

Migratory Birds as Disseminators of Avian Influenza Viruses to Egypt (2003 – 2023)

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E-mail address: hanan_24395@yahoo.com**Abstract**

Migratory birds are considered a vessel for preserving and transmitting microbes, which contribute with great force in transporting them from one place to another over long distances in the world. This would contribute to the emergence of disease pandemics globally and the most obvious example is the avian influenza (AI) epidemics over time. The most famous recent scenario is the AI pandemic that began in Hong Kong in 1990s. Natural resistance to AI helps migratory birds retain avian influenza viruses (AIVs) for long periods and transfer them from their source to many other countries during migration paths. Accordingly, they serve as a vector for the maintenance and introduction of both highly pathogenic and low pathogenic avian influenza (HPAI and LPAI). Through this study, the subtypes of high- and low-pathogenic avian influenza viruses found in migratory and wild birds in Egypt were identified, and the most important species of implicated birds were recognized since 1970s till now. In the view of geoepidemiology of AIVs transmitted by migratory birds, we highlighted the main entry, gathering places and movement routes encountered either with free-living or domesticated birds. By reviewing the outcomes of previous studies over the past two decades, a total of 108 AIVs including 70 of the LPAI and 38 of the HPAI were isolated from migratory and free-living birds. Migratory birds topped the list with 91% of the LPAI and 79% of the HPAI. Interestingly, Damietta province had the highest rates of virus isolation; 81.3% and 40% for LPAI and HPAI viruses respectively, followed by Sharkia particularly in HPAIV (26.7) and both borders are with Lake Manzala. Two Anseriformes-migratory birds; Northern shoveler & Green-winged teal harbored more than 50% of the isolated AIVs. Therefore, we call for conducting annual screening of AIVs in spring and fall. Focusing on Anseriformes and recently proposed quails as vessels for AIVs' mutations mainly in northeast Lakes' border provinces for early detection and quick intervene and avoid their spread became an urgent need.

KEYWORDS

Migratory birds, free-living birds, Anseriformes, Avian Influenza virus, Northern shoveler

Introduction

Egypt's significant location on the bridge between Africa and Eurasia reflects its important role in the migration paths of birds. There are two major overlapping pathways for migratory birds, (i) the Mediterranean/Black Sea and East Africa/West Asia flight path in which only light weight and small birds species migrate across the Mediterranean Sea, such as Anseriformes, falcons, quails, curlews, beavers, and many other species as they travel for a long time without stopping because there are no islands to stand on along the Mediterranean. (ii) The flight path of the Rift Valley/Red Sea, in which birds come from Palestine and cross Saudi Arabia crossing the Red Sea, then settled in Egypt around Jabal al-Zeit near Hurgada. Most birds here are heavy and large, such as eagles, hawks, egrets, pelicans, and other species that cannot fly for long distances and leave their wings carried by the wind (they do not flap their wings to help them fly), so Egypt authorities has built wind turbines in the Jabal al-Zayt area. The Mediterranean Sea migratory path overlaps with the flight path of the Rift Valley/Red Sea to northern Egypt and then winter along the Mediterranean coast, the Nile Valley, or the Red Sea coast, or

continue south into Africa. The most important bird species that follow this path are the order Anseriformes and Charadriiformes (Birdlife, 2018).

It is evidenced that low pathogenic avian influenza virus (LPAI) in migratory and free-living birds as early as 1970s (Hosny *et al.*, 1980; Amin *et al.*, 1980) and frequently isolated along this history till now (Naguib *et al.*, 2019; Nabil *et al.*, 2020; Abdien *et al.*, 2023). But highly pathogenic avian influenza virus (HPAI) was introduced via migratory birds in 2005 (Saad *et al.*, 2007). Sooner, H5N1 highly pathogenic AI virus was declared as endemic in Egypt (Aly *et al.*, 2006). Since 2006, the HPAI-H5N1 virus is regularly isolated with massive economic losses till 2015 from different poultry species (Aly *et al.*, 2006; Abdelwhab *et al.*, 2010; 2011; 2016; Hafez *et al.*, 2010; ElBakrey *et al.*, 2015; Mansour *et al.*, 2014, 2017; 2018).

