

H5 Influenza Viruses in Egypt

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For almost a decade, Egypt has been endemic for highly pathogenic avian influenza (HPAI) A(H5N1) viruses. In addition to being catastrophic for poultry production, A(H5N1) has also caused 359 human infections in the country (~40% of global cases), with 120 being fatal. From 2017, A(H5N1) viruses have been gradually replaced by HPAI A(H5N8) viruses seeded from Southeast Asia through Europe; no human cases have been reported since. This lack of human cases is not a consequence of fewer H5 infections in poultry. Despite governmental outbreak control, the number of avian influenza outbreaks has increased since 2006 partially fueled by noncompliance with preventive measures and suboptimal vaccination programs. Adherence to control measures is low because of social norms, especially among women and children—the main caretakers of household flocks in rural areas—and declining public awareness in the community. Egypt has thus become an epicenter for A(H5) virus evolution, with no clear resolution in sight.

Waterfowl and shorebirds are the major natural reservoirs for influenza A virus (IAV) (Webster et al. 1992). Historically, it was considered that within these reservoirs, IAV is maintained in the low pathogenic (LP) form, inducing only mild illness, if any. Following spillover to domestic gallinaceous poultry (including chicken and turkey), some viruses of H5 and H7 subtypes can transform to the highly pathogenic (HP) form and cause lethal infections (Alexander 2000) by altering the proteolytic cleavage site of the hemagglutinin (HA) protein and adjacent residues (Schmier et al. 2015). The high virulence of these HP viruses as well as their circulation within domestic species likely contributed to their relatively confined geographic distribution after their genera-

tion. This pattern of confined geographic distribution changed with the emergence of the HP A/goose/Guangdong/1/96 lineage (Gs/GD) A(H5N1) viruses clade 2.2 viruses, designated based on HA gene sequence, from this lineage were initially causing disease confined to China and southeast Asia (Guan et al. 2004; Saad et al. 2007). They later spread westward, concomitant with their detection in wild migratory birds and seeded outbreaks in countries along the way. The clade 2.2 viruses were first detected in poultry in Egypt in mid-February 2006 (Saad et al. 2007). Although most countries wherein Gs/GD A(H5N1) viruses have been isolated have had transient epidemics, others have not been as successful in eradicating the viruses and they have become endemic. Egypt is one such

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country in which these viruses have become endemic.

Egypt is in the northeast corner of Africa, spanning ~1 million square kilometers. As per United Nations estimates, the human population of Egypt is 100 million, with most living in the Nile Delta or in the narrow Nile Valley located south of Cairo. Poultry production is one of the fastest growing industries in Egypt, and most birds are found in commercial or backyard farms. Commercial farms include large and small farms that account for >75% of the total poultry production (Abdelwhab and Hafez 2011). Chickens are the most common bird raised, followed by ducks, geese, and turkeys. The backyard sector is critical for low-income households, and the cultural norm is for families in villages to raise mixed (e.g., chickens, pigeons, ducks, geese, and turkeys) backyard flocks for egg and meat consumption. The live poultry market (LPM) sector is also very active and plays a critical role in maintaining, amplifying, and disseminating avian influenza viruses (AIVs) among poultry species and from poultry to humans (Dinh et al. 2006; Zhou et al. 2009; Kandeil et al. 2010). Soon after their introduction, the clade 2.2 viruses were found in all major poultry sectors in Egypt.

The first human case of Gs/GD A(H5N1) in Egypt was detected in March 2006. Since then, Egypt has reported the highest number of human cases of H5 virus infections. As of September 2019, there have been 359 laboratory-confirmed human cases of H5N1 in Egypt reported to the World Health Organization (WHO), and the case fatality rate is >33.5% (WHO 2019).

The epidemiology of AIVs in Egypt has been influenced by two other events, both subsequent to the A(H5N1) introduction in 2006. In the first event in 2011, A(H9N2) G1-lineage viruses similar to those detected in neighboring countries were detected in poultry in Egypt. The A(H9N2) viruses are now geographically widespread across the country, and co-infection with A(H5) viruses is common (El-Zoghby et al. 2012a; Kandeil et al. 2014; Kayali et al. 2014; Naguib et al. 2015). In the second event, there was another introduction(s) of Gs/GD A(H5) viruses from Asia, this time of clade 2.3.4.4

