Epidemiology, ecology and gene pool of influenza A virus in Egypt: Will Egypt be the epicentre of the next influenza pandemic?

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Abbreviations: ELISA, Enzyme linked immunosorbent assay; IAV, influenza A viruses; HA, hemagglutinin; HI, hemagglutination inhibition test; HPAIV, highly pathogenic avian influenza viruses; LBM, live bird markets; LPAIV, low pathogenic avian influenza viruses; M, matrix; NA, neuraminidase; NAMRU-3, Naval Medical Research Unit–3; NLQP, National Laboratory for Veterinary Quality Control on Poultry Production; NS, non-structural; PA, acidic polymerase; PB, basic polymerase; WHO, World Health Organization.

Outside Asia, Egypt is considered to be an influenza H5N1 epicentre and presents a far greater pandemic risk than other countries. The long-term endemicity of H5N1 and the recent emergence of H9N2 in poultry call attention to the need for unravelling the epidemiology, ecology and highly diverse gene pool of influenza A virus (IAV) in Egypt which is the aim of this review. Isolation of a considerable number of IAV subtypes from several avian and mammalian hosts was described. Co-infections of poultry with H5N1 and H9N2 and subclinical infections of pigs and humans with H1N1 and H5N1 may raise the potential for the reassortment of these viruses. Moreover, the adjustment of IAV genomes, particularly H5N1, to optimize their evolution toward efficient transmission in human is progressing in Egypt. Understanding the present situation of influenza viruses in Egypt will help in the control of the disease and can potentially prevent a possible pandemic.

Introduction

Influenza A viruses (IAV) are segmented RNA viruses belonging to the family Orthomyxoviridae which infect a wide range of hosts including but not limited to 100 species in 12 orders of birds and several mammals.1,2 The genome of IAV consists of 8 gene segments where each segment represents an independent replication unit encoding one or more proteins. According to the surface glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA), 16 HA and 9 NA of IAV have been isolated from birds,3 while H17N10 and H18N11 have been recently identified in bats.4,5 The segmented nature of IAV allows the swapping of gene segments (reassortment or shift) between different influenza subtypes that infect the same cell. The resultant IAV reassortants differ compared to their parental viruses regarding virulence, adaptation and/or pathogenesis. Emergence of a novel reassortant virus in immune-naïve human populations may result in a pandemic with severe mortality.6 Another feature for IAV is the constant minor changes due to errors induced by the viral RNA-dependent RNA polymerase during replication inside the infected cells. The gradual changes (antigenic drift) in the antigenic sites or in the receptor binding domain enable the virus to escape from the vaccine-induced immune response or to expand the host range, respectively.7

A central dogma of influenza virus is that the wild birds are the reservoir for all IAV subtypes. The transmission of IAV from wild birds to domestic poultry occurs frequently,3,8 whereas the spillover of IAV from domestic birds to humans is less reported.9 In poultry, with the exception of some H5 and H7 viruses, all IAV subtypes are of low virulence. On the other hand, the severity of IAV in humans is not correlated with their virulence to poultry. Some highly pathogenic avian influenza viruses (HPAIV) induced up to 100% mortality in chickens, however it caused mild if any clinical signs in humans.10 Conversely, the recent low pathogenic avian influenza viruses (LPAIV) H7N9 in China and Malaysia showed no clinical signs in poultry but it killed 112 out of 355 (~32%) confirmed laboratory human cases since February 2013.11 Exceptionally, the evolving HPAIV H5N1 since 1997 caused devastating outbreaks in poultry and wild birds in several countries and it was able to kill 393 out of 667 (~59%) infected humans.12 Although many countries successfully eradicated the HPAIV H5N1 from poultry, Egypt, China, Vietnam, Bangladesh, Cambodia and Indonesia were declared as H5N1-endemic countries.12

Egypt is considered a hotspot for the evolution of a pandemic potential virus either via antigenic drift of the H5N1 to increase
its adaptation to humans\textsuperscript{13,14} or through reassortment with other IAV subtypes, especially H3N2 virus\textsuperscript{15} or H9N2.\textsuperscript{16} Egypt has experienced several waves of IAV since 1900s. Surveillance in the wild birds described the isolation of different IAV subtypes, some of these viruses found their way to infect domestic poultry as well as humans. In domestic poultry, H5N1, H9N2 and H7Nx viruses are circulating since few years. Other IAV subtypes have been reported from human, horses, donkeys, pigs and possibly cats and dogs. Therefore, this review highlights important aspects regarding the history, epidemiology, ecology and the gene pool of influenza viruses in animals and humans in Egypt.

