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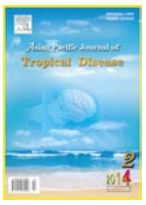

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Epidemiology of avian influenza H5N1 virus in Egypt and its zoonotic potential

Nahed Hamed Ghoneim, Khaled Abdel-Aziz Abdel-Moein*, Hala Mahmoud Zaher

Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

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ABSTRACT

Objective: To investigate the epidemiology of avian influenza H5N1 virus in domestic poultry and its zoonotic potential in Egypt.

Methods: Tracheal swabs were collected from two hundred and forty three domestic poultry (chickens, ducks and geese) from commercial farms and backyards, and thirty two blood samples from unvaccinated chickens. Fifty two throat swabs and twenty blood samples were collected from persons who are in contact with diseased and/or infected birds. Tracheal and throat swabs were examined for the presence of avian influenza virus H5N1 genome by real-time RT-PCR whereas blood samples were tested by competitive ELISA for the presence of avian influenza virus H5 antibodies.

Results: The overall prevalence of H5N1 in the examined birds was 5.3% while the prevalence rates among different poultry species were 9%, 4.7% and 0% for ducks, chicken and geese respectively. Moreover, we detected H5 antibodies in 12.5% of the examined backyard chickens. All examined humans were negative for both viral RNA and antibodies.

Conclusions: Our findings highlight the broad circulation of H5N1 virus among poultry in Egypt whereas it still has a limited zoonotic potential so far.

1. Introduction

The unprecedented spread of highly pathogenic avian influenza virus (A/H5N1) from Asia to Africa in 2005 was considered as a global epidemiological twist[1]. The emerging A/H5N1 in Egypt since mid-February 2006 caused enormous losses in poultry industry and the slaughter-campaign has overwhelmed the resources of the Egyptian veterinary and public health authorities[2]. Following the attack, the Egyptian authorities designed an integrated action plan in a trial to control the epidemic and to eliminate the circulating influenza viruses in 60 thousand poultry farms. Accordingly, the poultry farmers carried out active and effective programs including hygienic disposal of dead carcasses mostly by deep burying, vaccination of all poultry flocks using either H5N1 or H5N2 oil based vaccines and application of restricted bio-security measures including disinfection of all poultry houses using very effective and aggressive disinfectants. Despite of these control measures, the situation is still critical in Egypt and Indonesia where the risk of influenza H5N1 virus mutating into a major human threat remains high[3]. The long term endemic influenza virus infections in poultry increase exposure risks to surrounding human and in turn, create opportunities for the emergence of human-adapted strains with pandemic potential and severe illness with a high fatality rate among the known human cases[4,5]. Since 1997 there have been several outbreaks of H5N1 influenza viruses transmitted to the human population directly from poultry[6,7]. From 2003-2014, one hundred and seventy three confirmed human cases of infection with H5N1 viruses and 63 deaths have been reported to the World Health Organization[8]. However, avian influenza H5N1 virus is not efficiently transmitted from infected poultry to humans and direct transmission from man to man has been reported only in close family clusters, with very limited spread of the virus[9,10]. So, this study was carried out to better understand the epidemiology of the virus and to investigate its zoonotic potential.

2. Materials and methods

2.1. Detection of avian influenza H5N1 virus by using real-time RT-PCR (rRT-PCR)

Tracheal swabs were collected from 243 domestic birds from commercial farms and backyards from some

*Corresponding author: Khaled A. Abdel-Moein, Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
Tel: +20 1224986150
Fax: +20235725240
E-mail: Khal_105@cu.edu.eg
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governorates in Egypt (Figure 1), and throat swabs were obtained from 52 persons in contact with the examined birds. The swabs were pooled and the maximum size of pool consists of five samples, while highly suspected samples were tested without pooling according to method described by International Office of Epizootics[11]. Each swab was placed in a tube containing 0.5 mL sterile normal saline with gentamicin sulfate solution (50 mg/mL). The swab tip was cut off in the saline and the tubes were immediately transported to the laboratory on wet ice and stored at −80 °C for rRT-PCR examination. Firstly, the viral RNA was extracted from the collected swabs by using QIAamp viral RNA mini kit (Qiagen, Germany) and the procedure was conducted according to the kit instructions. Then, one step rRT-PCR was carried out using genesig real-time PCR kit (Primer Design Ltd) for qualitative and quantitative detection of avian influenza virus H5N1 genome. The amplification of *H5* and *N1* genes was done according to the manufacturer's protocol in Applied Biosystem 7500 (Applied Bio-systems, USA). Amplification protocol can be summarized as follow: reverse transcription at 55 °C for 10 min, enzyme activation at 95 °C for 8 min then 50 cycles including denaturation at 95 °C for 10 seconds and annealing/extension at 60 °C for 60 seconds. rRT-PCR is the technique of choice for avian influenza diagnosis as it requires swab sample completely machine dependant for preparation and reading of results, requires only 2.5 h, it minimize use of ethidium bromide dye and the capability to make both a qualitative and quantitative detection of the target[12].