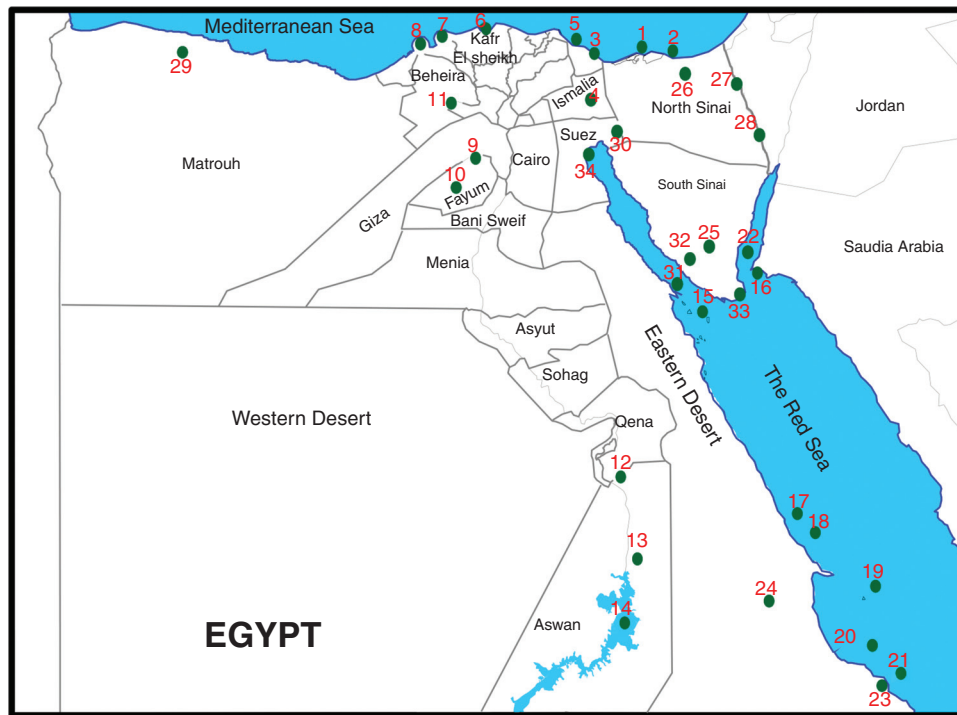
A(H5N8) viruses (Kandeil et al. 2017; Salaheldin et al. 2018a). These viruses have slowly displaced clade 2.2 viruses and have not been detected in humans, but reassortment with circulating A(H9N2) viruses has been observed (Hagag et al. 2019) (see below for more details).

In this review, we summarize the epidemiology and zoonotic characteristics of endemic A(H5) viruses circulating in Egypt and discuss some perceived weaknesses in response and control strategies that have contributed to their continued endemicity.

CURRENT EPIDEMIOLOGY OF A(H5) IN EGYPT

Emergence and Epidemiology of A(H5N1) Viruses in Egypt

The A(H5N1) virus was first detected in Egypt in the common teal (*Anas crecca*) in December 2005 during migratory bird sampling along the northern coastal region of the Damietta Governorate. Genetic analysis of this virus indicated a close relation to the A/bar-headed goose/Qinghai/65/2005 clade 2.2 virus detected months earlier during a large die-off of migratory birds in Qinghai Lake, China. Although definitive proof is lacking, the timing of events strongly suggests that wild bird migration led to transcontinental spread of the virus. Consistent with this hypothesis are the 34 stopover sites for migrating birds from the East Africa–West Asia and Black Sea–Mediterranean flyways present in Egypt (Fig. 1). After being identified in waterfowl, clade 2.2 viruses were isolated from poultry farms and backyard flocks in February 2006 across most governorates of Egypt. Control measures were implemented, including the culling of 40 million birds within a 10-mo period, causing serious losses to the poultry sectors. However, these efforts were not successful, and the virus became endemic. Once endemic, poultry outbreaks of A(H5N1) influenza settled into a mostly seasonal pattern, with sharp increases in activity during the colder months (November–March). The highest numbers of outbreaks have been recorded in Dakahliya, Monofiya, Kalyobiya, Sharqea, Gharbia, and Giza Governor-



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|------------------|--------------------------|---------------------|--------------------|
| 1- Lake Bardawil | 10- Wadi El Rayan | 19- Zabargad Island | 27- Quseima |
| 2- Zaranik | 11- Wadi El Natrun | 20- Siyal Islands | 28- WadiGerafi |
| 3- El Malaha | 12- Upper Nile | 21- Rawabel Islands | 29- El Qasr Desert |
| 4- Bitter Lakes | 13- Aswan Reservoir | 22- Nabaq | 30- Suez |
| 5- Lake Manzalla | 14- Lake Nasser | 23- Gabel Elba | 31- Gabel El Zeit |
| 6- Lake Burullus | 15- Hurghada Archipelago | 24- The Abraq Area | 32- El Qa Plain |
| 7- Lake Idku | 16- Tiran Island | 25- St. Katherine | 33- Ras Mohammed |
| 8- Lake Maryut | 17- WadiGimal Island | 26- Gabel Maghara | 34- AinSukhna |
| 9- Lake Qarun | 18- Qulan Island | | |

Figure 1. Locations of 34 wild bird stopover sites in Egypt.

ates, which are leading poultry-producing governorates in Egypt (Fig. 2A; El-Zoghby et al. 2013; Kayali et al. 2016; ElMasry et al. 2017; EMPRES 2019).