Simultaneously HPAI-H5N1 virus was frequently delivered from various migratory; Green-winged teal, Common tail, and Mallard in Damietta (Saad *et al.*, 2007; Kayed *et al.*, 2019), Northern shoveler (Naguib *et al.*, 2019) and free-living birds; Great Egret around Giza Zoo, Crow and Cattle Egret (Naguib *et al.*, 2019). In 2016, the second HPAI-H5N8 clade 2.3.4.4 hit Egypt

where, it was first detected in Green-winged teal in Port Said, common coot in Damietta (Kandeil *et al.*, 2017; Selim *et al.*, 2017). Within the year of 2016, HPAI-H5N8 continued to spread across various poultry backyard sectors and commercial farms of various bird species and different governorates in Egypt; Yehia *et al.* (2018) isolated the virus from backyard ducks in Cairo, Shehata *et al.* (2019) isolated it from chicken farms in Behaira and lately from the commercial chicken flock with typical respiratory symptoms, facial edema, and highly pathognomonic AI postmortem changes (Setta *et al.*, 2023).

Therefore, our primary goal was to provide data about the incidence of avian influenza viruses among migratory, wild, and free-living birds, that have had close contact with domesticated flocks and backyards during the last two decades. Furthermore, to highlight their role in introducing and maintaining LPAI and HPAI viruses in Egypt.

Immigration and migratory birds

The instinct for survival, searching for food, escaping from the cold, moving between breeding and non-breeding sites, and increase resources to live in some birds forces them to migrate in large groups and travel millions of miles around the world in a regular seasonal movement, in multiple directions called bird migration. There are at least 4,000 frequently migratory bird species, representing about 40 percent of the global total. Birds on these journeys do not easily reach their destinations, as they require a lot of effort, energy, rest, food, and water, so not all of them succeed, as some of them die. There are two main types of migration, long-distance migration, in which birds travel thousands of miles, as most birds in North America such as geese, black swans and Arctic tern and short-distance migration, in which birds usually travel tens or hundreds of miles for short distances, such as robins and waxwings. Among the most common migration patterns is latitude or longitude migration, in which migration occurs, either from east to west or from north to south, or vice versa. Altitudinal migration is for those birds that give birth at a higher area and are forced to migrate again because of the harsh conditions there. Seasonal migration occurs with the change of seasons, where birds migrate due to the unsuitable conditions of life to another place either from north to south in the fall looking for warmer regions or from south to north in spring for breeding. Finally, irregular pattern, also called nomadic migration, is for birds that migrate irregularly based on the much or lack of natural food sources in their area (Bianki, and Dobrynina, 1997).

Many types of migratory birds pass through Egypt, especially in the northern regions of the country, where the main migration pathways overlap, Black Sea/Mediterranean and East/African–West Asian. Migratory birds are concentrated mainly in and around the northern lakes where thousands of migratory bird's rests during their overwintering migration pattern to south and considered ideal places for migratory and resident birds. These birds are hunted in their gathering places, then, they purchased at bird markets within the cities surrounding those lakes. Among the most important types of these birds, Green-winged Teal (*Anas carolinensis*), Common teal (*Anas crecca*), Northern Shoveler (*Anas clypeata*), Northern Pintail (*Anas acuta*), Common coot (*Fulica atra*), Eurasian Teal (*Anas crecca*), Marbled Teal (*Marmaronetta angustirostris*), Red-back-shrike (*Lanius collurio*), Common-Blackbird (*Turdus merula*), Greater Flamingo (*Phoenicopteraidae*), Common moorhen (*Gallinula chloropus*), Woodcock (*Scolopax rusticola*), Brown Quail (*Coturnix ypsilophor*), Mallard duck (*Anas platyrhynchos*) and Purple gallinule (*Porphyrio martinicus*). Migratory birds depart in the fall from the breeding areas