**Prevalence of IAV in Domestic Poultry**

*Highly pathogenic avian influenza*

**Fowl plague (H7N1)**

The earliest quoted date for the beginning of the history of avian influenza in Egypt was as far back as 1912.\textsuperscript{17} Instances were also mentioned as a part of the global outbreak of fowl plague in 1923.\textsuperscript{18-20} From 1923, the disease was endemic in Egypt and nearly every poultry establishment was threatened\textsuperscript{17,21} and the virus was isolated from chickens, turkeys, waterfowls, peafowl, parrots and pheasants.\textsuperscript{22} At that time, control of the disease was mainly based on nationwide vaccination with whole-virus inactivated vaccines developed from circulating field strains in 1945 which was effective to protect chickens and turkeys from fowl plague.\textsuperscript{23} Between 1948 and 1950, the emergence of antigenically drift variants was described.\textsuperscript{24} In the early 1950s, the development of attenuated living vaccine from peafowl-origin virus after 400 passages in pigeon embryos was successful to prevent clinical signs and/or mortality against the variant strains under experimental conditions.\textsuperscript{25} By the 1960s, the disease was no longer mentioned and dramatically disappeared. Later the virus was classified as H7N1 (A/fowl/Egypt/45 or A/FPV/Egypt/45) with a typical HPAI multiple basic amino acid sequence at the HA cleavage site KKRRKR*GLF and intravenous pathogenicity index of 3.0.\textsuperscript{26-28}

**H5N1**

After the re-emergence of H5N1 virus in Southeast Asia in 2003, Egypt started a nationwide surveillance in domestic poultry and wild birds for a period of 3 y (2003–2006). A preparedness stated the intention to control the disease immediately by stamping out the infected poultry at the index farm and those located within a diameter of 3 km. It was further proposed that a buffer zone of 10 km diameter be created in which the movement of live poultry and eggs would be restricted. While no evidence of H5N1 was obtained during the surveillance, a few weeks before the end of the project, on February 10, 2006, the National Laboratory for Veterinary Quality Control on Poultry Production (NLQP) received chickens, ducks, geese and turkeys from 7 backyards and live bird markets (LBMs) with symptoms commonly seen in HPAIV infections in 3 different provinces in the Nile Delta (north of Cairo). Isolation and genotyping of HPAIV H5N1 were confirmed in cooperation between NLQP and the Naval Medical Research Unit–3 (NAMRU3), Cairo, Egypt. The isolation of the virus was officially announced to the public on February 16, 2006. The first introduction of the virus into Egypt was thought to be by legal or illegal trade of live poultry or imported feed components. This scenario was neither confirmed nor excluded, but later on introduction via wild birds\textsuperscript{29} was reported to be the most likely scenario (see below). Although, depopulation of the first cases was done according to the prepared contingency plan, the virus spread quickly, over a wide area within few weeks. As a result, inactivated vaccines were introduced in a trial to mitigate the severe socioeconomic impact of the disease which became officially endemic from 2008.\textsuperscript{30} Although the temporal pattern of the disease in the winter months was typical for the outbreaks in 2006–2008\textsuperscript{31,32}; in 2009–2012 the disease was reported year round particularly in the backyard sector.\textsuperscript{33} Since then, outbreaks have been reported nationwide and the disease is now concentrated in the Nile Delta region where high-density human and poultry populations exist.\textsuperscript{33,34}

As of the end of 2011, approximately most of the domesticated birds have been infected with the virus including chickens, ducks, geese, turkeys, guails, and ostrich as well as zoo birds.\textsuperscript{30,35,36} and evidence for the persistence of the virus in native-ducks in the backyards in mid-summer 2011 was reported.\textsuperscript{37} In the first outbreak in 2006 we could not detect the virus from pigeons,\textsuperscript{36} however in 2009–2010 the viral RNA was detected in one pigeon and in 2010–2012 in 36 samples.\textsuperscript{35,36} A recent study described an outbreak in a small backyard pigeons flock in 2011 with mortality up to 50%.\textsuperscript{38} Whether the virus progressively adapted in pigeons since 2006 in Egypt or it was surveillance bias should be studied. Other potential reservoirs of the virus have not been fully identified. The virus has been reported from egrets and crows which are the most common feral birds seen in close contact with poultry in Egypt,\textsuperscript{30,39} as well as herons from 2 different locations.\textsuperscript{40} So far the incidences of the virus in other feral birds – particularly sparrows, starlings, eagles, owls, black kite and/or Hoopoe (Upupa epops) that access poultry farms, feed stores/mills and litter disposal points and are in close contact with wild migratory birds - have not been investigated. Intriguingly, the viral RNA has been detected in several types of fish in some regions in the Nile Delta\textsuperscript{40} and in litter beetles (Alphitobius diaperinus) that were found in a commercial farm previously experienced H5N1 outbreaks (Hussein A.H., personal communication). Nevertheless, the significance of these potential hosts in terms of the epidemiology of H5N1 in Egypt is still to be demonstrated.