To better understand the ecology and evolution of viruses in Egypt, an active, longitudinal AIV surveillance program was implemented in 2009. Commercial and semicommercial farms, abattoirs, backyard flocks, and live bird markets (LBMs) were targeted across the country. The program had a 5% rate of AIV detection during 2009–2010, which was exclusively attributable to A(H5N1) infection, primarily in the commercial production sector (Kayali et al. 2011a). From 2010 to 2013, the detection rate increased to 10%, with A(H5N1), A(H9N2), and H5N1/

H9N2 co-infections detected. Importantly, in addition to an increase in prevalence, viruses were also detected in all poultry production sectors (Kayali et al. 2014). The AIV detection rate decreased to 4.6% from 2013 to 2015, and no A(H5N1) viruses were detected in the last quarter of 2015 (Kayed et al. 2016). Finally, from 2016 to 2018, the detection rate was 2.9%, with A(H9N2) being the most commonly isolated viruses; however, the A(H5) viruses have remained.

Genetic Evolution of A(H5N1) Viruses in Egypt

In the decade or more that A(H5N1) viruses have circulated in Egypt, they have predictably undergone substantial genetic evolution. As dis-

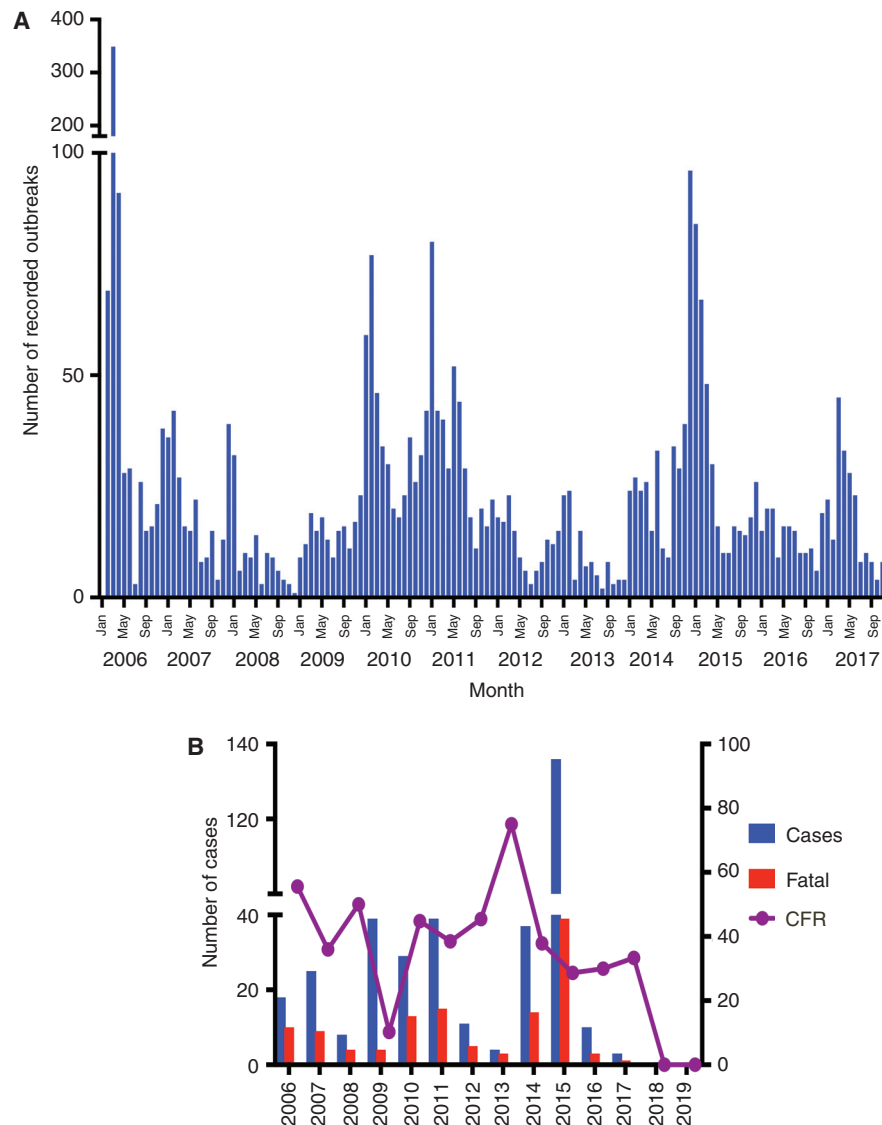


Figure 2. Epidemic curve for (A) poultry and (B) human cases of A(H5N1) in Egypt from 2006 to 2019.

