* (2013)
	A/avian/Libya/13VIR7225-2/2013	KM244121	Kammon *et al.* (2015)
Morocco	A/chicken/Morocco/SF1/2016	LT598512	El Houadfi *et al.* (2016)
	A/chicken/Morocco/SF2/2016	LT598513	
	A/chicken/Morocco/SF3/2016	LT598514	
	A/chicken/Morocco/SF4/2016	LT598515	
	A/chicken/Morocco/SF5/2016	LT598516	
	A/chicken/Morocco/19RS1944-1/2016	EPI1950238–EPI1950245	El Mellouli *et al.* (2022)
	A/chicken/Morocco/AS13/2018	MW165152	Sikht *et al.* (2022)
	A/chicken/Morocco/AS32/2019	MW165090	
	A/chicken/Morocco/2_924_21RS1333-2/2019	EPI1950230–233, 235–237 and EPI1950385	El Mellouli *et al.* (2022)
	A/chicken/Morocco/10_4437_21RS1333-10/2020	EPI1950285–EPI1950292	
	A/chicken/Morocco/24_520_21RS1333-28/2021	EPI1950201–EPI1950208	
Tunisia	A/chicken/Tunisia/12/10	JF323006	Tombari *et al.* (2011)
	A/migratory bird/Tunisia/51/10	JF323007	
	A/chicken/Tunisia/848/2011	JQ952591	Aouini *et al.* (2016)
	A/chicken/Tunisia/145/12	KP058446	
	A/chicken/Tunisia/812/2012	MN520599	Arbi *et al.* (2020)
	A/chicken/Tunisia/478/2013	MN520600	
	A/chicken/Tunisia/56/2014	MN520601	
	A/greater flamingo/Tunisia/121/2014	MW375808	Larbi *et al.* (2022)
	A/chicken/Tunisia/803/2015	MN51942	Arbi *et al.* (2020)
	A/chicken/Tunisia/40/2016	MN519422	
	A/black winged stilt/Tunisia/119/2018	MT609882	Larbi *et al.* (2022)
	A/gray headed gull/Tunisia/216/2018	MW375771	

In March 2013, an acute mixed avian respiratory disease occurred in Libya. The infection propagated rapidly throughout the country causing high mortality. A total of 17 tracheal and cloacal swabs were taken from affected poultry farms. AIV H9N2 infection was confirmed by RT- PCR. Kammon *et al*. (2015) were able to isolate LPAIV H9N2 viruses, named A/avian/Libya/13VIR7225-X/2013 (H9N2), where x = 2, 3 or 5 (Table 2). Phylogenetic analysis of the HA gene showed that the three Libyan H9N2 viruses isolated belonged to the G1-like lineage and were closely related to those isolated in Libya, the UAE, Qatar, Tunisia, and Saudi Arabia between 2006 and 2011 (Kammon *et al*., 2015).

Recently, a surveillance study was conducted in live bird markets in Tripoli, Libya and its neighbouring regions to assess AIV presence as well as its associated risk factors (Kammon *et al*., 2022). Between the months of February and March 2018, a total of 269 cloacal swabs obtained from different live poultry species, were tested using RT-PCR to detect AIV. The findings showed that 28 out of 269 (10.41%) tested positive for AIV, but the subtypes could not be determined. According to the results, only three poultry species-ducks, local chickens, and geese were found to be positive, with seropositivity of 21.5%, 12.24%, and 7.14%, respectively (Kammon *et al*., 2022).

Morocco

In January 2016, Morocco experienced an important AIV (H9N2) outbreak. During this outbreak, 11 poultry flocks from different Moroccan regions presented a high mortality rate (2 to 15%) with acute respiratory signs (sneezing, coughing, rales, and gasping), and an 80% drop in egg production. H9N2 AIV was detected in 10 of 11 samples using RT-PCR. Five of ten positive samples were isolated, sequenced, and named A/chicken/Morocco/SF1/2016 to A/chicken/Morocco/SF5/2016. According to phylogenetic analyses, the five H9N2 strains isolated in Morocco in 2016 belonged to the G1 sub-lineage of the Eurasian lineage and were nearly identical to those detected in the Middle East (El-Houadfi *et al*., 2016).