An important feature for the epidemiology of H5N1 in Egypt is the genetic variability of the virus: Two H5N1 viruses collected from birds in 2 neighboring backyards at the same day were genetically different.\textsuperscript{41} A similar observation was reported by El-Zoghby et al.\textsuperscript{34} who isolated 2 viruses from 2 adjacent poultry farms but belonging to 2 different genetic lineages whereas 2 viruses from LBM and commercial farms from 2 different provinces (about 800 km apart) clustered together. Another critical aspect in the epidemiology of H5N1 is LBM where the virus has
been detected year-round.\textsuperscript{32,42,43} It is worth mentioning with respect to this that in LBM (1) more than 70% of poultry are marketed alive, (2) there is no system for tracing back the infection and (3) veterinary inspection, cleaning and disinfection and disposal of offal are not adequately done. Also, commercial farms with low to null biosecurity measures are endemically infected with the virus. Birds in these farms have usually acquired the infection as a result of: part-time workers who keep infected backyard birds, contact with feral and wild birds, inappropriate cleaning and disinfection methods; and/or inadequate disposal of dead birds and/or infected litters.\textsuperscript{12,44}

A key feature of the dynamic of H5N1 in poultry in Egypt is the backyard birds that may play a significant role in human and poultry infections as summarized in Figure 1 (for more information the readers are referred to\textsuperscript{44,45}). Backyard birds are being infected mostly through (1) commercial farms through the selling of used equipment (i.e.: hoppers, drinkers, feeders, etc.), feed, infected birds and eggs or via (2) the introduction of live birds from markets. Actually, the keeping of backyard birds and livestock in the countryside and suburbs is a complex cultural, social and ecological phenomenon which is rooted in Egypt’s ancient past.\textsuperscript{46} Backyard birds have been a primary economic support for many villagers for thousands of years. In recent times, while apparently healthy poultry are usually sold in traditional LBM’s, 38% of households slaughter their birds once they become ill.\textsuperscript{30,47} Since the problems arising out of keeping birds in backyards, the Egyptian authorities banned keeping or trading of live birds and provided vaccination free-of-charge twice a year until 2009.\textsuperscript{48} H5N1-positive birds which were detected during the routine surveillance were culled but with very low, if any, compensation\textsuperscript{30,49} and there was no voluntary culling of infected birds. This strategy was not successful to eradicate the disease in backyard birds in 2006–2009. Since then, an alternate approach focusing on improving biosecurity measures through the implementation of sustainable health education and awareness programs in rural communities was suggested, but not acted on.\textsuperscript{30-52} More studies are required to identify the dynamic of H5N1 infections in poultry in Egypt including post-outbreak investigation in the commercial sector, period of persistence of H5N1 virus in LBM and transmission pathways from and to the

\begin{figure}
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Sites of interfaces of H5N1 virus of different hosts in Egypt. From the currently available data, backyard birds are an important source for the infection of birds in the commercial sector and LBM as well as for humans. Over 90% of infected humans probably acquired the infection through direct contact with backyard birds; whereas only 2 and 3 human-infections occurred due to contact with birds in the LBM or commercial sector, respectively. The confirmed transmission routes between different sectors and species are shown as straight lines while possible, but not yet confirmed-sources of infection are shown as dashed arrows. The virus was isolated from donkeys, feral and wild birds and the viral RNA was detected in fish. Only anti-H5 antibodies were detected in pigs, cats and dogs but no virus isolation or detection trials were reported.}
\end{figure}
backyard sector. The contribution of each sector of the poultry industry in Egypt to maintain and spread the virus merits further investigation.

Genetic analysis of the Egyptian strains and reverse genetic studies indicated several significant aspects due to the long-term evolution of the virus in poultry. During the last 8 years, viruses isolated from vaccinated birds clustered in clade 2.2.1.1 and 2.2.1.1a have accumulated over 20 amino acid substitutions in the HA protein alone and several fixed mutations in other viral proteins.55,56 A handful of mutations particularly in the HA-immunogenic epitopes57 or glycosylation sites (Abdelwhab et al., submitted) of these viruses were enough for the escape of these antigenic-drift variants from humoral immune response in vitro which may explain the lack or insufficient protection in poultry afforded by some vaccines either in the field or under experimental conditions.32,4,58,59 Presumably, the use of unsuitable H5 vaccine strains in Egypt might play a role in the evolution of immune-evasion viruses: Chinese Re-1/1996/H5N1 and Mexican H5N2 strains were used extensively until 2008, then local 2006 strains were used until 2009–2012, and updated to 2009 strain since 2012 onwards.56,60

Moreover, it is thought that the recent Egyptian strains become less virulent than the parent 2006-virus.61 A point mutation in the cleavage site resulted in a significant decrease in the virulence of the virus in chickens without affecting the transmission to contact birds.62 Moreover, these antigenic-variant strains accumulated glycosylation sites in the HA protein approximately each year. The predecessor 2006-virus had 6 potential glycosylated sites, whereas the variant strains since 2009 possessed 9 glycosylation sites.65 Recently, we found that this glycosylation pattern although did not significantly modulate the virulence or tissue tropism of the virus but it was necessary to increase and enhance virus replication, chicken-to-chicken transmission, virus excretion from infected birds, receptor binding affinity, neuraminidase activity and heat stability of the virus (Abdelwhab et al., submitted). Together, the virus continues to acquire genetic changes that are necessary to enhance the infectivity and transmission in poultry fostering its endemicity in Egypt.

