Seroprevalence of Avian H9N2 Influenza Virus in a Population of Iranian Domestic Dogs

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Abstract: The prevalence of H9N2 influenza virus in dogs was first time observed in Fars province of Iran. A total of 182 dogs were selected from the clinical cases at the Small Animal Clinic of Veterinary Medicine School, Shiraz University. After obtaining history, physical examination was performed and blood samples were obtained for serological examination (Eliza and HI assay) for the detection of H9N2-specific antibodies. Associated factors (age, breed, diet, place, presence of other dogs, general symptoms, respiratory and gastrointestinal signs) were also evaluated. The positive results showed that 81.7 % of ELISA positive cases had titer ≥ 32 for H9N2 influenza in HI test. Although positive result were found more in dogs with general or respiratory signs, no significant differences were observed in the evaluated factors and seropositivity. This research showed high seroprevalence of Ab against H9N2 in dogs and made this hypothesis that H9N2 may be important in dogs in virus persistence. Additional research is needed for detection of epidemiologic role of dogs in transmission and pathogenesis of H9N2 in dogs and humans.

Keyword: Canine influenza virus, Avian influenza virus, H9N2, Dog, Iran, Seroprevalence.

INTRODUCTION

Influenza A virus is a highly contagious pathogen that has been isolated from a wide variety of animals [1]. This virus is a member of the genus orthomyxovirus and divided in to three main types (A, B and C).

Influenza A viruses are classified into subtypes on the basis of the antigenicity of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA) [2, 3]. Influenza A is found in a wide variety of bird and mammal species [4].

Influenza A Viruses currently circulating in avian species represent a source of viruses capable of infecting mammals, and replication of avian influenza viruses in mammals may facilitate their adaptation in humans [5]. Cats and dogs, which come into close contact with humans, may also play a role in the interspecies transmission of influenza viruses. Examples of interspecies transmission of influenza viruses include: recent human and dog infections with the H5N1 subtype avian influenza virus [6]; canine infections with the H3N8 equine influenza virus in recent years [7, 8, 9]; transmission of a complete avian influenza virus (H3N2) to dogs and its successive intraspecies transmission between dogs [6, 10, 11]; or experimental infection of dogs with H9N2 (avian influenza) [12].

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Fars province is an active pole of the poultry industry in Iran, where 26, 14, and 10% of the broiler, layer, and broiler breeder farms of Iran are located [4].

Antigenic and genetic analyses of H9N2 viruses isolated during the last two decades indicated that these viruses are extensively evolving and have reassorted with other avian influenza viruses to generate multiple novel genotypes [13-15]. By the year 1997, H9N2 viruses had been isolated in multiple avian species throughout Asia, the Middle East, Europe and Africa [16, 17].

Amirsalehy *et al.* [12] experimentally showed that H9N2 can infect dogs and produce respiratory signs and antibody (Ab). However, there is no report about natural exposure of dogs to H9N2 viruses in the field conditions. Therefore, the objective of the study reported here was to determine the prevalence of H9N2 influenza virus in dogs referred to the Veterinary Teaching Clinic at Shiraz University.

MATERIALS AND METHODS

A convenient sample of 182 dogs referred to the Veterinary Teaching Clinic at Shiraz University was included in the study during October 2009 to September 2010. They were referred for various reasons such as routine check-up, vaccination or disease presence.

Only dogs older than 6 months were included in the study. Complete history including age, breed, gender,

ISSN: 1814-8085 / E-ISSN: 1927-5129/12

any clinical signs especially respiratory signs, like sneezing or coughing and gastrointestinal signs like vomiting or diarrhoea, type of housing (indoor, in home yard, shelter), feeding (cooked, raw chicken or both), presence of another dog and whether they were symptomatic or not were recorded. A physical examination which included: TPR (temperature, pulse and respiratory rate): assessment of depression or fever; thoracic auscultation; evaluation for coughing and/or nasal discharge including type of nasal discharge (e.g. serous or purulent); palpation of abdomen and checking for signs of vomiting and/or diarrhoea, were performed. A blood sample (4 ml) was obtained *via* cephalic vein puncture and placed in serum tubes. The serum was harvested and frozen at -20^{o}C. Samples were brought to room temperature prior to use. Positive samples for avian influenza virus antibodies (by using ELISA assays) [18] were analyzed for H9N2-specific antibodies using HI assays. For this purpose, 1% poultry RBC was used and the treatment protocol for dog serums was: suspension of dog serum with 1% chicken RBC (and 30 min at 37°C incubation and centrifuge 1500 rpm for 10 min) was performed and the fluid was used for examination. Serums with titer≥ 32 for H9N2 were considered positive [19].

