Avian influenza H5N1 in Egypt: What we know and what we have to know

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Abstract

Egypt has experienced endemic outbreaks of avian influenza viruses (AIV) of subtype H5N1 in poultry since 2008. Since then, these viruses continue to evolve. In 2006-2008, outbreaks were reported mostly during the winter season, but in 2009-2012 they occurred year round. H5N1 AIV succeeded in infecting not only domesticated birds but also many other avian species and mammals. Infections of humans were accompanied by mutations in the hemagglutinin (HA) protein which improved the binding affinity to human receptors but simultaneously retained its specificity for avian-receptors. Vaccines were applied nationwide to control the disease in poultry. Meanwhile, the viruses accumulated several point mutations in the HA immunogenic epitopes resulting in antigenic drift and the establishment of infections in vaccinated poultry. The Egyptian H5N1 viruses remain susceptible to oseltamivir, but genetic markers indicating resistance to amantadine have increased dramatically. In this mini-review, we discuss recent findings on the H5N1 viruses endemic in poultry and humans in Egypt, and suggest possible options to control the infection.

Key words: Avian Influenza Virus, H5N1, Egypt, Evolution, Reservoirs, Antivirals, Vaccination

Introduction

In February 2006, avian influenza virus (AIV) of subtype H5N1, clade 2.2.1, was introduced into Egypt presumably by wild birds (Saad et al., 2007; Aly et al., 2008). Since then, these viruses have caused devastating losses in the poultry industry and threaten public health (Abdelwhab and Hafez, 2011). Although data from surveillance and research on the Egyptian H5N1 viruses have been accumulating in the last few years, there are still large gaps in knowledge regarding the evolution and epidemiology of these viruses. In this short review, we focus on three aspects of H5N1 viruses in Egypt, virus evolution, potential reservoirs and susceptibility to antivirals and disinfectants. We also suggest options to combat the endemic situation of H5N1 viruses in Egypt.

Virus evolution

Time is the influenza virus' quest to adapt to a new host, environment and climate. Since 2006, H5N1 viruses in Egypt are circulating in unique niches that favour its entrenchment. The viruses circulate in backyard holdings where avian and mammal species including humans are in intimate contact (Abdelwhab and Hafez, 2011). Therefore, specific mutations may be acquired with high probability to alter receptor-binding affinity and/or increase the replication efficiency of the virus in a wide range of hosts, which has been recently confirmed in mice as a model for human infection (Watanabe et al., 2011). Likewise, speculations were raised about the acquisition of species-specific genetic signatures of the Egyptian H5N1 viruses in donkeys and ducks (Abdel-Moneim et al., 2011; Ibrahim et al., 2011). Also, genetic analyses indicated that the recent Egyptian H5N1 viruses possess mutations that could lead to a sustainable transmission from human to human by airborne droplets (Neumann et al., 2012; Perovic et al., 2013) which is of great concern and must be investigated in depth.

Circulation of these viruses in birds which had been vaccinated with H5 vaccines ranging from 78 to 98% HA protein homology to the field virus, as well as in non-vaccinated or inadequately vaccinated birds resulted in different evolutionary pathways (Abdelwhab et al., 2012; Arafa et al., 2012). Viruses isolated from vaccinated poultry showed stepwise acquisition of mutations in the immunogenic epitopes of the HA resulting in divergence at an increased evolution rate to escape from the immunity induced by the employed vaccine strains. This immune escape resulted in severe outbreaks despite continuing vaccination (Cattoli et al., 2011b; Arafa et al., 2012). In contrast, viruses isolated from non-vaccinated poultry had diverged at a lower evolution rate (Cattoli et al., 2011a; Abdelwhab et al., 2012) and developed a unique strategy to evade the innate immune response and replicate at higher levels in infected cells (Ahmed, A. S. et al., unpublished). Continuous update of the poultry vaccines to match circulating field viruses and establishment of AIV H5N1-free zones or compartments could help to ease this complicated epidemiological situation.

The adaptation of the H5N1 viruses to different environmental conditions in Egypt remains to be investigated. In 2006-2008, seasonal oscillation of H5N1 in poultry was reported with high prevalences in winter, very rare occurrence in early summer and absence in mid-summer (Aly et al., 2008; Hafez et al., 2010; Murray and Morse, 2011; Arafa et al., 2012). However, since 2009 the prevalence of the virus was high year round particularly in backyard birds and live bird markets (LBMs) (Arafa et al., 2012; El-Zoghby et al., 2013; Hassan et al., 2013).