Low pathogenic avian influenza

H9N2

Since the mid-1990s, H9N2 virus is endemic in poultry in the Middle East region,63,64 however the first isolation reports of H9N2 virus in Egypt appeared from December 2010 to May 2011 and concerned chickens,65,66 quails,67 and turkeys (Arafà et al., unpublished data). To the best of our knowledge, no H9N2 infections were reported in waterfowls in Egypt. It is important to mention that the detection of the viral RNA from birds in LBM had been done in 2006, but the virus was not isolated, which may indicate the presence of the virus in poultry in Egypt long time ago but was not detected for instance because of the testing regimes in the laboratories that were focused toward H5N1. Also, serological investigation revealed that the H9N2 virus was wide-spread in commercial farm settings between February 2009 and April 2012.68 About 50% (605/ 1225) of collected sera from 39 chicken flocks were tested positive for H9 subtype.68 In another study, 165 commercial flocks and only 3 cases of backyards were found positive.66 In 2011–2013, one study identified H9N2 in 24 out 60 broiler, one out of 5 layer and one out of 5 breeder flocks69 and the second study isolated the virus from 22 broiler flocks.70 While the majority of infected chickens showed respiratory distress66,68 and there was a decrease in egg production in breeder and layer flocks,68 infected quails and some breeder flocks were clinically healthy.16,67,70 Importantly, co-infection or prior infection with H5N1 and H9N2 was also reported in many cases in poultry in Egypt.16,36,66 Although predictable, the reassortment between H5N1 and H9N2 has not been yet reported.70 The highest incidence of H9N2 infections was in January 2012 (6.6%, 49/736) and concentrated mainly in the Nile Delta.16 The routes of the introduction of H9N2 into Egypt are not yet clear. The close genetic relatedness of the Egyptian H9N2 viruses to the viruses circulating in the Middle East region and the distribution of infected flocks along the main migration routes of birds in Egypt suggested that the virus was most likely introduced via wild birds.65 Vaccination using inactivated local field strains of H9N2 viruses has been recently implemented. Bivalent or trivalent vaccines with H5, Newcastle disease virus and/or infectious bronchitis virus have been also used firstly in the breeder and layer flocks then in the broilers. Thus, the acceleration of antigenic drift of H9N2 virus in Egypt due to vaccination pressure is expected similar to what has been reported for H5N1 virus.58,59,71

H6 and H7 subtypes

In the early 1990s, LPAIV H7N1 was isolated from turkeys72 and in the early 2000s, serological evidence for H6 among broiler and layer breeders was reported.73 In 2009–2010, antibodies against H7 virus were detected in 14/39 commercial chicken flocks. While the highest prevalence of positive samples was from layer flocks (9/18) followed by breeders (3/12) and broilers (2/9),68 there is no report for virus isolation in commercial or backyard poultry to date.

Prevalence of IAV in Wild Birds

Egypt lies at the crossroads of 2 major spatially-overlapping migration flyways; the Black Sea-Mediterranean flyway and the East African-West Asian flyway linking Africa, Europe and Asia. There are 34 defined migratory wetlands and stopovers where 7 and 15 regions are located along the coastlines of the Mediterranean and Red seas, respectively. The Nile Delta is an important wintering stopover for millions of birds between Eurasia and Africa. Therefore, attention has been paid to the possible role of wild birds in the introduction of different pathogens including IAV.29,34,74–79 While a considerable number of IAV subtypes have been isolated from wild birds in Egypt through several collaborative research projects (Table 1); nevertheless infections of
domestic poultry and/or humans with any of these viruses remain poorly defined.

A recent analysis of 15 IAV’s representing 8 different HA subtypes isolated from wild birds in Egypt from 2003 to 2007 indicated a close relation to Eurasian, African and/or central Asian lineages. Interestingly, it was found that viruses originated from 4 different flyways rather than the 2 flyways in Egypt. The most infected wild birds in Egypt were the northern shoveler, common teal and Egyptian goose (Table 1).