Statistical analysis was conducted using SPSS 11.5 software. Seroprevalence of infection was estimated with 95% confidence interval. Comparison of categorical and continuous variables between positive and negative dogs was performed by Chi-square and two independent samples t-test, respectively. Due to non-normal distribution of the age of animals, statistical comparison was done after logarithmic transformation of data. In all analysis, a P-value less than 0.05 was considered as statistically significant.

RESULTS

Of the 182 samples collected, 82 were positive for antibodies to AI virus [18]. Sixty seven samples or 81.7

Table 1: Distribution of Categorical Risk Factors for H9N2 Influenza Seroprevalence (By HI) in 182 Dogs Referred to Veterinary Clinic of Shiraz University (2009-2010)

Risk factors	Positive (n=67)		Negative (n=115)		P-value
	N	%	N	%	
Sex					
Male	38	34.5	72	65.5	0.43
Female	29	40.2	43	59.8	
Breed					
Pure	41	34.7	77	65.3	0.43
Mixed	26	40.6	38	59.4	
Keeping status					
Indoor	10	40	15	60	0.93
Yard	48	36.4	84	63.6	
Shelter	9	36	16	64	
Presence of other dogs					
Yes	33	32	70	68	0.13
No	34	43	45	57	
Type of food					
Cooked	45	35.4	82	64.6	0.56
Raw or cooked	22	40	33	60	
General signs and symptoms					
No	51	33.8	100	66.2	0.06
Yes	16	51.6	15	48.4	
Gastrointestinal symptoms					
No	62	38	101	62	0.32
Yes	5	26.3	14	73.7	
Respiratory symptoms					
No	62	36.5	108	63.5	0.71
Yes	5	41.7	7	58.3	

% of ELISA positive cases had titer≥ 32 for H9N2 influenza in the HI test. Frequency distributions of categorical variables based on HI test results are presented in Table **1**.

Among 110 male dogs, 38 (34.5%) were positive compared with 29 (40.2%) out of 72 female dogs (P=0.43). Nearly 35% (41/118) of pure breed and 41 % of mix breeds showed positive results (P=0.43). Regarding the housing status of animals, 40% (10/25) of indoor (pet) dogs and 36.4% (48/132) of yard dogs were positive compared with 36% (9/25) of shelter dogs (P=0.94). Thirty three out of 103 (32%) who were kept with other dog(s) and 34/79 (43%) with no dog had positive titre (P=0.13). Fifty one out of 151 (34%) dogs without general signs (depression and decrease appetite) and 16/31 (52%) with general signs (P=0.06), 62/170 (36.5%) of dogs with no respiratory signs or secretions and 5/12 (42 %) of dogs that had sneezing or coughing and nasal discharge (all of them had serous secretions) (P=0.72), 62/163 (38%) of dogs with no gastrointestinal signs, and 5/19 (26.3 %) of dogs with vomiting or diarrhoea were seropositive (P=0.31). Two dogs had vomiting and diarrhoea; both were found negative for Ab. Among the studied dogs 45/127 (35.4%) and 22/55 (40%) were fed cooked and raw (raw +/- cooked) poultry food, respectively (P=0.56). Mean age of seropositive dogs was 23.5 months compared to 18.4 months for seronegative dogs. The mean rectal temperature of positive and negative dogs was the same (39°C). No statistical difference was observed for temperature (P=0.53) or age (P=0.26) between the positive and negative groups (Table **2**).

DISCUSSION

During the last decade frequent reports of influenza A virus infections in dogs and cats resulted in considerable attention of veterinary practitioners and scientists in the fields of virology and epidemiology. There is no observational study on the epidemiology of H9N2 infections in dogs.

Epidemiological data suggest that H9N2 influenza viruses are prevalent in all continents and show that H9N2 influenza viruses are capable of infecting mammals, including humans [20].

Amirsalehi *et al.* [12] induced avian H9N2 infection by intranasal inoculation in dogs. They showed that avian H9N2 influenza virus subtype isolated from outbreaks in broiler farms can infect dogs and the affected animals shed the virus in the feces and nasal discharges. Therefore , animals in contact with contaminated surfaces can become infected.