Between July 2016 and October 2018, a further outbreak of acute respiratory infection was reported in Moroccan poultry. Significant mortality (30%) was declared with fowl aged between 19 and 43 days presenting severe respiratory signs. During this outbreak, a total of 105 swabs (organ and tracheal samples) were collected from 21 broiler flocks. According to Khantour *et al*. (2020), H9N2 AI infection was confirmed in 10 out of 21 flocks using RT-PCR. Sequence could only be obtained for four viruses; phylogenetic analysis showed that these belonged to the G1 sub-lineage of the Eurasian lineage. Furthermore, the nucleotide sequences of their HA gene show a high identity of 98% with A/chicken/Burkina Faso/17RS93-19/2017, and 96% to 98% with A/chicken/Dubai/D2506/2015. In addition, all the Moroccan isolates revealed high similarity of 97.9–99.9% to each other (Khantour *et al*., 2020).

During June 2018 and May 2019, Sikht *et al*. (2022) collected 151 samples (organs and tracheal swabs) from chickens with respiratory signs from 108 broiler flocks in eight Moroccan locations. After testing by RT-quantitative PCR (qPCR), 63 samples were identified as positive for AIV (58%). In this study, seven viruses, named AS13, AS14, AS29, AS32, AS71, AS76, and AS77, were fully sequenced. Based on phylogenetic analysis, the seven Moroccan H9N2 viruses detected clustered in the G1 lineage and close to the H9N2 viruses from the United Arab Emirates (UAE) in 2015, Morocco in 2016, Burkina Faso in 2017, and Algeria in 2017 (Sikht *et al*., 2022).

A recently published study was carried out on the evolutionary history and zoonotic potential of the H9N2 viruses between 2016 and 2021 in seven Moroccan regions. Organ samples, oropharyngeal, and cloacal swabs were collected from 81 poultry flocks. All samples were tested for IAV using RT-PCR, then the positive ones were analysed for the H9 gene. Samples were positive for AIV, specifically for the H9 subtype of IAV, from 76 of the 81 flocks. The complete genomes were sequenced for 22 positive samples, while only the partial genome could be sequenced for 6 viruses. Phylogenetic analysis of the HA gene confirmed that all the Moroccan strains isolated during the 2016 to 2021 period belonging to the G1-like lineage and grouped with H9N2 viruses detected in the UAE, Oman, Algeria, Burkina Faso, Senegal, Togo, Ghana, and Benin between 2015 and 2020. The study also suggested that the Middle East was the origin of the H9N2 virus in Morocco, and Morocco subsequently transmitted the virus to Algeria and West African countries. Consequently,

Morocco had a crucial role in H9N2 dissemination to other African nations (El-Mellouli *et al.*, 2022).

Egypt

During 2009 and 2010, Egypt experienced an avian respiratory outbreak. A total of 1,225 blood samples were collected from 39 poultry farms (9 broiler, 12 breeder and 18 layers flocks) between February 2009 and April 2010 (Afifi *et al.*, 2012). The HI test showed antibodies against the H9 subtype in around 54% of the sera (21 out of 39 flocks). The authors suggested that the first detection of the H9N2 virus in Egypt was due to the introduction of the virus by migratory wild birds, given the key role they play in the spread of the related H5N1 virus, which has been detected in Egypt since June 2006. Egypt's location on the migratory pathway of wild birds also supports this hypothesis (Figure 2) (Feare, 2010).

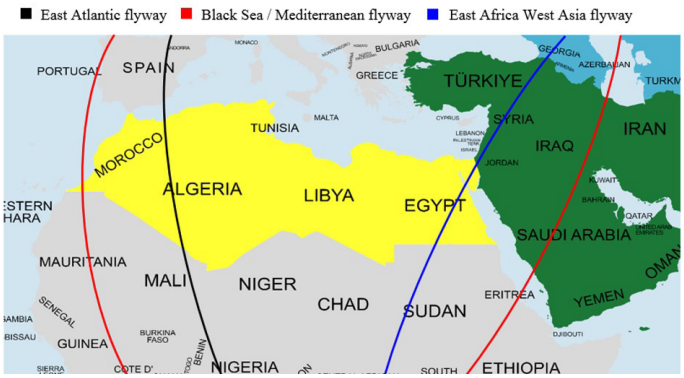

Figure 2: *Map representing the location of North Africa underneath the major flyways of wild birds (Feare, 2010) (Figure made using: https://www.mapchart.net/africa.html).*

LPAIV H9N2 was isolated and genetically characterized for the first time in Egypt, precisely in Giza, in May 2011. Ten tracheal and cloacal swabs were taken from commercial bobwhite quail flocks and tested for the H9N2 subtype using RT–PCR. In this study, an H9N2 virus was isolated and named A/quail/Egypt/113413v/2011 (H9N2). Phylogenetic analysis of the HA and NA genes showed that the Egyptian strain was highly like those isolated in the Middle East and clustered with them in the G1-like lineage (Arafa *et al.*, 2012; El-Zoghby *et al.*, 2012).