cussed, the first A(H5N1) viruses to enter Egypt had HA genes belonging to phylogenetic clade 2.2. After a period of circulation, a review of the nomenclature subsequently identified an Egypt-specific subgroup within clade 2.2, with a new 2.2.1 clade being proposed to cover it. The continued circulation of these clade 2.2.1 viruses in vaccinated (commercial sector) and nonvaccinated poultry sectors led to a within-country diversification of the viruses, likely through differential pressures of maintenance within flocks

with distinct immunities. Clade 2.2.1 viruses within the commercial sector eventually evolved to a point where they met the criteria for independent clade status. Clade 2.2.1 viruses were subsequently subdivided into clades 2.2.1.1 and 2.2.1.1a (commercial sector) in response to this increased diversity. Both clades cocirculated for several years until the 2.2.1.1a viruses, for reasons unknown, disappeared around 2014. Subsequent drift of clade 2.2.1.1 viruses eventually prompted a 2.2.1.2 clade designation of vi-

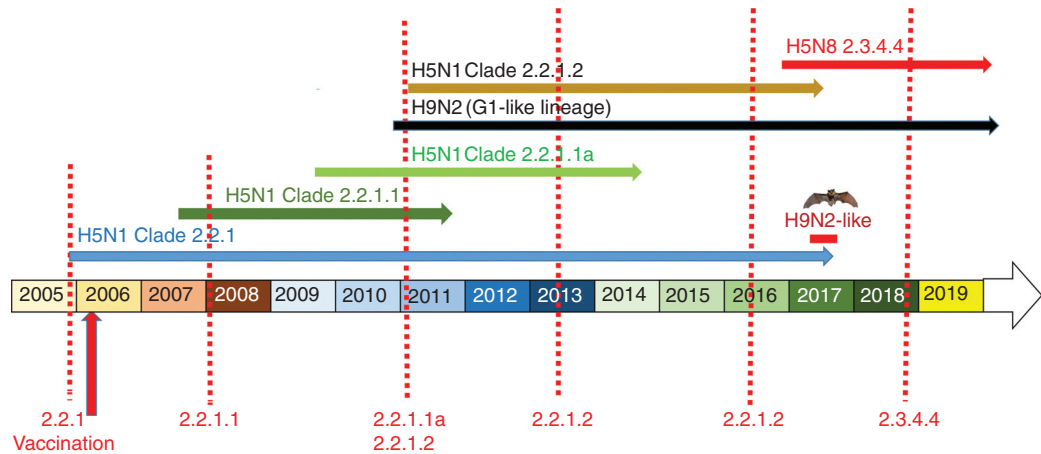


Figure 3. Timeline of the evolution of A(H5) clades of avian influenza in Egypt from 2005 to 2019.

ruses in the country (Fig. 3; Kayali et al. 2011b, 2016; Abdelwhab et al. 2012a, 2016; El-Shesheny et al. 2014; Arafa et al. 2015).

Evolution of the H5 gene of Egyptian viruses not only led to changes in their classification but also to noticeable antigenic changes (El-Shesheny et al. 2012; Kayali et al. 2016). Viruses drifted antigenically over time through glycosylation changes as well as mutations in key HA antigenic sites. El Shesheny et al. evaluated the antigenic cross-reactivity of HPAI H5N1 viruses from 2006 to 2017 by using a panel of antigenic site A and B reactive monoclonal antibodies raised against HAs of A/Viet Nam/1203/04 (H5N1; clade 1) and A/bar-headed goose/Qinghai lake/1A/05 (H5N1; clade 2.2). Viruses fell into three clusters: A, B, and C. Cluster A included isolates from 2006 to 2007 (clade 2.2.1), cluster B included isolates from 2008 to 2010 (clade 2.2.1.1 and clade 2.2.1.1a), and cluster C included isolates from 2011 to 2012 (clade 2.2.1.2) (El-Shesheny et al. 2012). The alignment of genetic and antigenic clustering suggested that immune pressure may have driven virus evolution, as has been postulated for other A(H5N1) viruses (Cattoli et al. 2011). HA amino acid mutations (74, 140, 141, 144, 156, 162, and 184) were observed in clade 2.2.1.1 viruses and were associated with antigenic drift and reduced vaccine effectiveness (Abdelwhab et al. 2012a; El-Shesheny et al. 2014). It has been suggested that the emergence of new antigenic var-

iants can be partially explained by inadequate vaccines and vaccination policies driving drift rather than control.