### Table 1. Infections of birds, animals and humans with different influenza A virus in Egypt from 1910s to 2014

| Pathotype | Subtype | Host | Year | Reference |
|-----------|---------|------|------|-----------|
| HPAI      | H7N7    | Chickens, turkeys, waterfowls, pigeons, peafowl, parrots, pheasants | 1910s-1960s | 17, 20, 22 |
|           | H5N1    | Wild ducks (common teal), Chickens, ducks, geese, turkeys, quails, ostrich, pigeons, great egrets, crews, herons, humans, donkeys, pigs*, dogs*, cats*, fish, beetles | 2005       | 174 |
|           | H5N2    | Wild birds (Shoveler) | 2003, 2005, 2006 | 39 |
|           | H2N8    | Wild birds (Shoveler) | 2007 | 39 |
|           | H3N1    | Wild birds | 1970s | 175 |
|           | H3N2    | Human | 2002-2007 | 175 |
|           | H3N8    | Wild birds (Ducks) | 1990s | 144 |
|           | H4N1    | Wild birds | 1989-1990, 2000, 2008 | 150, 176 |
|           | H4N6    | Wild birds (Ducks) | 1970s | 180 |
|           | H5Nx    | Rock dove | 2005 | 44 |
|           | H5N2    | Wild birds (Shoveler) | 2003 | 39 |
|           | H5N7    | Wild birds (Shoveler) | 2006, 2007 | 39 |
|           | H5N9    | Wild birds (Ducks) | 2005 | 72 |
|           | H7N1    | Turkeys | 1990s | 72 |
|           | H7N3    | Wild birds (Shoveler) | 2004, 2006 | 39, 78 |
|           | H7N7    | Wild birds (Shoveler and Green wing teal) | 2007 | 39 |
|           | H7N9    | Wild birds (Shoveler) | 2006, 2007 | 39 |
|           | H9N2    | Quails, chickens, turkeys | 2011 to date | 182-184 |
|           | H9N9    | Wild birds (Pintail) | 2005 | 39 |
|           | H10N1   | Wild birds (Teal) | 2003, 2005 | 39 |
|           | H10N4   | Wild birds (Shoveler) | 2007 | 39 |
|           | H10N7   | Wild birds (Teal, Shoveler) | 2004, 2007 | 39 |
|           | H10N9   | Wild birds (Teal, Shoveler) | 2007 | 82 |
|           | H11Nx   | Wild birds (Teal) | 2004 | 39 |
|           | H11N6   | Wild birds (Ducks) | 1976 | 39 |
|           | H11N9   | Wild birds (Teal) | 2004 | 78 |
|           | H13N8   | Wild birds (Teal) | 2005 | 78 |

*p* indicates positive serological results only.

H5 subtypes
While the isolation of H5N1 from wild birds in Egypt was not reported before or after 2005, however, LPAIV H5N2 was isolated from shoveler in 2003 and from a common teal (*Anas crecca*) in October 2005. The first and only report of isolation of H5N1 from wild birds was in December 2005, a few weeks before the epidemic in domestic poultry. Out of 1304 sampled migratory birds, one swab from a common teal (*Anas crecca*) trapped in the Nile Delta was positive for HPAIV H5N1
(A/Teal/Egypt/14051-NAMRU3/2006). This report was published at the end of 2006 due to failure of the virus isolation in the original sample. The teal-virus was genetically close to H5N1 viruses isolated from domestic poultry and humans in Egypt suggesting that the introduction of the virus into domestic poultry was by wild birds.49

H10 subtypes

H10 has been the most prevalent IAV subtype in wild birds in Egypt since 2003. It has been isolated in combination with N1, N4, N7 and N9 NA-subtypes (Table 1). Five H10N7 viruses were isolated from wild birds (teal ducks and shoveler) between 18 and 22 April 2004, in a market of hunted migratory birds.82 Other H10N7 viruses were also isolated in 2007.59

H7 subtypes

Since 2003, H7 subtype has been the second most prevalent subtype in wild birds in Egypt (Table 1). An H7N7 was isolated from a black kite "Milvus migrans" in migration-season in the 2005 from South Sinai.83 The virus has been also isolated from shoveler, green-wing teal and Egyptian goose in the period 2004–2006.38,84 Moreover, H7N1, H7N3 and H7N9 were identified between 2004 and 2007 (Table 1).

Other subtypes

From 1970s to 2007, IAV’s of the subtypes H1, H2, H3, H4, H6, H9, H11 and H13 were identified in wild birds in Egypt as summarized in Table 1.75,78,84-88

Prevalence of IAV in Animals

Pigs

While pigs are frequently infected by H1 and H3 in combination with N1 or N2 subtypes however, they are susceptible to many other IAV subtypes 89 and hence they act as a “mixing vessel” for different influenza viruses.90 Egypt was one of the first countries to domesticate and intensively produce pigs and has been doing so from at least the fourth millennium BC.91 That said, the official number of pigs in Egypt did not exceed 40000 head in 2011.92 In recent years, they have been found concentrated primarily in slums, rural and peri-urban areas with insufficient biosecurity measures or veterinary inspection. Pigs in Egypt are fed organic waste, including dead birds, and are in a regular contact with wild and feral birds.54,93,94 Little is known about the prevalence of IAV in pigs in Egypt that may be related to the low number of pig populations in the country.