Also in May 2011, another AIV H9N2 strain was isolated and characterized but this time in Alexandria (Egyptian governorate). A flock of broiler breeders aged 42 days suffered from respiratory signs with morbidity (70%) and significant mortality (15%). Ten paired tracheal and cloacal swab samples were taken and tested using RT–PCR. The positive sample obtained was sequenced and named A/chicken/Egypt/BSU-CU/2011 (H9N2). Based on HA and NA phylogenetic analysis, the Egyptian H9N2 virus detected clustered in the G1 lineage and was similar to the H9N2 strains circulating in the Middle East (Abdel-Moneim *et al.*, 2012).

During the months of December 2011 and April 2013, 22 samples (cloacal and oropharyngeal swabs) were taken from broiler chicken farms in Egypt. Based on phylogenetic analysis, the H9N2 viruses showed a high similarity with those viruses from neighbouring Middle Eastern countries and clustered together with those of the G1-like lineage (Kandeil *et al.*, 2014).

From 2017–2020, 44 H9N2 positive samples (cloacal and oropharyngeal swabs) tested by RT-PCR were selected for sequencing. According to the results of phylogenetic analysis, all 44 H9N2 strains isolated in Egypt belonged to the G1-like lineage within genetic subgroup B and exhibited a high degree of amino acid sequence similarity (ranging from 96.5% to 99.9%) with the first strain discovered in Egypt (A/quail/Egypt/113413v/2011), as well as 94.7% to 96.2% sequence identity with strains isolated between 2013 and 2016 (Adel *et al.*, 2021).

Recently, molecular characterization of LPAIV H9 was carried out between 2020 and 2021 in Ismailia in Egypt. In this study, the LPAI H9 was isolated from commercial broiler flocks. Samples from 14/34 farms (41.17%) were positive using RT-qPCR. Three representative samples were selected for partial HA gene sequencing and named A/chicken/Ismailia/X/2021 (H9) where X is the unique laboratory identifier (82, 88, and 92). Phylogenetic analysis showed a close similarity with those isolated in the Middle East in 2020 with sequences clustering in the G1-like lineage (Shelkamy *et al.*, 2022).

Algeria

A study on mixed avian respiratory diseases was performed in Algeria between February 2012 and August 2013. Tracheal and cloacal swabs were collected from poultry flocks that presented respiratory signs and mortality of 5% to 47%. Tracheal swabs were used to confirm the presence of AIV for the first time in Algeria using RT-qPCR, but due to poor transit conditions, no AIV was isolated, nor could the strain

be characterized (Sid *et al*., 2015).

In 2017, H9N2 AIV was isolated for the first time in Algeria. Two studies were carried out on the 2017 avian influenza outbreaks, one in earlier 2017, and the other in later 2017 (Jeevan *et al*., 2019; Barberis *et al*., 2020). In April 2017, an avian respiratory disease occurred in the North-East of Algeria, precisely in Batna province, and it quickly spread to nearby regions (Setif and Msila). Around 300 chickens were lost from each flock every day, accounting for 40 to 60% of all mortality in flocks older than 45 days of age. Avian influenza infection was confirmed by RT-qPCR in 20 organ pools. Ten virus isolates were identified as AIV H9N2 by sequencing the HA and NA and were named A/chicken/Algeria/XBBD/2017 (H9N2), where X is the unique laboratory identifier (6, 8, 9, 10, 12,13, 15, 17, 17 and 19). According to phylogenetic analysis of the HA and NA genes, it was confirmed that the ten viruses belonged to the G1 sub-lineage of the Eurasian lineage (Barberis *et al*., 2020). Later in 2017, Algeria experienced another avian respiratory disease but this time in north-central Algeria, precisely in Fouka in Tipaza province. During the months of October and November 2017, three poultry flocks were affected. In the two flocks in which the chickens had not received an H9N2 vaccine, morbidity was 57% in chicks aged 23–26 days. In the third flock, in which chickens were vaccinated with a monovalent H9N2 vaccine, the morbidity was between 17% and 34%. Fifteen H9N2 avian influenza viruses were isolated from broiler-chicken tracheal swabs, and after HA and NA sequencing, it was determined that all H9N2 AI strains detected were clustered in the G1 sub-lineage of the Eurasian lineage. In addition, the nucleotide comparison revealed that the H9N2 Algerian strains were nearly identical to each other (99% to 100%) and showed a high similarity with those isolated in Morocco, Burkina Faso, and the United Arab Emirates (Jeevan *et al*., 2019). In this study, it was also shown that these viruses can infect and replicate in the upper respiratory tract of ferrets and transmit from experimentally inoculated ferrets to in-contact ferrets (Jeevan *et al*., 2019).

