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The Potential Role of Animals in the Epidemiology of Avian Influenza Virus H_5N_1 and Its Public Health Implications

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Abstract: In the last few years, Avian influenza virus H5N1 (AIV H5N1) was known to infect several mammalian species. Much remains unknown about the role of animals in the epidemiology of the virus. The current study was carried out to investigate the possible role of animals in the epidemiology of AIV H5N1. For this purpose, nasal and pharyngeal swabs as well as blood samples were obtained from 30 stray dogs, 39 stray cats, 42 donkeys and 50 rats. All animals were collected from villages heavily concentrated with poultry farms. Nasal and pharyngeal swabs were examined for the presence of AIV H5N1 genome by real-time reverse transcriptase polymerase chain reaction whereas blood samples were tested by competitive enzyme linked immunsorbent assay for the presence of AIV H5 antibodies. Of the examined animals only dogs and cats showed seropositive results with high seroprevalence 50% and 56.4% for dogs and cats respectively while none of donkeys and rats was seroreactors. On the other hand, viral RNA of AIV H5N1 was not detected in both pharyngeal and nasal swabs from all examined animals. Therefore, Dogs and cats seems to be important mammalian reservoirs for AIV H5N1and may have a potential role in the epidemiology of the virus with great public health burden.

Key words: Avian Influenza H5N1 · Animals · Epidemiology

INTRODUCTION

The emergence of highly pathogenic avian influenza virus H5N1 (AIV H5N1) in 1990s and its implications in both veterinary field and public health community created a bad need to better understand the epidemiology of this virus specially when a great attention to the virus was drawn after its direct transmission from birds to persons who came in contact with affected poultry leading to the appearance of human cases of the disease in Hong Kong 1997 [1, 2]. Since this date, AIV H5N1was rapidly spread across Asian countries and the virus found its way toward Europe and Africa to produce the disease everywhere. Nowadays, AIV H5N1was considered as a serious threat to poultry industry in many countries as well as it possessed a great zoonotic potential resulting in severe illness with a high fatality rate among the known human cases [3]. However, AIV H5N1was thought to be an avian pathogen, the virus showed the ability to jump the species barrier to infect wide range of animals as well as humans. In the last few years, several reports and researches were released to document experimental and/or natural infections with AIV H5N1 among domestic animals such as pigs, dogs, cats, rodent, ferrets, donkeys and even some wild animals as tigers [4, 5]. Nonetheless, much remains unknown about the natural infection with AIV H5N1 among different animals. So, the current study was carried out to learn more about the occurrence of AIV H5N1 in some animal species in order to better understand the possible role of these animals in the epidemiology of AIV H5N1to improve the knowledge required for the control of such pathogen and its impact on both veterinary and public health fields.

MATERIALS AND METHODS

Nasal swabs and pharyngeal swabs as well as blood samples were obtained from 50 rats (Rattusnorvigacus), 42 donkeys, 30 stray dogs and 39 stray cats. All animals were collected from the same areas in the vicinity of poultry farms from villages heavily concentrated with poultry farms in Giza Governorate of

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Egypt. Blood samples were processed to get sera by centrifugation at 2500 r.p.m for 10-15 minutes [6] and serum samples were then stored at – 20°C for further examination. On the other hand, nasal and pharyngeal swabs were collected in phosphate buffer saline and stored at – 80°C.

Direct Detection of AIV H5N1 by Using Real-Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR): Firstly, the viral RNA of the AIV H5N1 was extracted from the collected nasal and pharyngeal swabs by using QIAamp® viral RNA mini kit (Qiagen, Germany) and the procedure was performed according to the directions of the manufacturer's. The extracted RNA underwent one step real-time RT-PCR amplification protocol using genesig real-time RT-PCR advanced kit for quantification of AIV H5N1 genomes (PrimerDesign Ltd). The kit contained all components required for the test including H5 primer and probe set which are designed to detect all influenza H5 sequences from the avian H5N1 lineage and also contained N1 primer and probe set; positive controls for both H5 and N1 templates. The test was done according to the directions of the kit to amplify H5 and N1 genes into 2 separate one step reactions one of them using H5 primer and probe set and the other one using N1 primer and probe set. The amplification conditions can be summarized as: reverse transcription at 55°C for 10 minutes; enzyme activation at 95°C for 8 minutes followed by 50 cycles of 95°C for 10 seconds and 60°C for 60 seconds. The amplification step was carried out in Applied Biosystems 7500 Instrument (Applied Biosystems, USA).