H1N1 and H3N2

In 1979–1980, a total number of 480 pig serum samples were tested for anti-influenza antibodies. Of these, about 52.5% were found positive against swine influenza H1N1 and 10.4% showed seroconversion to human H3N2.95 Detection of the 2009 pandemic H1N1 (pH1N1) antibodies in pigs in Egypt was also reported96 but no further details are available.

H5N1

Generally, pigs have low susceptibility to H5N1 viruses with limited virus excretion from the respiratory tract upon artificial infection, therefore it is stated that pigs do not play a remarkable role in the epidemiology and evolution of H5N1.97,98 In Egypt, 1.6% (4/250) and 4.4% (11/250) serum samples in 2008 were found positive when tested against H5N2 and local H5N1 antigens, respectively.93 More recently, 4.3% (4/93) of tested pigs sera were found positive to H5N1.99 The two independent studies showed higher rates of H5N1 infection of pigs in Egypt than those recorded in China and Vietnam100,101 that possess a very high number of pigs in comparison to Egypt. These findings raised an alarm on the potential role that might be played by pigs in the epidemiology and evolution of H5N1 virus in Egypt. The panic was aggravated after the emergence of pH1N1 in 2009 which harboured reassortant genes from IAV of human, avian and swine origins. To reduce the risk of reassortment between pH1N1 and H5N1, the Egyptian authority slaughtered over 300000 pigs and planned to transfer pig “farms” to locations outside densely populated areas96; an action, which in retrospect, was considered a misguided attempt to control the disease.102

Equine

Equine influenza is caused by 2 distinct IAV subtypes: H7N7 and H3N8. Both subtypes cause an acute respiratory tract infection of horses, donkeys and mules. Equine H7N7 subtype has not been isolated since 1978103 but H3N8 virus is still evolving. It crossed the species barrier to dogs104-108 and probably to humans as well.109-111 Swapping of gene segments between equine H3N8, avian, swine and humans IAV has been reported.111-113 Egypt is an important center for raising and marketing of the pure breed of Arabian horses.114 The number of equine in 2011 exceeded 3.4 million head; 64000 horses, 3.4 million asses and 1160 mules.92 Therefore, the contribution of equine influenza in the expansion of the gene pool of IAV in Egypt should not be neglected.

H3N8

So far, there have been 3 outbreaks of equine influenza H3N8 in horses, mules and donkeys in Egypt. The first outbreak was in October 1989 in Monofiya governorate in the Nile Delta.115,116 This outbreak was thought to be caused by equine H7N7 virus115 or H7N7 mixed with H3N8.114 The second outbreak in 2000 was reported from the Nile Delta117 as well as from Upper Egypt.118 The most recent and most severe outbreak, this time involving H3N8 was in 2008.115 The infections spread to several provinces within short time.114,120,121 The virus had 98% genetic identity with H3N8 viruses from USA and Japan.114 Trials to develop inactivated vaccines for this virus were described.122,123 It is notable that there are gaps in the epidemiology of equine influenza in Egypt particularly the source of infection and post-outbreak surveillance, although severe economic losses occurred due to suspension of horseracing or death of animals.114,120
H5N1

In March 2009, H5N1 was isolated for the first time from donkeys suffering from respiratory distress and evidence of exposure to H5N1 was recorded in 27 out of 105 (25.71%) serologically tested donkeys. The epidemic occurred one week after infection of household poultry at the same village. In another independent study, antibodies against H5N1 were detected in 23.8% (38/160) and 30.6% (11/36) of the tested horses and donkeys, respectively. The equine isolate possessed viral genes that were closely related to other Egyptian avian HPAIV H5N1 indicating a common origin of these viruses. It had Q226 and G228 in the HA protein which are typical for avian and equine viruses but not for human ones. It also possessed a deletion in the receptor binding domain (serine at position 145) which enhanced the affinity of the Egyptian H5N1 viruses to human-type receptors and lysine at position 627 of PB2 which is a marker for efficient replication of IAV in human. Uniquely, it had 5 amino acids in PB2 (S529M and S530A), PA(472S), NS2 (66L) and M2(29T). Still needing an answer is the question of whether such peculiar amino acids play a role in the virulence and/or transmission of H5N1 virus to equine or not.