Tunisia

In late 2009, an H9N2 AI outbreak occurred in Tunisia for the first time. LPAIV H9N2 was isolated during the second AI wave (July–October 2010). In this study, two H9N2 AIV strains were detected (A/chicken/Tunisia/12/2010 (H9N2) and A/migratory bird/Tunisia/51/2010 (H9N2)) from a commercial chicken and a migratory bird, respectively. Phylogenetic analysis showed that the two 2010 Tunisian isolates were related to the Middle Eastern viruses and grouped with them within the G1-like lineage (Tombari *et al*., 2011, 2016).

Between October 2010 and May 2011, the agriculture ministry of Tunisia conducted a surveillance study on commercial farms in 20 governorates to estimate the prevalence of AI. The study included 187 layers, 453 broiler, 58 breeder broiler, and 102 turkey farms (a total of 800 flocks). Antibodies against AIV infection were found in 28.7% (223 out of 800) of flocks. Virological analysis was done on 400 flocks–swab samples (cloacal and tracheal) taken from 20 birds in each flock were tested. Samples from 40 (10%) of the flocks were positive by RT-qPCR for the M gene. According to phylogenetic analysis of HA and NA, all sequences identified between 2010 and 2011 clustered in the G1-like H9N2 lineage and revealed a high similarity with those identified in 2010 (Tombari *et al*., 2013).

In 2012, an H9N2 LPAIV was isolated and characterized in the South Tunisian outbreak. The HA and NA amino acid sequence of the 2012 Tunisian strain revealed that A/chicken/Tunisia/145/2012 (H9N2) grouped in the G1-like lineage and showed high identity with viruses detected in Libya and the Middle East (Aouini *et al*., 2016).

From April 2012 to January 2016, an AIV surveillance programme was carried out in 23 Tunisian governorates during which 660 samples (organ samples and tracheal and cloacal swabs) were taken from commercial poultry farms to detect the AIV virus. In this study, five H9N2 LPAIV were isolated and sequenced (A/chicken/Nabeul/812/2012, A/chicken/Tunis/478/2013, A/chicken/Tunisia/56/2014, A/chicken/Mahdia/803/2015, and A/chicken/Sfax/40/2016). According to phylogenetic analysis, the HA, NA, M, and NP segments belonged to the Eurasian G1-like lineage, while the PB1, PB2, PA, and NS segments were related to G1-like, Y280-like, and Korean-like lineages, indicating reassortment (Arbi *et al*., 2020).

Between 2014 and 2018, Larbi *et al*. (2022) conducted the first study on H9N2 AIV from wild birds and lagoon water in Tunisia. During 2014–2015, tracheal

and cloacal swabs were collected from two healthy young greater flamingos, ten organ samples (one black-winged stilt and nine gray-headed gull) were taken in 2018 from Mamoura Lagoon and Kuriat Island, and Maamoura Lagoon water was also collected. Eleven out of 13 samples tested positive for the H9 gene using RT-qPCR and virus was successfully isolated from 5 of the 11 positive samples. According to HA and NA phylogenetic analysis, the five H9N2 AIV isolates clustered in the G1-like sub-lineage of the Eurasian lineage. According to this study, the five isolates and those found in the Middle East, North, and West Africa had a high similarity (Larbi *et al.*, 2022).

Implications of the epidemiology of H9N2 viruses in North Africa

Tracing the path of the H9N2 virus shows that it emerged for the first time in North America (Homme and Easterday, 1970), spread to China (Shortridge, 1992), Asia (Guan *et al.*, 1999), the Middle East (Nili and Asasi, 2002; Banks *et al.*, 2000), and finally arrived in North Africa (Alexander, 2007; El-Mellouli *et al.*, 2022). North Africa has experienced a series of waves of H9N2 since its first detection in Libya in 2005 (Al-Garib *et al.*, 2007). Morocco is considered a potential route of entry for H9N2 due to its location along the Mediterranean flyway, which is one of the major migratory bird routes in the world (Figure 2). It is possible for a new emergence of subtypes with distinct characteristics with another NA subtype and not just with H9N2, as has been seen with H5N1, H5N5, and H5N8 (Lewis *et al.*, 2021). To date, only G1-like H9N2 virus detected in Africa (Figure 3) (Awuni *et al.*, 2019).