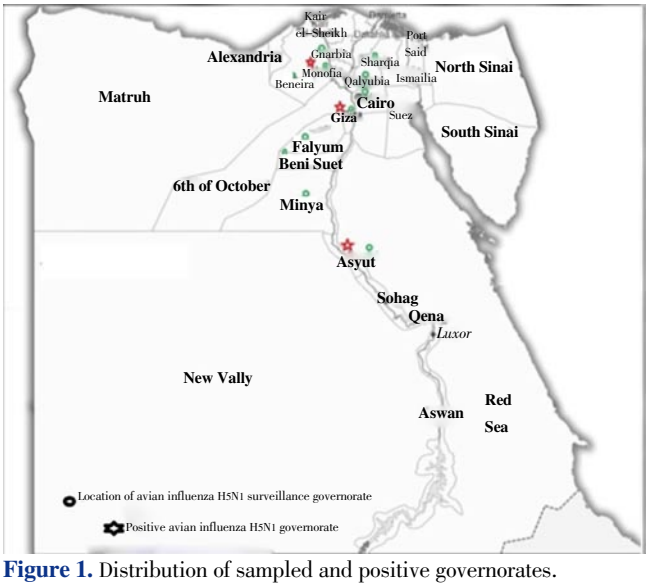

Figure 1. Distribution of sampled and positive governorates.

2.2. Detection of avian influenza H5 antibodies by using competitive ELISA

Blood samples were collected from 32 backyard (un vaccinated) chickens and 20 persons in contact with infected birds. Blood samples were centrifuged at 2500 r/min for 10-15 min and serum samples were then stored at −20 °C until processing[13]. Serum samples were tested for the presence of H5 antibodies by using competitive ELISA kit (ID-screen®, IDvet, France). Competitive ELISAs are easy to perform and scale up to accommodate the screening of large numbers of sera from various species, so it could be effective for large-scale surveillance of avian influenza virus in avian flocks or herds of other species[14].

3. Results

H5 antibodies were detected in 12.5% of the examined backyard chickens while H5N1 genome was detected in 13 out of 243 examined birds, giving a ratio of 5.3%. The species wise distribution of rRT-PCR results was 9.0%, 4.7%, 0.0% for ducks, chickens and geese respectively (Table 1). The highest positivity rate came from vaccinated farmed flocks (6.4%) followed by un vaccinated backyard flocks (2.7%) (Table 2). Among chickens, 10.5% of the samples from layers, 9% from breeders, and 4.6% from broilers were positive as shown in Table 3. Moreover, the infection of poultry appears to be seasonal, and the highest positive results were recorded in the winter. Of all sampled governorates, positive results were only recorded in three of them (Al-Monofia, Giza and Asyut) (Figure 1). On the other hand, all human samples yielded negative results when examined for both H5N1 genome and antibodies.

Table 1
Prevalence of avian influenza H5N1 virus among the examined chickens, ducks and geese by using one-step rRT-PCR.

Type of bird	Number of the examined birds	Positive birds *n* (%)
Chickens	191	9 (4.7)
Ducks	44	4 (9.0)
Geese	8	0 (0.0)
Total	243	13 (5.3)

Table 2
Prevalence of avian influenza H5N1 virus among the examined vaccinated farmed and unvaccinated backyard chickens, ducks and geese by using one-step rRT-PCR.

Type of bird	Farmed (vaccinated)		backyard (unvaccinated)	
	Number of the examined birds	Positive birds *n* (%)	Number of the examined birds	Positive birds *n* (%)
Chicken	159	9 (5.6)	32	0 (0.0)
Duck	11	2 (18.1)	33	2 (6.0)
Geese	0	0 (0.0)	8	0 (0.0)
Total	170	11 (6.4)	73	2 (2.7)

Table 3
Prevalence of avian influenza H5N1 virus among the examined farmed broiler, layer and breeder chickens by using one-step rRT- PCR.