Other animals

H5N1

In 2013, few H5N1 positive samples from other animals in Egypt using hemagglutination inhibition test (HI) and enzyme linked immunosorbent assay (ELISA) were obtained. Of those samples, about 28% (7/25) of cats and 12% (3/25) of dogs were found to have anti-H5 antibodies. Meanwhile no evidence for virus infections in cattle, buffaloes, sheep and goats was reported until now. It is worth pointing out that the application of traditional serological tests (i.e.; HI and ELISA) in different animal species particularly pigs, donkeys, cats and dogs has not be extensively validated for application in these species. Therefore, it is important to conduct surveillance based on virus isolation to avoid misinterpretation or overestimation of the biological role, if any, of these animals in the spread of the virus in Egypt. Moreover, under laboratory conditions ferrets survived the infection after eating of heavily-infected chicken meats with an Egyptian H5N1 virus showing mild meningoencephalitis without neurological signs or viral antigen detection in the brain. It was stated that the Egyptian virus was less lethal than the Asian H5N1 virus. In mice, a virus isolated in 2006 was less virulent in comparison to generated mutants resembling the human-origin virus from subclade 2.2.1/C since 2009. The latter was able to infect mice at lower titers inducing moderate to severe bronchial necrosis and inflammatory cell infiltrates indicating progressive adaptation on mammals.

IAV in Humans

Seasonal infection of humans occurs often by H1N1 and H3N2. In the case of pandemics fatalities are estimated to be several millions of people. In the last century, 4 pandemics were documented: H1N1 in 1918, H2N2 in 1957, H3N2 in 1968 and H1N1 in 1977. In 2009, pH1N1 emerged in Mexico then the USA and then worldwide causing about 148000–249000 deaths. So far, there is scanty information on the history of influenza in humans in Egypt and most of the available literature briefly described some epidemiological and/or immunological aspects.

Human IAV

Although it is difficult to confirm, the first reported epidemic of human influenza in Egypt took place sometime between 1650 BC – 1550 BC and resulted in thousands of deaths. In the 19th century (1889–1892), 2 influenza epidemics were recorded in Egypt where fever, respiratory distress and renal disorders were described among infected patients. There are no available data on the situation of IAV pandemics in Egypt during this period. In the 1918–1919 pandemic, infections ranged from mild illness to 50% fatality in the British expeditionary forces stationed in the country. More information was published on the seasonal influenza in human in some regions in Egypt in 1952–1953, 1963–1965, 1966–1972, 1973–1974.

In 1983, a novel H1N2 resulted from the reassortment between the contemporary circulating H3N2 and H1N1 in humans in China without further spread to other regions. In 2001–2002, this H1N2 subtype was reported in over 41 countries in 4 continents including Egypt. Infected persons in Egypt were from 5 to 15 year-old with classic upper respiratory tract infections. Further, H3N2 was reported in Egypt in 2002–2007 but no specific details are available. The 2009 pH1N1 was first detected in Egypt in June and became the predominant influenza virus in human in mid-November of the same year. To December 2010, less than 17,000 infected cases, among them 281 confirmed related fatalities, were officially reported. From June 2009 to the end of 2011, the case fatality rate among 13782 children under 10 y was 0.5% (71 deaths) most of them were in 2009 (46 deaths) then 2010 (20 deaths) and 2011 (5 deaths). In another study, 47.5% of 2–60 months old children (n = 1200) were positive in 2010–2011 in Egypt. In addition to the flu-like illness induced by the virus, ocular manifestations were also reported.

Zoonotic transmission of IAV in Egypt

Hitherto, 3 IAV sub-types have successfully jumped from animals to humans in Egypt; H1N1, H5N1 and H10N7 (Table 1).

H1N1

The only available report on transmission of IAV from animals other than birds in Egypt was from pigs in 1979–1980, since 20 out of 200 human sera (10%) reacted positive against the swine H1N1 virus.

H5N1

Egypt, after Indonesia, is the country worst affected by H5N1 and the country with the highest reported human infections since 2009. The first reported human infection was in March 2006, a few weeks after the suspected date of the infection of domestic...
poultry. As of August 02, 2014, the fatality rate of H5N1 in Egypt was 36% (63/173) compared to 59% (386/650) global fatality rate. The low case-fatality rate in Egypt was probably due to (1) rapid identification of a large number of infected children who were successfully treated and recovered (2) infection of humans with low-virulent virus clade (3) pre-infection with H1N1 virus induced non-specific cross immunity to H5N1 (4) some deaths from H5N1 might not have been reported or (5) probably due to genetic traits of the population. To March 2009, only 1% (36/6355) of suspected cases of H5N1 in Egypt was positive for H5N1 virus and 3 family clusters were described. In another surveillance study, 14% (42/299) of tested individuals were subclinically infected with H5N1 where the number of infected females was higher than males. Unexpectedly, 12% of Cairo residents investigated in that surveillance were positive while 8.4% of the farmers in the country side were found to be infected. These findings might be explained by inadequate surveillance procedures.