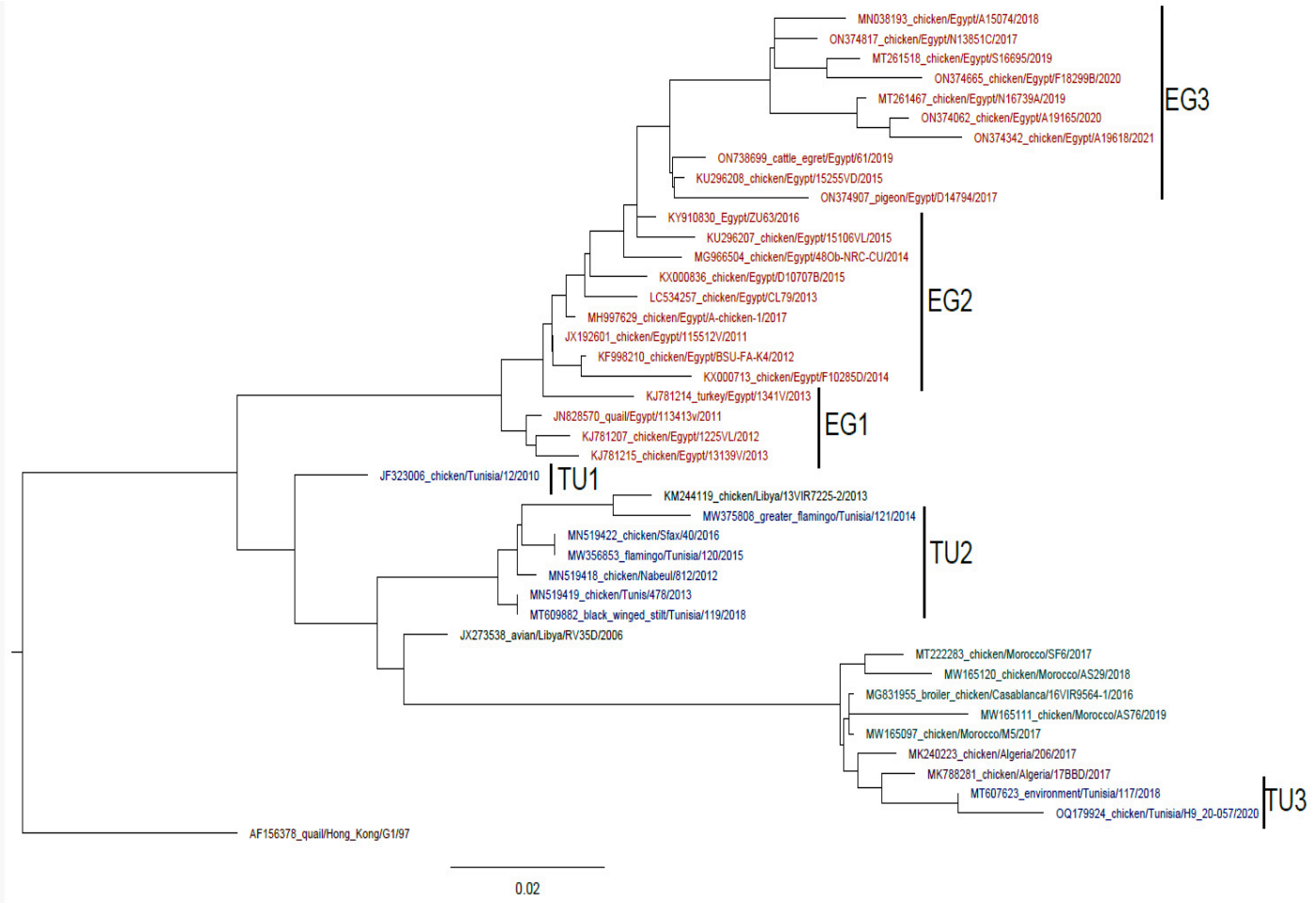

Figure 3: *Phylogenetic tree of the H9N2 HA sequences identified in North Africa from 2010 until 2021. A total of 41 North African sequences were retrieved from GenBank (see Table 2 for accession numbers) and aligned with the G1 lineage reference sequence (A/quail/Hong Kong/G1/97; GenBank accession number AF156378) using MUSCLE. Phylogenetic analysis was performed with MegaX software using the maximum likelihood model. The tree was edited using Figtree. The North African sequence names are colour-coded according to country of origin: Algerian viruses (green), Egyptian (red), Libyan (brown), Moroccan (purple), Tunisian (blue). Three distinct clusters of Egyptian (EG) and Tunisian (TU) viruses are indicated.*