Breed of chicken	Number of the examined chickens	Positive chickens *n* (%)
Broilers	129	6 (4.6)
Layers	19	2 (10.5)
Breeders	11	1 (9.0)
Total	159	9 (5.6)

4. Discussion

Long-term endemicity of avian influenza H5N1 virus in poultry and continuous sporadic human infections

in several countries has raised the concern of another potential influenza pandemic. Suspicion of the avian origin of the previous pandemics results in close investigation of the mechanism of interspecies transmission[15]. It was obvious from this study that the presence of H5 antibodies in substantial proportions of unvaccinated chickens was probably due to exposure to the field virus either a low pathogenic avian influenza H5 virus or a high pathogenic avian influenza H5N1 virus of lower pathogenicity, resulting in birds surviving infection and maintaining immunity[16]. The highest positive result by rRT-PCR was recorded in ducks. This may reveal that the avian influenza become more endemic in ducks in Egypt, as domestic ducks play an important role in the epidemiology of highly pathogenic H5N1 avian influenza. The ducks shed the virus without showing any symptoms of illness, making them the ultimate natural reservoir[17]. Free-ranging ducks are implicated in the transmission of virus to the environment and subsequently to other ducks or other species, since water in which ducks swim, drink, and eat presents a high exposure risk to humans and other birds. Therefore the risk is greatest in rural areas of affected countries, where domestic ducks and chickens often mingle, frequently sharing the same water supply where the viruses may be potentially transmitted to chickens under these conditions[18]. The positivity rate was higher for vaccinated farmed flocks than for unvaccinated backyard flocks. This may be attributed to the fact that in Egypt, commercial farms are major reservoirs for influenza (H5N1) virus, and because the sampled poultry at commercial farms were vaccinated with commercially available subtype H5 vaccines, whose effectiveness becomes highly questionable[19]. So the detection of avian influenza virus in vaccinated farmed birds showed that the used vaccines as well as a vaccination program against high pathogenic avian influenza H5N1 in Egypt could not totally prevent the circulation of the virus in vaccinated birds[20]. Therefore, the long-term circulation of the virus under immune pressure from natural infection or vaccination, or both, may result in both genetic and antigenic changes in the virus[21]. These antigenic changes can enable a virus to better escape the host's ability to control infection, resulting in less protectiveness of vaccines over time or total failure of the current vaccine[22]. Hence enforcement of bio-security measures and systematic vaccination coverage by regions, and quality control of the current vaccines could interrupt the continuous circulation of the virus in poultry[20]. The lower positivity rate among backyard poultry may be explained by the fact that the growers slaughter these birds at the first sign of disease[19]. Moreover, the layer chickens recorded higher result than broilers and breeders, which was in accordance with Kayali *et al*. in 2011[19]. On the other hand the peak of poultry infection with avian influenza was in the winter because the avian influenza virus is more active in lower temperature[23]. It is worth mentioning that the Asyut Governorate recorded higher percentage of avian influenza infection than other governorates. From our investigation, this may be due to that farms in Asyut are very near to each other, so when avian influenza outbreak occurs in one farm, it is easy to spread rapidly to other farms.

On the other hand, all human serum and swab samples yielded negative results although five of them were in intimate contact with two H5N1 positive backyard ducks. Moreover, no signs of avian influenza infection appear on those five persons. These findings were in accordance with Kandeel *et al*. in 2010[24], who reported that during February 2006-March 2009, none of 3 941 asymptomatic persons exposed to avian influenza (H5N1) from infected poultry were tested positive by using a RT-PCR, and also with Vong *et al*. in 2006[9], who found that 351 participants from 93 households exposed to poultry suspected of having H5N1 infection were tested negative for H5N1 antibodies. This means that despite frequent and direct contact with H5N1 infected birds, none of persons acquired the infection, so H5N1 influenza virus may have a low zoonotic potential. This concept was supported by many previous studies which concluded that viral hemagglutinin protein of human influenza virus differs from that of avian ones; hence the transmission of avian influenza H5N1 to humans needs mutations in such protein[25-29]. In conclusion, our findings indicated broad circulation of the endemic avian influenza virus H5N1 among poultry in Egypt. Domestic ducks play an important role in the epidemiology of H5N1 avian influenza in Egypt. In addition, the infection of poultry with H5N1 virus appears to be seasonal; the peak of virus activity was in the winter. In Egypt, commercial farms are major reservoirs for influenza (H5N1) virus. Circulation of avian influenza virus H5N1 in vaccinated birds continues to devastate the poultry industry in Egypt, so updated poultry vaccine should be considered in endemic countries like Egypt. Continuous surveillance to elucidate the spread of avian influenza virus H5N1 among commercial farms and backyards, integration of multifaceted strategies and global collaboration are needed to control the disease in poultry in Egypt. Finally, the highly pathogenic avian influenza H5N1 virus still has a limited zoonotic potential to human being, even in circumstances in which human-poultry interactions are regular, intimate and frequent. Therefore its transmissibility to human being needs further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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