To date, there have been 173 human infections reported to the WHO; over 90% were linked to close contact with backyard birds. On the other hand, only 3 workers in the commercial sector were infected and they were reported to have recovered. In addition, 2 poultry sellers acquired the infection from LB and the source of infection in 2 other cases remained unknown. Two important features in the epidemiology H5N1 infection in humans in Egypt have been found to be the sex and age of infected people. The prevalence of infection was remarkably high in women in the age group 20–39 y and children less than 10 y. Keeping, slaughtering, defeathering and evisceration of birds are the common sources of infection in women whereas close contact, cleaning of infected premises and playing with infected birds (or poultry wastes) are considered the main reasons for the disease in toddlers and children.

The most common symptoms in 81 confirmed H5N1 human cases in Egypt from March 2006 to June 2009 were fever, acute respiratory distress, shock, muscle and joint pain and to a lesser extent, sore throat and renal failure. Nevertheless, there is an expectation that subclinical (or mild) infection of H5N1 is widespread in humans in Egypt and that the number of infected persons are actually higher than the detected prevalence. Like the seasonal prevalence of H5N1 outbreaks in poultry, the increased infection rate in humans is associated with winter months where high humidity and low temperature probably favor the droplet transmission of the virus. The infection is concentrated mainly in the Nile Delta, the influenza epicentre in Egypt.

Fortunately, the vast majority of the Egyptian strains are sensitive to the NA inhibitor oseltamivir which may also explain the lower case-fatality rate of H5N1 in human in Egypt. Nevertheless, the continuous evolution of H5N1 in Egypt makes it a potential candidate for being a possible pandemic virus. The H5N1 virus of human-origin (2.2.1/C subclade) is dominant in backyard birds and has been also isolated in small-scale commercial poultry. Interestingly, there is an assumption that selection pressure from vaccination of poultry in the commercial sector has created escape mutants that were unable to infect humans. Two reverse genetic studies showed that the Egyptian viruses acquired 2 mutations in the HA protein (seen only in 2.2.1/C) which enhanced binding affinity to human-type receptors but retained its specificity to the avian-type receptors as well. Also, these mutations increased the attachment of the virus to the epithelial cells of the lower respiratory tract of human. Moreover, it is now known that only 5 mutations are required for the airborne transmission of H5N1 viruses in mammals. Some Egyptian viruses encode 3 of these mutations: HA-220K, HA-N154D and PB2–627K which is a real matter of concern. Additionally, the virus was positive while 8.4% of the farmers in the country side were found to be infected. These findings might be explained by inadequate surveillance procedures.

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**Concluding Remarks and Outlook**

This review has thrown the light on the history, prevalence and diversity of IAV in animals and humans in Egypt where many puzzles must be solved to reduce the potential of the emergence of pandemic influenza virus. Egypt is on 2 of the main flyways of migratory birds linking Eurasian and Africa, therefore influenza viruses in wild birds in Egypt merit more in-depth investigation. Wild birds were claimed to be the source for the infection of domestic poultry with HPAIV H5N1 and humans with H10N7 virus. In addition, the majority of IAV HA subtypes were identified from wild birds in Egypt. The endemic HPAIV H5N1 became established in birds and jumped from poultry to humans and some animals. Thus, surveillance to identify the potential reservoir hosts of HPAIV H5N1 in Egypt is important to gain a better understanding of the virus epidemiology and to develop effective control strategies. Target and active surveillance in poultry in the commercial sector should be rigorously conducted because many factors foster the undetected spread of the virus such as (1) vaccination of commercial poultry in Egypt, in addition to masking the clinical disease, decreased the passive outbreaks reporting, (2) the virus acquired mutations to reduce virulence but enhance transmission in poultry and (3) misdiagnosis due to mutations in the PCR primer sites may influence the overall incidence of the disease in different poultry sectors. Innovative approaches to eradicate the infection in backyard birds particularly in the Nile Delta should
be developed and must consider the cultural, social and economic aspects of the household poultry. Egypt has many “Trojan horses” in the epidemiology of IAV including asymptomatic vaccinated birds, subclinically infected ducks, quails, equine, pigs and even humans. There are several scenarios for the potential reassortment of IAV in Egypt from animals and human sources (Fig. 2) which must be extensively studied to predict the gene constellation of viruses with public and animal health implications. There are substantial gaps in the epidemiology, genetic information and pathobiology of IAV in human including the H5N1 virus. Nationwide surveillance to map the distribution of subclinical and mixed infections of IAV is highly desired. Monitoring of IAV in humans, birds, and different animal species should be conducted regularly. Continuous evolution, jumping from host to host and diverse gene pool of IAV in Egypt (resembling the situation in China) warrant more global attention to control the disease in animals before it becomes a serious worldwide public health problem.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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