Surveillance of the H9N2 virus should be conducted periodically by sampling domestic and wild bird populations, with a focus on certain geographic areas such as poultry farms, live bird markets, and migratory bird flyways, which are major sources of H9N2 transmission (Afifi *et al.*, 2012; Kammon *et al.*, 2022).

Vaccines are still the most effective means for the prevention and control of all diseases of viral origin worldwide. In North Africa, despite the earliest detection of H9N2 AIV (Libya, 2006), not all North African countries practice a vaccination protocol properly (Slomka *et al.*, 2013). In fact, some countries, such as Libya, have not implemented any vaccination programmes at all (Kaboudi, 2019). However, several North African countries have adopted vaccination programmes that include inactivated virus vaccines against the H9N2 virus for the poultry industry. The specific types of flocks that are vaccinated may vary from country to country and may include breeder, layer, turkey, and broiler flocks (Kaboudi, 2019; El-Mellouli *et al.*, 2022). To achieve greater vaccine efficacy, it is preferable to use locally produced vaccines as they have been shown to provide better results compared to imported ones. In addition to those which include both LPAIV (H9N2) and HPAIVs (H5N1 and H5N8) viruses, can provide full protection at a lower cost (Gomaa *et al.*, 2019; Ebrahim and Seioudy, 2020).

Risk assessment and surveillance of H9N2 AIV

Risk assessment of H9N2 AIV should be conducted globally, and mitigation efforts should be instigated to stop virus outbreaks. There is a high risk of H9N2 spread in poultry markets, especially with the high number of birds that increase the risk of slaughter area contamination. The high number of birds kept in a restricted space may be exposed to contaminated surfaces, which could raise the risk of infection (Kung *et al.*, 2003). The solution includes estimating the required daily number and following an "all-in-all-out" approach that could reduce the virus spread. The presence of the virus in the waste is a high-risk factor with the possibility of its transmission to people handling the waste and stray animals. This highlights the importance of proper disposal of waste from poultry shops (Sun *et al.*, 2013). This should give the seller enough time to clean the slaughter area and interrupt the infection cycle.

There is a high risk of AIV transmission between backyard birds and backyard poultry via water bodies (Yuan *et al.*, 2014). Keeping the birds outside the poultry shops poses a risk of AIV spread by contacting other birds and stray animals (Iqbal *et al.*, 2013; Dixit *et al.*, 2024). Other factors like clean water availability, housing of birds in shops, and sharing equipment between shops have a significant role in AIV transmission (Cardona *et al.*, 2009; Garber *et al.*, 2007; Chaudhry *et al.*, 2015; Gompo *et al.*, 2020).

Active surveillance and control measures are very important to control AIV control. Unfortunately, the H9N2 outbreak during 1992–1998 was missed and the virus spread to several countries. The spread was reduced because of the restriction of bird movement, disinfection, stopping poultry trading, and culling of infected birds (Sagong *et al.*, 2023).

Despite the implementation of vaccine strategies against H9N2, which reduced H9N2 virus isolation and virus spread, the virus continues to circulate, and new antigenic variants arise. This might be the result of vaccine mismatch, low doses, or inadequate application of vaccines (Sun *et al.*, 2012; Cui *et al.*, 2021). Differentiating between infected and vaccinated birds (DIVA) is an important strategy for enabling trade of poultry (Umar *et al.*, 2016). Surveillance is important for determining influenza incidence and evaluating the risk factors associated with the disease (Derrar *et al.*, 2019). Since 1948, the World Health Organisation (WHO) has established the Global Influenza Programme. This initiative was developed to enhance the monitoring, prevention, and control of influenza on a global scale (Barakat *et al.*, 2011). In North Africa, the Institute Pasteur has assisted National Influenza Centers in establishing influenza monitoring systems (Derrar *et al.*, 2019).