in Eastern Europe and Western Asia south to the north and center of the African continent in search of warmth and food, crossing Egypt twice a year and returning in the spring, making Egypt one of their main routes and representing a land bridge between three continents. It is also the second most important migration route for birds globally. There are also free-living/ wild birds in Egypt, such as bobwhite quail (*Colinus virginianus*), Hooded Crow (*Corvus cornix*), Cattle egret (*Bubulcus ibis*), Turtle Dove (*Streptopelia turtur*), Sparrow and many other species live freely and with a direct or indirect contact with other domesticated backyard and commercial birds (Altizer *et al.*, 2011; Mansour *et al.*, 2014).

All these birds (migratory birds, especially wild waterfowl, or free-living/ wild birds) play an important role in the transmission, dissemination, and spread of pathogens including avian influenza viruses between countries and across continents on an ongoing basis. Therefore, viruses can be transmitted to domesticated poultry, in neighboring countries, or over long distances along migration paths. Consequently, free-living/ wild birds have a major role in preserving these viruses and the emergence of new evolution with high virulence even for the reservoir itself (El-Bakrey *et al.*, 2016; Yang *et al.*, 2023). Such naturally resistant wild birds might serve as vectors for introduction of HPAI viruses into new locations. This became clear in the case of the first induction of H5N1 to Egypt via migratory birds in 2005, then spread among domesticated birds causing devastating outbreaks (Aly *et al.*, 2006; Saad *et al.*, 2007; Soliman *et al.*, 2012).

Avian influenza viruses

Avian influenza virus is an enveloped virus belonging to the Orthomyxoviridae family and has a single-stranded, eight-segment RNA genome. It is subtyped based on the two proteins hemagglutinin (HA) and neuraminidase (NA) on its coated surface into 18 known types of HA and 11 known types of NA (Tong *et al.*, 2013). It is classified according to its pathogenicity into HPAI and LPAI. It infects a wide range of birds and mammals and poses a potential and unexpected threat to humans due to the fragmented, mutating nature of the genome. LPAI viruses constantly infects birds asymptotically and is considered a primary latent source for virus shedding and spreading silently, allowing it to cross-spread with other subtypes, genetic reassortment, and mutations occur which increase their virulence, and emerge new evolutionary strains of the disease at intervals. HPAI viruses infect the poultry sector in massive outbreaks, accompanied by high mortality and severe economic losses, but it may appear masked in wild free-living, and migratory birds without any signs or lesions (Kawaoka, 2006; Klenk *et al.*, 2008; Tong *et al.*, 2013).

The intercontinental transmission of HPAI-subtype H5 virus waves included H5N1 clade 2.2 (2005–2006), H5N1 clade 2.3.2.1c (2009–2010), H5N8 clade 2.3.4.4a and H5N1 clade 2.3.2.1c (2014–2015), and H5Ny clade 2.3.4.4b (2016–2017) (Bi *et al.*, 2016; Lycett *et al.*, 2020). Additionally, during 2020–2021, a new upsurge of HPAIV H5N1/H5N8 clade 2.3.4.4b outbreaks was reported in wild and domestic birds in Eurasia (Sobolev *et al.*, 2021; Okuya *et al.*, 2022) and Africa (WAHIS, 2023) including Egypt (Mosaad *et al.*, 2023).

Migratory and free-living birds as transmitting vessels of AIVs in Egypt

Low pathogenic avian influenza viruses

Regarding LPAI viruses, the incrimination of migratory and

wild birds in their introduction as early as 1970s H3N1, H4N1 and H11N6. The H4N6 LPAI virus was detected in domesticated ducks in 1976 (Hosny *et al.*, 1980; Amin *et al.*, 1980). Three decades later a continuous almost yearly record of different subtypes of LPAI viruses in various migratory and wild free-living birds. H3N8 was detected in 1990s from domesticated ducks (El-Said *et al.*, 1998).

