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RESEARCH ARTICLE

Prevalence of Antibodies to H9N2 Avian Influenza Virus in Backyard Chickens around Maharlou Lake in Iran

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ABSTRACT

Backyard chickens play an important role in the epidemiology of H9N2 avian influenza virus infection. Close contact of backyard chickens with migratory birds, especially with aquatic birds, as well as neighboring poultry farms, may pose the risk of transmitting avian influenza virus, but little is known about the disease status of backyard poultry. A H9N2 avian influenza virus seroprevalence survey was carried out in 500 backyard chickens from villages around Maharlou lake in Iran, using the hemagglutination-inhibition (HI) test. The studied backyard chickens had not been previously vaccinated and showed no clinical signs of disease. The overall HI titer and seroprevalence against H9N2 were 7.73 and 81.6%, respectively.

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INTRODUCTION

There is increasing evidence that H9N2 avian influenza viruses are endemic in chickens and other land-based poultry, such as quail, pheasant, chukar and other minor domestic poultry in many Asian and European countries (Guan *et al*., 1999; Cameron *et al*., 2000; Liu *et al*., 2003; Kim *et al*., 2010; Park *et al*., 2011). Avian influenza disease due to H9N2 subtype in poultry during later part of the 1990s has been noticeably increased worldwide. The H9N2 subtype outbreaks occurred in domestic ducks, chickens and turkeys in Germany during 1995 and 1998, in chickens in Italy in 1994 and 1996, in pheasants in Ireland in 1997, ostriches in South Africa in 1995, turkeys in the USA in 1995 and 1996 and in chickens in Korea in 1996 (Naeem *et al*., 1999; Bano *et al*., 2003; Capua and Alexander, 2004). More recently, H9N2 viruses have been reported in Middle Eastern countries and have been responsible for widespread and serious disease problems in commercial chickens in Iran, Pakistan, Saudi Arabia and United Arab Emirates (Naeem *et al*., 1999; Banks *et al*., 2000; Pourbakhsh *et al*., 2000; Nili and Asasi, 2002; Vasfimarandi and Bozorgmehrifard, 2002; Alexander, 2003; Nili and Asasi, 2003; Capua and Alexander, 2004; Aamir *et al*., 2007; Naeem *et al*., 2007). In 1998 an outbreak of low pathogenic avian influenza virus (H9N2 subtype) has occurred in Iranian poultry industry (Pourbakhsh *et al*., 2000; Vasfimarandi and Bozorgmehrifard, 2002; Nili and Asasi, 2002, 2003). Backyard (village) chickens throughout the world especially in Middle East countries play an important role in people nutrition due to meat and egg production. Maharlou lake has been situated at the Southeast of Iran. Numerous and different types of birds (especially water-birds) migrate to this lake and resident around it for several months a year. As village chickens have more contacts with these birds, they may play a role in transmission and amplifying the virulence of the field viruses. Therefore, village chickens are probably very important for survival of AIV in the environment and may also play an important role in the spread of the virus among industrial poultry flocks, but little is known about the disease status of backyard poultry. The aim of this study was to record serological evidence of exposure of the village chickens around Maharlou lake to H9N2 avian influenza virus by hemagglutination inhibition test.

MATERIALS AND METHODS

Serum samples and HI assay

A total of 500 blood samples were randomly collected from the wing vein of backyard chickens (unvaccinated, mature and healthy chickens) belonging to five villages around Maharlou lake. Total population of backyard chickens in these five villages, were about five thousands. Samples were taken from 100 backyard chickens in each village, then centrifuged and frozen at -20°C before being submitted to the laboratory. Antibodies against H9N2 avian influenza virus (A/chicken/Iran/772/99 (H9N2)) present in the serum were evaluated

using the hemagglutination inhibition (HI) assay. The HI assay was performed using 96 'U'-well microtiter plates, doubling dilution in PBS, 1 % v/v red blood cells (RBC), and 4 HA units of AIV antigen (Alexander *et al*., 1983).