For the virological surveillance system, oropharyngeal and nasopharyngeal swabs should be collected and placed in cryovials containing 2 ml of virus transport medium. These samples must be stored at 4°C and transported daily to the National Influenza Center (NIC) or Regional Reference Laboratories (Yazidi *et al.*, 2019).

LPAIV surveillance is more challenging than for HPAIV because low pathogenicity avian influenza is not a notifiable disease and causes few human cases. Surveillance is rare or never conducted in low and middle-income countries (LMIC), so the H9N2

AIVs are probably more widespread than is recorded (Peacock *et al*., 2019).

Three primary populations can be targeted for AIV surveillance: wild birds, farmed poultry, and occupationally exposed populations. The awareness of the persistence, intraspecies and interspecies transmission, and evolution of AIVs is largely dependent on the surveillance programmes in those groups. Although wild birds are the main reservoir, AIV surveillance is lacking in wild birds limited to the last outbreak virus. From 2009 to 2011, active AIV surveillance was conducted in the Indian state of West Bengal on wild and domestic poultry (Pawar *et al*., 2012). Researchers found low pathogenicity H9N2 and H4N6 viruses in domestic ducks and chickens throughout the period (Pawar *et al*., 2012). Two surveillance programmes conducted in Egypt between 2010 and 2013 and between 2012 and 2013 in a variety of regions and poultry production sectors revealed frequent co-infection of the same avian host in addition to co-circulation of subtype H9N2 and subtype H5N1 viruses. These co-circulating viruses raise the possibility of reassortment (Kayali *et al*., 2014; Shakal *et al*., 2013).

From 2008 to 2010, a surveillance study was carried out in Shanghai, China, on workers who were exposed to poultry during their work. There was evidence showing that a significant proportion of workers had anti-H9 antibodies. In addition, over 700 influenza viruses (H3N2, H1N1, B) were identified from the nasal and throat of people exhibiting influenza symptoms, and over 200 H9N2 AIVs were detected in the cloacal and tracheal swabs taken from live poultry (Wang *et al*., 2015). The likelihood of reassortment between avian and human influenza viruses is increased by this co-circulation. Thus, it is crucial to monitor AIVs over an extended period in workers who are exposed to poultry (Rahimi-Rad *et al*., 2016).

Passive surveillance programmes require significant investment; identifying patterns in the epidemiology of outbreaks in resource-poor countries can help focus these efforts. The similarity of many of the characterised viruses to other Middle Eastern viruses supports the hypothesis that viruses are introduced in the east via wild bird flyways that cross Egypt (Table 3). This begs the question whether the H9N2 virus emerged in Libya, where it was first identified or if it might have first emerged in Egypt and gone undetected. In Egypt, surveillance efforts could be focussed on the time that migratory birds, in particular ducks, are passing through and in the region of the four major stopover sites (known as Ramsar sites) in Egypt (Naguib *et al*., 2019). There was no apparent seasonal trend in the occurrence of outbreaks previously reported in North African countries (Table 3), but this could be because earlier smaller outbreaks were missed. Different flyways cross Morocco and Algeria to the west. If surveillance could be co-ordinated across the North African countries, this could confirm whether the LPAI viruses are introduced via wild birds and help monitor for patterns of spread. Understanding risk of introduction and patterns of spread are important as outbreaks of diseases of concern can lead to countries imposing bans on trade of poultry and poultry products. According to Trend Economy (https://trendeconomy.com/), the main export markets for Egypt in 2023 were Malawi and Cameroon. Most imported live poultry were from the United Kingdom, France and the USA, further supporting the notion that wild birds are the source of initial introduction of LPAI.

Antigenic variability of AIV

The progressive accumulation of mutations over time, mainly in the HA gene alone or in both the HA and NA genes, is attributable to the low fidelity and lack of corrective activity of the viral RNA-dependent RNA polymerase. This set of phenomena is the basis of 'antigenic drift (Hannoun, 1980). In contrast, a complete change in antigenic properties occurs by replacing a gene segment encoding the HA of one subtype with that of another, which may or may not be accompanied by the replacement of the NA gene, inducing an 'antigenic shift' (Webster *et al*., 1982). This is due to the segmented nature of the influenza virus genome meaning that simultaneous co-infection of a host cell with two different of influenza A viruses can result in the reassortment of the segmented viral genes (Webster *et al*., 1992; van Maanen and Cullinane, 2002). In 2013, a novel avian influenza A H7N9 virus that infects humans was identified in China. It is believed that this H7N9 virus emerged from multiple reassortment events involving two distinct groups of H9N2 avian influenza viruses. The HA gene likely came from avian influenza viruses in ducks, while the NA gene appears to have originated from migratory birds infected with avian influenza viruses traveling along the East Asian flyway (Liu *et al*., 2013).