Since 2000s, many active surveillance studies on circulating AIVs in migratory and wild/free-living resident birds which revealed many subtypes of LPAIV introduced to Egypt particularly around Lake Manzala including LPAI-subtypes (H1, H2, H4, H6, H7, H9, H10, H11, H13 and LPAI-H5N2) from apparently healthy Common teals (*Anas crecca*), Northern shoveler (*Anas clypeata*), Green-Winged Teal (*Anas carolinensis*), Northern Pintail (*Anas acuta*), Black Kite (*Milvus migrans*), and Mallard (*Anas platyrhynchos*) in Damietta, Port-Said, Abu-Simble, and South Sinai (Saad *et al.*, 2007; Aly *et al.*, 2010; Soliman *et al.*, 2012; Naguib *et al.*, 2019). Interestingly, H1N1 was annually introduced till 2012. The LPAIV subtypes H7Nx were the most isolated from migratory birds since 2004–2016 which could be threatening through host adaptation and possibility of highly pathogenic evolution, particularly H7N7 was detected in domesticated geese (Soliman *et al.*, 2012; Naguib *et al.*, 2019). Other subtypes of LPAIV of several permutations (HxNx) continuously introduced by migratory birds to Egypt (2003–2019) based on available literature oriented to migratory bird threat as a vector for avian influenza viruses. The subtypes H7Nx and H10Nx were the top list isolates; 23 and 14 respectively in which H7N3 was the predominant one (12/23) followed by H10N1 (7/14) and H7N1 (4/23) while, H7N7 and H10N1 were three isolates/each, followed by H7N9 and H10N9 (two isolates/each), meanwhile, H10N4, H10N6 (one isolate/each). However, the N gene was not applicable in two H7Nx isolates. The dominant species infected with these isolated were Northern shoveler (n=34) and Green-winged teal (n=21) which categorized as waterfowl and considered a main reservoir of influenza viruses as previously mentioned (Lebarbenchon *et al.*, 2009) (Figure 1).

show the intermingling of bird species with each other and clear the role of migratory waterfowl in introducing LPAIVs allowing its transmission, maintenance, preservation and antigenic diversity and emerge of a new more virulent mutant.

LPAI-H9N2 subtype (A/quail/Hong Kong/G1/97-like (G1-like) first appeared in China 1996, and according to the assembly scheme described by Fusaro *et al.* (2011), the G1-like strain was shown to be the most geographically widespread from East Asia to the Middle East. Through follow-up studies of the virus, it was found that there were two main hotspots of its spread: China, Saudi Arabia, and the UAE, in the years 1996, 1997, and 1999, respectively. It spread quickly through wide bird migration pathways, as well as poultry industry trade, and rapidly spread to the rest of the world (Li *et al.*, 2020). Its first introduction into Egypt is crucial, since its detection was proved only in domesticated birds; an apparently healthy commercial bobwhite quail flock in Egypt and commercial chickens with respiratory distress in 2011 (El-Zoghby *et al.*, 2012; Monne *et al.*, 2013) and from this date it has been recognized as endemic among Egyptian poultry populations, creating serious economic loss in poultry industry. Later, researchers detected H9N2 in migratory birds in 2015 in three wild ducks from Lake Manzala in Damietta during ongoing AIV surveillance studies (Kayed *et al.*, 2019).