The genetic evolution of A(H5N1) viruses in Egypt was also associated, somewhat concerningly, with phenotypic differences in virus receptor binding. Clade 2.2.1.2 viruses evolved an HA deletion at residue 129 and also amino acid substitutions D43N, S120N/D, and I151T, and these changes resulted in enhanced binding to the host cell receptor that is preferred by human influenza viruses (Kayali et al. 2011b; Watanabe et al. 2011; Abdelwhab et al. 2012a; El-Shesheny et al. 2014). Laboratory adaptation studies of earlier A(H5N1) viruses from Vietnam and Indonesia showed that a limited number of genetic changes are needed for airborne transmission among ferrets (Herfst et al. 2012; Imai et al. 2012). One of the identified HA mutations, T156A, resulted in loss of a potential glycosylation site and is also common to all viruses of clade 2.2.1.2. A mutation at PB2-E627K was also required for efficient airborne transmissibility and was common in Egyptian A(H5N1) viruses isolated from infected poultry and humans. Additional studies have shown many HA substitutions, most present at some level in Egyptian A(H5N1) viruses, which can enhance binding to the human virus receptor (Chen et al. 2012; Schmier et al. 2015).

Anti-influenza drugs, including adamantanes (amantadine and rimantadine) and neur-

aminidase (NA) inhibitors (oseltamivir and zanamivir) are available for treating IAV infection. In Egypt, many human cases of A(H5N1) have been treated with oseltamivir. Resistance to oseltamivir through an NA N295S mutation was confirmed in two fatal cases within the same family (Earhart et al. 2009). Luckily, most Egyptian human and avian A(H5N1) viruses did not have NA inhibitor-associated resistance markers (Kayali et al. 2011b; El-Shesheny et al. 2014). In contrast, the amantadine resistance-associated S31N mutation was commonly observed in viruses belonging to clades 2.2.1.1 and 2.2.1.2 (El-Shesheny et al. 2016; Abdelwhab et al. 2017). The development of an M2 V27A substitution, which was also associated with amantadine resistance, was detected in two human 2.2.1.1 A(H5N1) isolates (Younan et al. 2013).

Biological Properties of A(H5N1) Viruses in Egypt

The A(H5N1) viruses have been isolated from a wide range of hosts in Egypt, including chickens, ducks, quails, pigeons, ostriches, great egrets, crews, donkeys, pigs, wild birds, and humans (Saad et al. 2007; Aly et al. 2008; Abdel-Moneim et al. 2010; Abdelwhab and Hafez 2011; Kayali et al. 2011a, 2016; El-Zoghby et al. 2012b; Kayed et al. 2019). This diverse range facilitates spread of the virus and can contribute to viral diversity. Despite this and an increase in the number of human cases, there have been limited studies evaluating the biological properties of Egyptian A(H5N1) viruses in mammalian models. Nine poultry viruses isolated between 2014 and 2015 from clade 2.2.1.2 were evaluated for their ability to transmit in ferrets (Arafa et al. 2016). As has been typical for A(H5N1) viruses from other regions, these viruses could not transmit efficiently among ferrets. Despite limited transmission of A(H5N1) viruses among ferrets (and among humans), zoonotic infections have occurred, many within LPMs, suggesting that unique conditions occur in these environments (Lai et al. 2016). Bertran et al. (2017) experimentally demonstrated that naive chickens and ferrets exposed to the same air space in which chickens were slaughtered became infect-

ed and died. Consistent with these results, AIV has been detected and isolated from air samples in LPMs (Wu et al. 2018).

The pattern of A(H5N1) virus pathogenicity in poultry in Egypt changed from the first wave of 2006 viruses. The observed mortality increased (up to 100%) in poultry flocks in 2006–2007 and then dropped to 20%–60% in 2008–2017 (Kayali et al. 2011b). These field observations were supported by laboratory data showing a range of intravenous pathogenicity indexes (1.8 to 2.9) for these viruses (Abdelwhab et al. 2016). Field observations showed that in contrast to chickens, ducks exhibit mild or no clinical signs after infection with A(H5N1) viruses. Pekin ducks experimentally infected with a clade 2.2.1.2 virus showed no clinical signs or mortality (Salaheldin et al. 2018b; Samir et al. 2018), suggesting a potential role for ducks in maintaining clade 2.2.1.2 viruses as the dominant virus in Egypt.