Table 3: *Summary of H9N2 avian influenza outbreaks in North Africa (2005–2021).*

Country	Outbreak period	Regions affected	Detection method	Phylogenetic lineage	Related strains
Algeria	February 2012 - August 2013	Various	RT-qPCR		
	April 2017	Batna, Setif, Msila	RT-qPCR	G1 Eurasian	Morocco, Burkina Faso, UAE
	October - November 2017	Tipaza (Fouka)	RT-qPCR	G1 Eurasian	Morocco, Burkina Faso, UAE
Egypt	2009 - 2010	Various	HI Test	G1-like	Middle East
	May 2011	Giza, Alexandria	RT-PCR	G1-like	Middle East
	December 2011 - April 2013	Various	RT-PCR	G1-like	Middle East
	2017 - 2020	Various	RT-PCR	G1-like	Middle East
	2020 - 2021	Ismailia	RT-qPCR	G1-like	Middle East
Libya	March - May 2005	Northwest Libya	RT-PCR		
	March 2013	Various	RT-PCR	G1-like	Libya, UAE, Qatar, Tunisia, KSA
	February - March 2018	Tripoli, Neighbours	RT-PCR		
Morocco	January 2016	Various	RT-PCR	G1 Eurasian	Middle East
	July 2016 - October 2018	Various	RT-PCR	G1 Eurasian	Burkina Faso, Dubai
	June 2018 - May 2019	Various	RT-qPCR	G1 Eurasian	UAE, Algeria, Burkina Faso, Morocco 2016
Tunisia	Late 2009 - October 2010	Various	RT-qPCR	G1-like	Middle East
	October 2010 - May 2011	Various			Middle East
	2012	South Tunisia	RT-qPCR	G1-like	Libya, Middle East
	April 2012 - January 2016	Various	RT-qPCR	G1-like	Middle East
	2014 - 2018	Various	RT-qPCR	G1-like	Middle East, North Africa

Current studies depend on the HA for identifying new clusters of H9N2 based on the HA inhibition test which is limited to sample size, spatial, and temporal scale. Zhai *et al.* (2024) developed the H9N2 prediction model (PREDAC-H9) combined with HA sequencing results to reveal the H9N2 antigenic distribution and variation. The PREDAC-H9 uses antigenic epitopes, glycosylation, and antigen-binding sites to predict H9N2 viruses. The model showed that the widely prevalent G1 lineage developed only two clusters, while the h9.3-BJ94 lineage developed five clusters. Vaccine pressure against the h9.3-BJ94 lineage might have accelerated antigenic drift.

Antiviral therapy is essential to control influenza infections, especially in animals at higher risk of serious infection and complications (Doll *et al.*, 2017). Peramivir (sialic acid analogue), Oseltamivir and Zanamivir are the most common neuraminidase inhibitors (Young *et al.*, 2001; Magano, 2009), while Rimantadine inhibits the M2 proton channel of the influenza virus (Rees *et al.*, 1999). Baloxavir Marboxil (BXM) is an inhibitor of the cap-dependent endonuclease of the polymerase acidic protein (PA) in influenza A virus (Nemoto *et al.*, 2019).

Conclusions and Recommendations

Despite their low pathogenicity, Influenza virus H9N2 poses a significant threat to the poultry sector due to the high level of morbidity and mortality observed during each outbreak, which leads to economic losses worldwide. Surveillance of the H9N2 virus in domestic and wild bird populations is essential. This surveillance should include live bird markets, as they play a crucial role in the spread of AIV. This regular surveillance can give more information about the prevalence, transmission, and potential risks associated with H9N2 and other influenza viruses. Performing sequencing and phylogenetic analysis on the viral genome is important to know the genetic characteristics, evolution, and potential virulence factors of H9N2.

Novelty Statement

This review article is novel in its focus on the situation in North Africa, highlighting the need for continued surveillance.

Author's Contribution

HK: writing - original draft, writing - review & editing, visualization;
OE: writing - review & editing, visualization;
JMD: writing - review & editing, supervision.

Conflict of interest
The authors have declared no conflict of interest.

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