Sustained isolates of LPAI-H9N2 occurred in 2018 from four Northern shovelers in the Gamasa area of Damietta (Nabil *et al.*, 2020), followed by two isolates, one from migratory Dukams and one from migratory Turtle doves at Port-Said, in addition to one isolate from Cattle egret from Ismailia Governorate in 2019 (Abdien *et al.*, 2023). Ten isolates of LPAI-H7N3 were isolated, two in 2015 and three in 2016 from Green-winged teal beside 5 from Northern shoveler in 2016 and one H7N9 in 2016 in Damietta (Kayed *et al.*, 2019). Meanwhile, LPAI-H6N2 started to emerge again in 2017 and 2018 from Eurasian teal in Behira around Lake Idko and from Northern shoveler in Gamasa beside Damietta respectively (Zanaty *et al.*, 2019; Nabil *et al.*, 2020). When looking at the emergence of LPAI-H9N2 (A/Quail/Hong Kong/G1/97) from healthy quails potentiate the interest to understand the importance of its role in epidemiology of H9N2 as a highly susceptible host vessel to the avian influenza virus is clear, where scientists have considered them to be another mixing bowl, like pigs which they express receptors for avian-type a2,3-galactose and human a2,6-galactose receptors for the generation of reassortant viruses from mammalian and avian sources with possibly novel antigenic and genetic features (Guan *et al.*, 1999; Guo *et al.*, 2000; El-Zoghby *et al.*, 2012).

Highly pathogenic avian influenza viruses

The appearance of HPAIVs in migratory birds in Egypt began three decades after the emergence and persistence of LPAI viruses. The HPAI-H5N1 subtype was first detected in Green-winged teal and Common teal migratory ducks at Lake Manzala in Damietta Egypt 2005 (Saad *et al.*, 2007). In the same year, the HPAI-H5Nx virus was stated in the free Rock dove (*Columba livia*) (Zhang *et al.*, 2017; Naguib *et al.*, 2019). A year later, it was isolated from resident Cattle Egert (*Arden alba*) freely living in the area surrounding the Giza Zoo (Soliman *et al.*, 2012). As well as from Crows (*Corvus* spp.) in 2007 (Zhang *et al.*, 2017). It then detected again in the migratory Northern shoveler in 2012 and in the Cattle egret in 2014 (Zhang *et al.*, 2017) and in the mallard duck (*Anas platyrhynchos*) with two clades (2.2.1.1 and 2.2.1.2) in Damietta 2015 (Kayed *et al.*, 2019) and finally in resident waterfowl Geese with clade 2.2.1.2 in Monofiya and one native duck in Giza, 2016 (Hamouda *et al.*, 2019). Comparing the timing of the

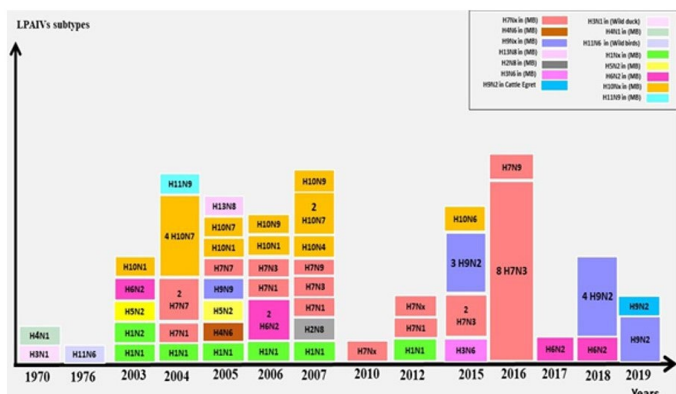


Figure 1. LPAI viruses were recorded in both migratory and free-living resident wild birds in Egypt yearly based on recorded data in the available literature.

Although there was no evidence about the introduction of LPAI-H9N2 to Egypt which is endemic among domesticated birds since 2011 (EL-Zoghby *et al.*, 2012), it was detected in migratory birds 2015 – 2019 (Saad *et al.*, 2007; Soliman *et al.*, 2012; Kayed *et al.*, 2019; Zanaty *et al.*, 2019; Naguib *et al.*, 2019; Nabil *et al.*, 2020; Abdien *et al.*, 2023).