Detection of Avian Influenza H5 Antibodies by Using Competitive Enzyme Linked Immunosorbent Assay (cELISA): Competitive ELISA was found to be a highly sensitive, specific and reliable test for screening of avian influenza antibodies among avian and other species [7, 8]. Serum samples from the examined animals were tested for the presence of antibodies against avian influenza virus H5 by using (ID-screen® competitive ELISA kit, ID.VET, France). The test was conducted according to the manufacturer's protocol and the result was expressed for each sample as a competition percentage which was calculated according to the formula (optic density of the sample/mean value of negative control optic density) ×100 regarding samples with competition percentages ≤ 35% as positive samples.

RESULTS

The seroprevalence of avian influenza virus H5 antibodies was 50% for dogs and 56.4% for cats while the sera from rats and donkeys were negative (Table 1). Moreover, most of dogs and cats showed strong seropositive results which were reflected by lower competition percentages (Figure 1) whereas, the peak sero prevalence of AIV H5 antibodies was found in winter and spring (Table 2). On the other hand, neither H5 nor N1genomes was detected in all of the examined animals when their nasal and pharyngeal swabs were tested by rRT-PCR.

DISCUSSION

Recently, the occurrence of highly pathogenic AIV H5N1in different mammalian species and the expansion of the virus host rang was attracted the worldwide attention because of it is considered as a great threat for public health [9]. The results of the current study revealed that overall examined mammalian species (dogs, cats, donkeys and rats) which co-inhabited the same areas only dogs and cats were sero positive for AIV H5 antibodies in extremely high sero prevalence 50%, 56.4% for dogs and cats respectively. Such prevalence was found to be higher than that obtained in other previous studies 25% for dogs in Thailand [10]; 1.8% and 2.6 % for cats in Germany and Austria respectively [11]. 0% for dogs in Nigeria [12]; 4% and 8% for dogs and cats respectively in Egypt [13]. So, our results may be the highest so far. Moreover, the competition percentages of the obtained cELISA results showed high antibody titers among positive dogs and cats which may indicated a recent infections with AIV H5N1 specially when most of them were concentrated in winter and spring seasons which are the peak seasons of AIV H5N1 among birds in Egypt [14]. This leads us to suggest the circulation of the virus from birds to dogs and cats and may be vice versa. This concept was strongly supported by Amonsin *et al*. [15] who documented that the complete genomic sequences of avian influenza H5N1viruses obtained from dogs and cats during 2004 and 2005 outbreaks in Thailand were closely related to that isolated from avian species in the same period to prove the natural transmission of the virus from poultry to dogs and cats. Meanwhile, other papers described the disease in dogs and cats and the shedding of the virus in their nasal discharges and feces to contaminate water, soil

Table 1: Seroprevalance of AIV H5 Antibodies among the examined animals

Animal	Number of the examined animals	Number of positive Animals	Percentage (%)
Dogs	30	15	50
Cats	39	22	56.4
Rats	50	0	0
Donkeys	42	0	0

Table 2: Seasonal distribution of avian influenza H5 antibodies among the examined dogs and cats

	Dogs			Cats		
		Positive			Positive samples	
Season	Number of the examined animals	Number	Percentage (%)	Number of the examined animals	Number	Percentage (%)
Winter	15	11	73.3	25	15	60.0
Spring	6	3	50.0	7	5	71.4
Summer	5	1	20.0	4	2	50.0
Autumn	4	0	0.0	3	0	0.0
Total	30	15	50.0	39	22	56.4