Virus reservoirs

Household poultry. In Egypt an estimated 250 million of poultry is kept in backyard holdings, which amounts to about 23% of the total poultry production (Abdelwhab and Hafez, 2011) kept by over 10% of the human population (Meleigy, 2007). Surveillance showed that, in contrast to commercial poultry, the virus is endemic in backyard birds which play an important role in further transmission of the virus to other birds in the commercial sector and in LBMs (Aly et al., 2008; Hafez et al., 2010; El-Zoghby et al., 2013). Moreover, most of the human H5N1-infections had been associated with contact to backyard poultry (Kandeel et al., 2010). To investigate the role of this possible reservoir, a targeted surveillance of backyard holdings was recently conducted in 111 households during the mid-summer season in five provinces with the highest H5N1 incidence in 2010-2011. Only in one province, asymptomatic infections of native duck breeds were reported in three different backyards while chickens and imported duck breeds were negative (Hassan et al., 2013). This low incidence rate in backyard ducks may indicate a minimal contribution as a reservoir. Therefore, until now it is not clear whether ducks are the “Trojan horse” of H5N1 AIV in Egypt serving as endemicity reservoir or whether they are victims being infected from another reservoir. Nevertheless, the rapid and random movement of live poultry in Egypt may result in widespread dispersal despite a limited infection at source. Also, slaughtering or disposal of sick or dead chickens and native ducks can influence the results of such surveillance. Thus, it is useful to investigate susceptibility of native and foreign avian breeds to the Egyptian AIV H5N1.

From 2006 until 2009, a vaccination policy was established using commercially available vaccines of the H5 serotype but not necessarily matching the circulating field virus. However, this vaccination strategy was not successful in reduction of the prevalence of the virus due to low vaccination coverage (Peyre et al., 2009; El Masry et al., 2013). Moreover, vaccination can suppress symptoms and, thus, mask the infection, while excretion of the virus from infected birds is not prevented. These asymptomatic virus shedders pose a serious risk not only for maintenance of infection in poultry, but also for human infections. Hence, vaccination of backyard birds in Egypt had been stopped in 2009 (El Masry et al., 2013). However, alternative strategies to control the disease in the backyard sector were not established. Therefore, there is an urgent need for innovative approaches to control the infection and to better understand its dynamics in the backyard sector.

Live bird markets. In Egypt, marketing of poultry depends mainly on trade of live birds. Commercial poultry from several provinces is usually gathered in collection points and then redistributed throughout the country. In contrast, backyard birds are usually sold in local or traditional LBMs. In Egypt, around 16000 LBMs sell live or freshly slaughtered birds to consumers, and over 4300 small slaughtering and de-feathering points sell freshly slaughtered or chilled birds (Hosny, 2009). Surveillance has revealed that H5N1 AIV is endemic in LBMs year round (Abdelwhab et al., 2010; El-Zoghby et al., 2013). Consequently, LBMs are considered an important reservoir of H5N1 viruses in Egypt. The majority of LBMs contain birds from the commercial sector (mostly chickens) and from backyard holdings (mainly waterfowl). The H5N1 incidence rate was particularly high in LBMs which sold ducks (Abdelwhab et al., 2010; El-Zoghby et al., 2013). The exact pathway of transmission of infections from/to LBMs is unknown, also because recording of the source of birds and tracing back the infections are very difficult (El Nagar and Ibrahim, 2007). The infection may be perpetuated in the LBMs or introduced with each new batch of poultry. Although transport of poultry without prior laboratory testing to confirm an AIV H5N1-free status is officially prohibited, illegal transport of birds is common practice. Although the efficiency of closing LBMs to control AIV infections has been proven in Hong Kong and USA (Bulaga et al., 2003; Kung et al., 2003; Lau et al., 2007; Yee et al., 2008; Fournie et al., 2011), total closure of LBMs is difficult to achieve because the capacity of slaughterhouses is limited to a maximum of 30% of the total poultry production (Abdelwhab and Hafez, 2011). There are several potential options to control the infection in LBMs. (1) To change consumer preferences by selling frozen birds at lower prices can be supported by the government or the poultry industry. (2) To invest into the establishment of slaughterhouses which may be encouraged through reduction of customs fees or taxes. (3) To establish a one-day rest each week or month for cleaning and disinfection of LBMs.

(4) To trace the infection of poultry in LBMs. (5) To provide fair compensation of the owners for the depopulation of infected birds.

Humans. As of December, 19, 2013 173 human infections with AIV H5N1 were recorded in Egypt of which 63 were fatal (WHO, 2013). All infected humans but two had documented contact with poultry and the vast majority of infections was due to direct contact (e.g. keeping, slaughtering) with backyard birds (Kandeel et al., 2010). Nonetheless, the actual number of infected humans in Egypt is probably higher than the laboratory confirmed cases. Asymptomatic infection of humans in Egypt is of great concern (Anon, 2009) and there are suggestions that infection of humans with viruses of lower virulence, which had evolved from highly pathogenic precursors, may have occurred (Fasina et al., 2010; Perovic et al., 2013). Recently, evidence for seroconversion of apparently healthy people in Egypt was obtained (El-Sayed et al., 2013). Hence, the role of putative asymptomatically infected humans may be worth elucidating.