Interestingly, LPAI-H7N7 was isolated in 2004 from both Northern shoveler and Green-winged teal around Lake Manzala and in 2005 from Black kite (Aly *et al.*, 2010; Soliman *et al.*, 2012), followed by its isolation from free living Egyptian geese in Aswan around Lake Nasser in 2006 (Soliman *et al.*, 2012), and as previously proven isolation of LPAIVs from wild ducks in 1976, this may

appearance of the Gs/GD/HPAI-H5N1 subtype in migratory, wild, and resident birds in Egypt with those in the world, it becomes clear that it always started in Asia, particularly China, where the first outbreak occurred in 1961, then in Guangdong 1996, Hong Kong in late 2002 and early 2003, with deaths including wild and captive aquatic birds (waterfowl, herons, gulls, flamingos).

Outside Hong Kong, via wildlife and migratory life, continuous periodic outbreaks have happened in eight countries, including bird infections and human mortalities in 2004. Followed by a huge outbreak of the virus in wild waterfowl including many Bar-headed geese, Brown, and Great-headed gulls in Qinghai Lake, China in 2005. Later in the same year, extended to Europe, then after spread throughout including 38 countries in Asia, Africa, and the Middle East through the period 2006–2009 associated with human fatalities (Ramey *et al.*, 2022). Therefore, it is clear that the peak spread of the deadly, highly pathogenic avian influenza virus H5N1 in many wildlife and migratory birds besides humans in the world was the period when it began to be monitored and discovered in migratory birds in Egypt, which proves its arrival via migration, and confirms that waterfowl especially ducks are the main and important reservoir in its transmission via Countries of the world. At the same time, its detection in Egypt in healthful migratory birds (Mallard ducks, Northern shoveler) in the wetland areas of the Menoufia near the infected Cattle egret living freely around the Giza Zoo and infected Crows supports the idea of co-habitation of the viruses between them and explains the effective role of these birds in the spread of the virus over long distances and miles due to its ease of contact with various domestic poultry flocks, especially those live freely in backyards around lakes in villages. In 2017, the highly pathogenic subtype H5 was isolated from Northern shoveler and Eurasian teal in Sharkia Berket Akyad but they failed to determine the type of neuraminidase gene (Zanaty *et al.*, 2019).

In 2016, a new subtype of HPAI-H5N8 clade 2.3.4.4 was detected in two hot spots northeastern Egypt (Port-Said and Damietta) around Lake Manzala in four Green-winged teal ducks, two Common coots, and one Northern shoveler. Then, the virus was isolated in 2018 (from four Northern shovelers in Gamsa area in Damietta), 2019 (from a Turtle Dove in Port-Said as well as from a free-living Crow in Dakahlia and Cattle Egret in Ismailia), and 2020 (from migratory Common moorhen in Sharkia) (Kandeil *et al.*, 2017; Nabil *et al.*, 2020; Abdien *et al.*, 2023) In the years 2021 and 2022, the HPAI-H5N1 virus reappeared again but with clade 2.3.4.4 (the same H5N8 clade), with high fatal rate (neurological symptoms, pathological changes, and deaths) in both migratory and wild birds, such as Greater-flamingo (Phoenicopteridae); Common-blackbird (Turdus merula); and Mallard duck in Birkat El-Boalwa in Ismailia 2021, and Damietta 2022, as well as from White Ibis in Giza 2021. Evidence is mounting that HPAI Goose/Guangdong/96 viruses of all subtypes that were introduced and spread in Egypt through ideal hosts (wild and migratory birds) from 2005 to 2022 are continuing to replicate, maintain their presence, and mutate (either through antigenic shift/or drift) in these tanks (vessels), especially aquatic ones, are characterized by exchange of AIVs in both directions (the mechanism of persistence). This is also what researchers have achieved in their monitoring studies of avian influenza virus globally (Ramey *et al.*, 2022). (Figure 2).