Zoonotic Infections of A(H5N1) Viruses

More than 860 zoonotic infections of A(H5N1) viruses have been reported in 16 countries, with a case fatality rate of >50% (WHO 2019). Egypt accounts for a substantial proportion of these infections, with a current laboratory-confirmed case count of 359 and a case fatality rate of 33.5% (Fig. 2B). During the winter of 2014–2015, there was a sharp increase in human cases of A(H5N1) associated with clade 2.1.2.2 viruses. Although startling, the reason for the rise in human cases is unclear, and since 2016 only 13 new cases have been reported. In the 11 years of A(H5N1) virus circulation in Egypt, human cases have been detected with clade 2.2.1 and 2.2.1.2 viruses, which were dominant in the backyard sector. Correspondingly, 70% of human cases have been from individuals who reported exposure to backyard poultry (Kayali et al. 2016). Although the number of human infections in Egypt has been relatively high, screening for A(H5) infection is based on a case definition of influenza-like illness and a history of poultry contact 2 wk prior to disease onset (Refaey et al. 2015). It is very likely that the official case count is an underestimate, because



many infected people might have milder symptoms. Such individuals are unlikely to seek medical care in Egypt. A 3-yr seroprevalence study found that ~2% of Egyptians exposed to poultry showed evidence of prior A(H5N1) exposure (Gomaa et al. 2015). A further study conducted in the Beheira Governorate showed a 4% seroprevalence in poultry workers (Samaha et al. 2015). These studies confirm that the number of A(H5N1) human cases in Egypt is greatly underreported, and consequently the case fatality rate is greatly overestimated. Interestingly, there was an increase in the number of human cases reported in 2014–2015, when the Egyptian Ministry of Health actively collected additional respiratory samples in communities having poultry outbreaks (Kayali et al. 2016).

EMERGENCE OF HPAIV H5N8

For more than a decade, the majority of Gs/GD lineage A(H5) viruses had been associated with the N1 NA. However, in 2010, this trend changed with the detection and spread of A(H5N8) viruses in China. These viruses were initially reported in wild birds and poultry in Southeast Asia (primarily China). The association of these viruses with wild birds was likely a key factor in their subsequent dissemination to Korea and Japan and then to Siberia, Europe, and the United States (Harder et al. 2015; Lee et al. 2017). In 2016, there was another wave of A(H5N8) virus dissemination from Asia (Beerens et al. 2017; Fusaro et al. 2017; Kleyheeg et al. 2017; Pohlmann et al. 2017; Scoizec et al. 2018). In November 2016, these A(H5N8) viruses were detected in Egypt, likely introduced via Europe; these viruses are still present and represent the dominant A(H5) currently circulating (Fig. 3; Kandeil et al. 2017). The A(H5N8) virus situation has been further complicated by multiple introductions of A(H5N8) virus into Egypt (Yehia et al. 2018). Although it is premature to suggest their disappearance, as A(H5N8) virus detections have increased, A(H5N1) virus detections have dramatically decreased. As A(H5N8) viruses have not yet been associated with human infections, the displacement of A(H5N1) viruses with

A(H5N8) viruses may well be beneficial from a public health perspective.

REASSORTMENT OF HPAIV H5Nx IN EGYPT

Cocirculation of HPAI (H5N1, H5N8) and LPAI (H9N2) viruses in poultry provides an opportunity for reassortment. Co-infection of birds with A(H5) and A(H9N2) viruses in Egypt is not uncommon, yet few reassortments have been detected to date (Abdelwhab el et al. 2010; Kayali et al. 2016; Hassan et al. 2019). A(H5N1)/A(H9N2) reassortants have been detected in other countries where these viruses cocirculate, suggesting that there is not complete incompatibility between their gene segments (Monne et al. 2013; Marinova-Petkova et al. 2016). Lack of detection of reassortant viruses in Egypt may also be due to lack of sensitivity of current surveillance measures for identifying such viruses or an associated fitness defect (Moatasim et al. 2017; Naguib et al. 2017; Arai et al. 2019). Although no A(H5N1)/A(H9N2) reassortants have been described, a novel A(H5N8)/A(H5N1) reassortant has been detected in a duck. This virus contained seven A(H5N8) virus genes with the N2 from the A(H9N2) virus (Hagag et al. 2019). The complete significance of this or similar viruses remains unknown.