Influenza is a relatively new disease and no vaccine was available for any subtype of these viruses in dogs. So dogs were unlikely to have background titres as a result of vaccination. Present study showed sixty seven samples (81.7 %) of ELISA positive cases had titer≥ 32 for H9N2 influenza in HI test. Influenza virus subtype and its prevalence could be different in each country. Seroprevalence of antibodies to the H3N8 virus in dogs in Ontario, Canada were 0.4% [21], whereas Holt *et al*. [19] found 31 out of 74 (42%) dogs from shelters in Philadelphia, USA which were seropositive for H3N8. In South Korea, Lee *et al*. [10] observed avian origin canine infected influenza (H3N2) more significantly in farm dogs than pet dogs (19% vs. 0.5%). In our study, no significant difference was observed between indoor (pet) dogs and shelter dogs.

Age and gender differences as well as the presence of other dogs were not significant. More positive results were found in mix breeds (41 %) than pure dogs (35%), maybe because of more mix breeds with low level of food and hygiene in shelters. But the difference was not significant.

Similarly, Amirsalehy *et al.* [12] found no significant relation between fever and induced infection with H9N2 [12]. Holt *et al*. [19] found no significant difference for nasal discharge, coughing and rectal temperature between seronegative and seropositive (for H3N8) dogs. Although low grade fever, nasal discharge, coughing and sneezing are common clinical signs in

Table 2: Comparison of Continuous Risk Factors for H9N2 Influenza Seroprevalence in 182 Dogs Referred to the Veterinary Clinic at Shiraz University (2009-2010)

	Age[a] (months)					Temperature (ºC)	
	Mean	SD	Median	Min	Max	Mean	SD
Positive (n=67)	23.6	29.9	12	1.5	156	39.0	0.47
Negative (n=115)	19.1	21.9	12	1.5	120	39.1	0.52

[a]Logarithmic transformation was performed for comparison of means.

canine influenza but 20-25% of infected dogs were asymptomatic [22].

In this study, dogs with general signs such as depression and decrease appetite had higher seroprevalence (52%) than dogs without general signs (34%) and the difference was tending to be significant (P=0.06)

The most important part of the epidemiological characteristics of influenza is an interspecies transferring of virus. Before influenza occurrence in dogs, direct interspecies transmission and establishment of host-specific lineages of influenza virus have been reported in domestic poultry, pigs, horses and humans [23]. Dogs can be infected with many strains of influenza virus [19]. Infection of a dog with avian H5N1 (Song *et al*. 2008), H9N2 [12] and H3N2 [6, 10, 11] have been reported in recent years. This raises the possibility of co-infection of dogs with two strains and the generation of novel reassortment viruses. Currently, influenza infections in dogs are not considered to be a zoonotic disease, but due to close contact between dogs and humans or poultry farms, the disease outbreak could be occurred.

The H9N2 infection persists in poultry farms in Iran [24] and some farms have dogs that are fed with dead chickens. Although the role of H9N2 infection in dogs in transmission or epidemiology of this infection in poultry farms is not clear, some consideration especially for farm workers or shelter staff is advised. Influenza virus has been found in faeces [12], aerosol and fomites, including clothing [22]. Staff often do not use or cannot change barrier gowns and gloves after cleaning each cage or handling each animal, so the virus can spread throughout a shelter or poultry farm.

Although, some dogs can shed the virus after infection without concurrent clinical signs of respiratory tract disease [22, 25], isolation of dogs with suspected respiratory tract infections from the general population is recommended [22]. Influenza virus is inactivated on the hands of a person *via* washing with soap and warm water for > 20 seconds or by application of an alcohol-based hand sanitizer. It is inactivated on bedding *via* routine laundering with detergent and can be inactivated on surfaces through the use of many commercially available disinfectants [22]. Although interspecies transmission of influenza viruses between poultry and dogs has not been proven yet, but keeping dogs far away from the aviary, feeding them with healthy cooked or commercial food and good sanitary programs in farms could be eligible.

Based on our data, this was the first survey on H9N2 influenza seropositivity for dogs in Iran. Conducting further studies in this area of research are recommended.

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Received on 28-05-2012 Accepted on 13-06-2012 Published on 02-07-2012

http://dx.doi.org/10.6000/1927-5129.2012.08.02.15