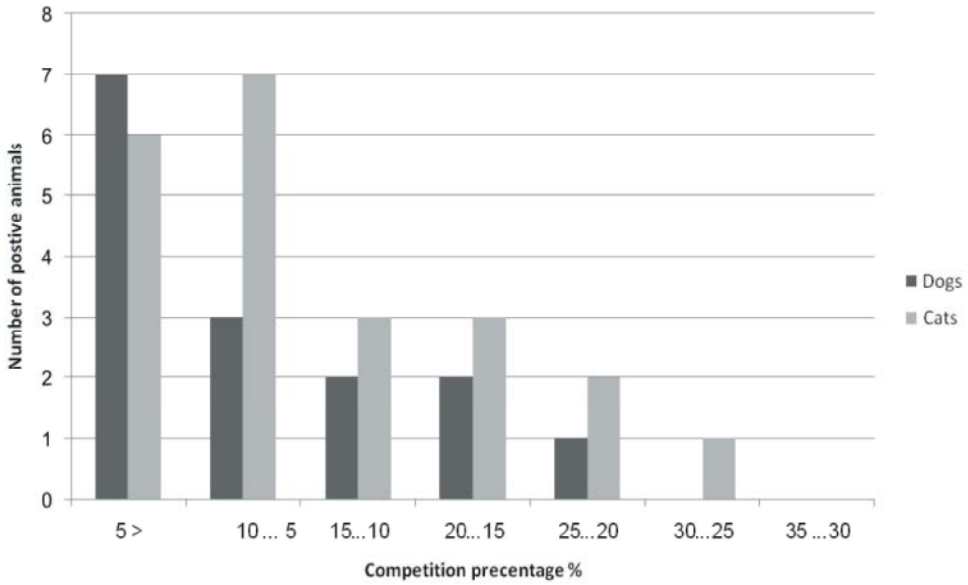

Fig. 1: Distribution of the positive cELISA results in dogs and cats according to the competition percentage

and environment [16, 17] and thereby sharing in the epidemiological cycle of the virus which may then return back to poultry a matter which highlighted the potential role of dogs and cats in the epidemiology of avian influenza upon the veterinary field. Furthermore, our findings in the current study goes beyond the veterinary field to involve the public health community as this high seroprevalence of avian influenza virus H5 antibodies recorded in the present study in dogs and cats may indicate the dissemination of this virus among them and the transmission of the virus from dog to dog and/or cat to cat may have been happened. This process enables the virus to be more adapted to the mammalian tissues and so dogs and cats may be considered as bridging hosts or vehicles for the interspecies transmission of AIV H5N1 to magnify the threat for humans being dogs and cats as popular companion animals usually come in close contact with humans particularly children in rural areas, villages and everywhere [18, 17]. Moreover, dogs and cats were found to be susceptible to the infection with some human influenza viruses including H1N1 with high communicability between humans causing great outbreaks and epidemics [19, 20, 21, 22, 23]. Accordingly, AIV H5N1 and other human influenza viruses as H1N1may co-colonize dogs and cats giving the chance for reassortment to take place and release of new strains of influenza viruses which may cause massive epidemics.

It is noteworthy that in spite of high seroprevalence of AIV H5 antibodies among the examined dogs and cats we could not detect AIV H5N1genomes in the examined nasal and pharyngeal swabs of these animals by using rRT-PCR which may be due to all examined animals were apparently healthy in addition to short period of AIV H5N1 shedding in dogs and cats [24, 11, 25, 26]. Finally, neither AIV H5N1 genome nor antibodies could be detected in the examined donkeys and rats although they inhabited the same areas from which dogs and cats were caught a subject which underlined that these animals may have limited role in the epidemiology of AIV H5N1. In conclusion, the present study brings a new insight to the epidemiology of avian influenza virus H5N1as it highlighted the crucial role that may be played by dogs and cats in the circulation of the virus. So, the controlof stray dogs and cats specially around the poultry farms and in villages should be considered as a cornerstone in the implementation steps for control of avian influenza virus H5N1to combat its existence, mutation and evolution.

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