Statistical analysis

Data from each sampling stage for HI titer were analyzed statistically by one-way analysis of variance (ANOVA). While to know the difference of seroprevalence among village, Chi-square test was applied.

RESULTS

Results of the investigation revealed that all five villages had chickens that were positive for antibodies to H9N2 avian influenza virus. Samples were considered negative if titers were ≤8. Positive flocks had at least one serum sample with titer >8 or at least 3/15 with titer = 8. The mean antibody titer of H9N2 avian influenza virus in backyard chickens sera was 7.5, 6.9, 8.2, 7.47, 8.59 and the seroprevalence was found 83, 76, 84, 78 and 87% in five villages (Table 1). The overall antibody titer and seroprevalence of H9N2 avian influenza virus were recorded 7.73 and 81.6%, respectively. No significant variation (P>0.05) in H9N2 avian influenza virus antibody titer and seroprevalence were found among the five villages. In spite of presence of high antibody titers among chickens in each group, no clinical symptoms were observed.

Table 1: Seroprevalence of H9N2 in backyard chickens in Iran

Village #	Seroprevalence	
	Positive #	%
1	83	83
2	76	76
3	84	84
4	78	78
5	87	87
χ^2 Value	0.550	
df	4	
P Value	0.968	

From each village 100 samples were collected.

DISCUSSION

In the present study, in each village the H9N2 AIV antibody titer was found in the range of zero to 10 $\log_2$ HI (P<0.05). This could be due to non-intensive rearing system in village chickens that resulting in different stages of infection in these chickens. The absence of clinical signs of influenza in backyard chickens, in spite of high antibody titers in some birds, could be due to persistent exposure and acquired resistance of these birds to influenza virus in the environment, and therefore, these birds would be naturally vaccinated against this virus. Van Kammen *et al*. (1982) reported influenza antibodies in sera of free village fowls. Cheng *et al*. (2002) was found H9N2 avian influenza antibody titer in 26% of human sera and only in 7% of chicken sera and concluded that the human H9N2 virus infection probably derived from chicken H9N2 virus. An investigation was undertaken by Naeem *et al*. (2003) in selected broiler-breeder, broiler and layer flocks, nine H9N2 AIV isolates were recovered. Serological data from this investigation indicated that chickens in flocks with a previous history of respiratory tract infection and some without overt clinical respiratory signs had seroconverted to H9N2 AIV. In Hong Kong during 2001-2003, H9N2 avian influenza virus has highest prevalence among live poultry market (Choi *et al*., 2004). Another study was conducted by Li *et al*. (2004) anti-H9N2 antibody was found in 12.8% of the chickens and 5.1% of the poultry farm workers. Al-Natour *et al*. (2005) reported that the seroprevalence of avian influenza was 71% among broiler-breeder flocks in Jordan. The number of positive sera correlated with flock size and in farms located within the migratory route of migratory wild fowl.

Further study was conducted by Nooruddin *et al*. (2006) in Bangladesh, the overall seroprevalence of avian influenza was recorded 9.82%. In molecular identification of agents causing respiratory infections in chickens from southern region of Pakistan, out of 50 tracheal samples, 28 samples were positive for AIV and serotyping of these isolate showed that 6 samples were positive for H9 subtype (Ahmed *et al*., 2009). In the study areas, the backyard chickens were reared under semi-scavenging system and were allowed to scavenge with ducks in the yard, in the crop fields near to water reservoirs where domestic ducks, wild ducks and migratory birds used to scavenge over there. This factor may contribute in natural infection to the backyard chickens (Alexander, 2003; De Marco *et al*., 2003; Senne *et al*., 2003; Vander *et al*., 2003; Capua and Alexander, 2004).

Conclusions

Higher seroprevalence rate were observed in the present study than the other study showed the close and frequent contact of village chickens with numerous and different types of migratory water-birds in the survey region. According to the results of this study, H9N2 avian influenza virus was endemic in backyard chickens of Iran with various degrees of infection and these birds can be asymptomatic carriers of influenza virus.

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