CHALLENGES IN CONTROLLING HPAIV H5N1 IN EGYPT

In Egypt, thousands of A(H5) outbreaks have been recorded in poultry since February 2006 and have resulted in severe economic losses for the poultry industry. These losses have affected the incomes of 1.5 million people whose livelihoods depend on this industry. The first wave of the disease in 2006 resulted in the culling of 40 million birds in an attempt by the veterinary authority in Egypt to control the infection in commercial farms (Peyre et al. 2009). Further attempts at control have included elements of vaccination, ongoing active surveillance, rapid diagnosis, biosecurity enhancement, regular training, awareness programs for poultry workers, and further culling of infected poultry (Swayne 2012).

Vaccination

Much of the funds made available for AIV control in Egypt have been directed toward vaccination (Peyre et al. 2009). Vaccination of backyard and commercial poultry began with the first wave of viruses in 2006, and different types of inactivated H5 vaccines, mainly based on North American H5N2 viruses and reverse genetic modification of GD/GD viruses (H5N1, Re-1), were used (Hafez et al. 2010). More than a quarter of a billion doses of several commercial vaccines were imported by the Egyptian government. As discussed above, from 2007 to 2011 antigenic drift variants of A(H5N1) viruses were detected in vaccinated chicken (Balish et al. 2010). In July 2009, the government-supported vaccination of backyard poultry was terminated (Peyre et al. 2009). Ten years later, implementation of AI vaccination in Egypt has had a limited impact on the presence of the virus.

At least 24 commercial inactivated AI H5 vaccines have been licensed for use in poultry in Egypt as per General Organization of Veterinary Services files. These vaccines have contained a diverse range of antigens ranging from older H5 lineage viruses to several Gs/GD lineage viruses. Older H5 vaccine antigens include A/turkey/Minnesota/3689–1551/1981 (H5N2), A/chicken/Mexico/232/1994 (H5N2), A/duck/Potsdam/1402/1986 (H5N2), A/chicken/Italy/22A/1998 (H5N9), A/turkey/England/N28/1973 (H5N2), and A/turkey/Wisconsin/1968 (H5N9). Reverse genetics–derived antigens include Re-1 (A/goose/Guangdong/1996 (H5N1) clade 0), A/chicken/VN/C58/2004 (H5N3) (clade 1), Re-5 (A/duck/Anhui/1/2006 (H5N1) clade 2.3.4), Re-6 (A/duck/Guangdong/S1322/2006 (H5N1) clade 2.3.2), Re-Egy (A/chicken/Egypt/18-H/2008 (H5N1) clade 2.2.1.1), A/chicken/Egypt/Q1995D/2010 (H5N1) clade 2.2.1.1, and A/chicken/Egypt/M2583D/2010 (H5N1) clade 2.2.1.2. These vaccine antigens represent a considerable amount of antigen space and have had variable homology to circulating viruses ranging from limited to high. The HA homology of the most abundantly used vaccines to circulating Egyptian viruses (at the time of the study) had been estimated to range from

78% to 94% (Abdelwhab and Hafez 2011). Efficacy studies on these commercial vaccines in poultry have shown incomplete protection (Kim et al. 2010; Kilany et al. 2011; Kayali et al. 2013). Continual reappraisal of the efficacy of available vaccines needs to become an integral component of response measures (Pfeiffer et al. 2010).

In addition to vaccine mismatch, the main factors contributing to vaccination failure in Egypt include the presence of maternal immunity during vaccination (Kim et al. 2010; Abdelwhab et al. 2012b), absence of intensive country-wide vaccination programs, age of birds during vaccination (Peyre et al. 2009), number of vaccination doses required for each type of poultry (Peyre et al. 2009), immunosuppression due to concurrent cryptosporidiosis (Eladl et al. 2014), inflammatory bowel disease live vaccines, mycotoxicosis (Abdelwhab et al. 2016), and reluctance to vaccinate breeder and backyard poultry. Clearly, vaccination must be an integral component of Egypt's continued control efforts, but several variables and confounders need to be fully evaluated to optimize the effects.