Globally, HPAI H5N8 clade 2.3.4.4 was first introduced through sporadic outbreaks that occurred in Asia and Europe during 2010–2013, where the virus was isolated from both domestic and wild birds (Marchenko *et al.*, 2011). This clade became adapted to asymptomatic waterfowl and showed limited ability to transmit among mammals (Kaplan *et al.*, 2016). Then it

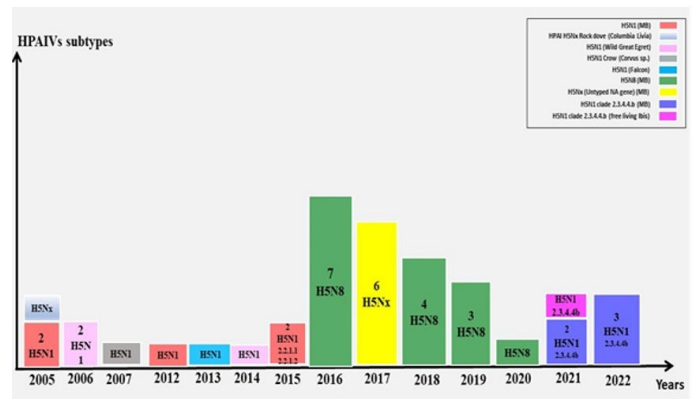


Figure 2. HPAI viruses were recorded in both migratory and free-living resident wild birds in Egypt yearly based on recorded data in the available literature.

spread to South Korea in 2014 (Jeong *et al.*, 2014), where it was isolated from Eurasian wigeons, and extended to Russia during autumn migration in September 2014. It was then discovered in wild birds living in the United States of America and Canada (Lee *et al.*, 2015). Through 2015, subsequent outbreaks of attacked wild waterfowl, Raptors, domestic chickens, and turkeys in areas throughout western and central USA and Canada in which losses of an unknown number of wild birds and more than 50 million domesticated birds (Bevins *et al.*, 2016). During this, America alone lost \$3 billion through mass culling of bird farms to eradicate the disease. Despite this, HPAI-H5N8 subtype continued to emerge and can be isolated from wild birds in the wetlands of North America until the end of November 2016, and also spread widely in Europe, Asia, Africa, Uganda and Germany until 2021 in most species of wild birds (crows, ducks, geese, swans, and gulls) with no cases in human (Harder *et al.*, 2015; Lee *et al.*, 2017; Abolnik *et al.*, 2019; Poen, *et al.*, 2019). This scenario explains the origin of the HPAI-H5N8 virus and why it is spreading around the world.

In Egypt, the analysis of the data related the migratory bird species delivering AIVs along the period of study, revealed that the aquatic migratory species which known as mixing vessels and reservoirs for AIVs are the commonly recorded. The extreme charge of two main species; Northern shoveler (n=34) and Green-winged teal (n=21) was interesting (Figure 3) particularly around Lake Manzala.

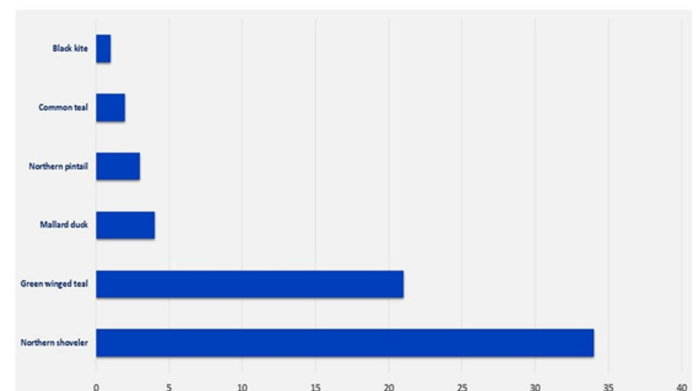


Figure 3. Species and number of migratory birds infected with AIVs during 2003-2022 in Egypt.

Intermingling spread of HPAI and LPAI subtypes in migratory, wild, free-living, and domesticated birds

Concerning the mixed annual spread of HPAIV and LPAIV subtypes in migratory, wild, free-living, and domesticated birds in

itor AIVs in the fall and spring with a focus on aquatic birds for early and update identification to perceive any new emergent quickly intervene to control them before passing their hazard to the poultry industry and public health in Egypt.

Conflict of interest

The authors declare that they have no conflict of interest.

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