Other animate and inanimate reservoirs. In addition to domesticated birds e.g. chickens, turkeys, ducks, geese, pigeons, ostriches and quails, presence of Egyptian H5N1 viruses has also been reported from common egrets, crews and herons, the most common feral birds in Egypt. Moreover, the virus caused morbidity and mortality in zoo birds. In mammals, H5N1 viruses have been isolated from humans and donkeys, and serological surveillance showed anti-H5N1 antibodies in pigs, dogs and cats (Abdelwhab and Hafez, 2011; Eissa et al., 2012; El-Sayed et al., 2013). Recently, AIV H5N1 genomes were detected by RT-PCR in freshwater fish. This contamination was probably acquired through fertilization of fish ponds with infected poultry manure, feeding of fish with infected poultry carcasses or presence of infected feral/backyard birds around the aquatic habitats (Eissa et al., 2012). The potential spread of the H5N1 viruses into other birds and livestock in Egypt was not investigated, so far. Likewise, there is little knowledge on the persistence of H5N1 viruses in the environment, particularly in poultry manure and water (e.g., ponds, canals, lakes and seas), although it has already been considered a risk for increased H5N1 AIV infections of poultry in Egypt (Sheta et al., 2013). The role of other animal species as reservoirs (e.g. rodents, wild birds) or vectors (e.g. mosquitoes, houseflies) for H5N1 viruses in Egypt should be elucidated.

Susceptibility to antivirals and disinfectants

In poultry, the incidence of the mutation in the M2 protein, S31N, resulting in amantadine-resistance has dramatically increased since 2008 (Kayali et al., 2011). Almost all resistant strains were isolated from commercial poultry farms in sector 3 which have minimal biosecurity measures, if any. Whether those viruses are naturally selected or whether there is a positive selection by illegal application of amantadine in poultry in Egypt is currently under investigation. It is worth mentioning that amantadine is the cheapest anti-influenza drug and, thus, financially attractive also for use in poultry. However, the extra-label use of amantadine in poultry is totally prohibited to minimize emergence of amantadine-resistant strains in human (Abdelwhab and Hafez, 2012).

Luckily, the neuraminidase inhibitor oseltamivir is still effective in treatment of infections of humans with Egyptian H5N1 viruses as only a few strains were shown to be resistant (Earhart et al., 2009; Kandeel et al., 2010; Kayali et al., 2011; Younan et al., 2013). Zanamivir is also effective against the Egyptian H5N1 viruses (Rashad et al., 2010b). Many synthetic chemicals and herbal extracts have been shown to inhibit replication of H5N1 AIV. Synthetic chemical compounds such as thiophene, thienopyrimidine and pyrazolopyrimidine nucleosides, pyrazole, pyridazinone, oxadiazole, triazole, thiazolidine and thioxopyrimidine derivatives had variable antiviral activities against strains of H5N1 AIV in Egypt (Rashad et al., 2010a; Rashad et al., 2010b; Flefel et al., 2012). Extracts from Capparis sinaica Veill, Aloe hijazensis and the Red Sea grass Thallasodendron ciliatum have successfully inhibited replication of some Egyptian H5N1 viruses in vitro (Abd-Alla et al., 2012; Ibrahim et al., 2013a; Ibrahim et al., 2013b). Also, fractions of esterified whey proteins exhibited a potent antiviral activity against the Egyptian H5N1 viruses (Taha et al., 2010). However, none have so far been examined rigorously under field conditions.

Moreover, commonly used chemical disinfectants e.g. Formalin, TH4, Virkon S and Glutaraldehyde were effective to eliminate infectivity of the Egyptian H5N1 viruses even in the presence of organic matter when used in the appropriate concentrations for two minutes at room temperature (Marzouk et al., unpublished data). Nevertheless, the resistance of the Egyptian H5N1 viruses to environmental conditions (e.g. temperature, humidity, salinity, dryness, PH) is still unknown.

General remarks and recommendations

In the foreseeable future the elimination of the H5N1 AIV infection endemic in poultry in Egypt is unlikely. Therefore, more efforts are required to better understand changes in the evolution and epidemiology of the virus. Several genetic analyses indicated that the Egyptian H5N1 viruses carry several molecular markers that are linked to (1) adaptation to humans, (2) increased virulence in mammals, and (3) human-to-human transmission. Thus, there is an urgent need to study the virulence and transmissibility of these viruses in mammals e.g. ferrets, mice, guinea pigs as a model for the human situation. Although oseltamivir currently remains effective against H5N1 AIV in

Egypt, constant monitoring for the possible emergence of resistant variants is required. Rigorous evaluation of the potency of vaccines against Egyptian strains of H5N1 is essential to effectively control the disease in poultry. Ideally, this should be conducted frequently either annually or biennially under representative field conditions (e.g. efficacy against new emerging viruses and/or in the presence of maternal immunity). Surveillance should continue in LBMs, backyard holdings and humans to understand the ecological and biological reservoirs of the virus. Tracking the infection in LBMs, establishment of new slaughterhouses and changing the consumer preferences are a real challenge for the control of H5N1 AIV in Egypt which must be considered by the policymakers. Control of the disease in the backyard sector should take into account the societal and economical needs of the owners. Last but not least, continuing awareness programmes are helpful to prevent acquisition and spread of the infection, particularly in the rural areas and suburbs.

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Conflict of Interest

The authors declare no conflict of interests regarding the publication of this article.

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