Biosecurity

Biosecurity is the first line of defense against infectious diseases. Several practices are needed to limit the introduction of infections into a poultry production unit. These practices are divided into three major categories: segregation, to keep contaminated people, animals, and materials away from uninfected birds; cleaning, to remove most of the contaminating organic matter; and disinfection, to decrease microbial load (De Benedictis et al. 2007; Wanaratana et al. 2010). Many technical recommendations on biosecurity practices have been issued for large- (Newell et al. 2011) and small-scale producers (Negro-Calduch et al. 2013). According to the production scale and level of biosecurity measurements, Egyptian poultry production systems are classified into four sectors. Sector 1 consists of integrated broiler and breeder farms that have typically implemented standard operating procedures for biosecurity. Sector 2

encompasses commercial poultry production systems with moderate to high biosecurity, birds or products usually marketed commercially, and strict prevention of contact with other poultry or wildlife. Poultry in sectors 1 and 2 in Egypt are reared in modern, high-standard biosecurity buildings equipped with air filtration systems, pretreated feeding systems, clear protocols for cleaning and disinfection, and strict entrance policies. Overall, farms in these sectors have been less affected by A(H5) infection (Abdelwhab and Hafez 2011). Sector 3 covers small commercial poultry production systems with low to no biosecurity and represents the major element for poultry production in Egypt. Most sector 3 farms are located near to or even within villages, are close to each other, and are the main source of live poultry for LBMs. Sector 4 consists of backyard production with no or minimal biosecurity, and birds or products are consumed locally or sold at LBMs (Negro-Calduch et al. 2013). Sectors 3 and 4 are the riskiest factors for virus evolution and transmission to humans.

Most poultry trade in Egypt depends on retail LBMs and street hawkers, which has been a major hurdle for A(H5) control. Because of the limited number of abattoirs (309 units in 2015) and the traditional Egyptian cultural preference for selling freshly slaughtered birds, LBMs are widespread in all rural and urban regions of Egypt. Different poultry species, including live-captured wild birds, are housed together in these environments, sometimes in the same cage, where they stay for several days. Stray pets have free access and direct contact with waste and wastewater generated during slaughter, and cleaning runoff enters the main sewage system without treatment.

Different approaches to enforce biosecurity measures and reform practices in poultry farms are urgently needed and have been planned by the Egyptian government in cooperation with the Food and Agriculture Organization (Abdelwhab and Hafez 2011).

Elimination of Infected Poultry

During the first wave of A(H5N1) virus infection in Egypt, depopulation of affected flocks

(within 3 km of the initial outbreak), quarantine and movement controls on poultry (within a 7-km radius of the outbreak location) were implemented. Veterinary authorities provided partial compensation for affected farms. Subsequently, the incidence of A(H5N1) outbreaks decreased, although it is unclear whether that was the effect of rigorous culling strategies, administering inactivated vaccines, or a combination thereof. The estimated compensation during the first wave of H5N1 introduction in Egypt was \$29,375,000. After 2008, many farmers and breeders stopped reporting the presence of bird mortality within their flocks, especially after compensation was stopped and social stigma increased and due to other social aspects. It became almost impossible to control the virus under these circumstances.

Education and awareness are essential components of elimination strategies. Providing information about the virus and its effect to employees in poultry production helps provide justification and lower resistance for depopulation efforts. In Egypt, education and awareness programs in rural communities have been suggested but unfortunately not implemented (Ismail and Ahmed 2010).

Active Surveillance

An important component of AIV control is to implement active surveillance programs. Such programs not only provide an early alert for the incursion of emerging viruses but also allow monitoring the evolution and prevalence of endemic viruses, which can inform other control measures. Several surveillance studies on AIVs have been conducted in Egypt (Rabinowitz et al. 2012; Soliman et al. 2012; Afifi et al. 2013; Kayali et al. 2014) despite challenges in maintaining them. Inadequate financial and overall support from the state poses constant problems for surveillance activities in Egypt, with some governorates being very underrepresented. Although surveillance activities are one of the hardest aspects of control programs to maintain under resource constraints, their value cannot be underestimated.



CONCLUDING REMARKS

There are substantial challenges to the successful control of A(H5) viruses in Egypt and other endemically infected countries. Maintaining control strategies after the initial introduction of AIV into a country is daunting, and even more so after 13 years of continued virus circulation. Egypt is one of the countries hardest hit by A(H5) viruses, and there is no obvious end in sight. Although the frequency of human infections reported from Egypt has reduced with the introduction of clade 2.3.4.4 viruses, stasis is an unlikely long-term outcome. Vaccination must remain a central component of A(H5) control in Egypt, but by using a more concerted, controlled, and regulated approach than what has been done to date. Without doubt, viruses will continue to evolve in Egypt, and the threat of new introductions from Asia will remain. However, there is uncertainty about the future impact of virus evolution and introductions and in how to best use the limited existing control resources. Egypt must continue to leverage internal and external opportunities and partnerships to continue monitoring of and research into AIV and associated control measures.

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