

Serological Evidence of Inter-Species Transmission of H9N2 Avian Influenza Virus in Poultry, Iran

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Abstract: Ducks and in-contact backyard chickens on 20 smallholder backyard farms in 4 districts of Shiraz, Southwest of Iran, were monitored for antibodies against H9N2 avian influenza virus using hemagglutination-inhibition (HI) test. A total of 200 unvaccinated ducks and backyard chickens were sampled. The mean HI titers and seroprevalence in ducks and backyard chickens were 8.3, 5.7 and 78.4, 62.9%, respectively. Results of this study revealed that the Scavenging ducks are the natural reservoir of avian influenza viruses and play an important role in the epidemiology of H9N2 avian influenza virus infection.

Key words: Backyard chickens, ducks, H9N2, transmission

INTRODUCTION

In 1998 an outbreak caused by a low pathogenicity avian influenza virus (LPAIV, H9N2 subtype) occurred in the Iranian poultry industry (Nili and Asasi, 2002, 2003). Backyard chickens and aquatic birds such as ducks play an important role in dissemination of LPAIV viruses to neighboring poultry farms. Low pathogenic AIV (LPAIV) are widely distributed in wild avian species around the world. They have been most frequently identified in waterbirds of the orders Anseriformes (including ducks, geese and swans) and Charadriiformes (particularly gulls and terns) (Fereidouni *et al.*, 2010). Waterfowl are the natural reservoir of avian influenza viruses (Webster *et al.*, 1992), and experimental research indicates that ducks may play a role in the maintenance of LPAI (H9N2) viruses. Infected ducks may exhibit no clinical signs yet can excrete high concentrations of virus that are pathogenic to other poultry species. It is now well recognized that global influenza virus surveillance in wild birds is important in understanding the role of wild birds in the epidemiology and ecology of these viruses (Sturm-Ramirez *et al.*, 2005, Keawcharoen *et al.*, 2008). In particular North American and European countries gathered massive epidemiological information regarding circulation of AIV in wild birds. Yet, little is known about the prevalence of AIV in wild birds in West and Central Asian countries and the Middle East (Fereidouni *et al.*, 2010). Backyard (village) chickens throughout the world, especially in Middle Eastern countries, play an important role in people nutrition due to meat and egg production, but little is known about the disease status of backyard chickens in Iran.

Possible risk factors for LPAI spread in Iran include duck movements, contacts between ducks and other poultry and animal species, poor poultry husbandry,

inadequate handling of sick and dead ducks and backyard chickens by flock owners, and poor awareness of control strategies among poultry farmers. However, no analytical study assessing risk factors for LPAI infection has been conducted in Iran. As in many other Asian countries, domestic ducks on smallholder farms in Iran are allowed to scavenge freely during the day around houses, in the villages, duck owners supply little or no feed (Henning *et al.*, 2010). To assess the hypothesis that ducks contribute to the maintenance and transmission of avian influenza (H9N2) viruses, we conducted a longitudinal investigation describing temporal patterns of antibodies against LPAI (H9) and virus prevalence in unvaccinated scavenging ducks and chickens that have contact with these ducks (in-contact chickens) in Shiraz, Iran. This study provides more informations about the serological status of ducks and backyard chickens against LPAI in West and Central Asia and the Middle East.

MATERIALS AND METHODS

Serum samples and HI assay: During April 2010 to October 2010, Ducks and in-contact chickens on 20 smallholder backyard farms in 4 districts of Shiraz, Southwest of Iran, were monitored for antibodies against H9N2 avian influenza virus using Hemagglutination-Inhibition (HI) test. A total of 200 serum samples were randomly collected from the wing vein of ducks and backyard chickens (unvaccinated, mature, and healthy birds) and stored at -20°C until tested. Serum samples were tested in veterinary research laboratory in Shiraz for antibodies to H9N2 avian influenza virus (A/chicken/Iran/772/99(H9N2)) by using the hemagglutination inhibition (HI) test according to methods recommended by the World Organisation for Animal Health (2009).

RESULTS AND DISCUSSION

In all studied farms the birds were clinically normal and unvaccinated against AIV. 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 (Nooruddin *et al.*, 2006). Results revealed that all flocks had birds that were positive for antibodies to H9N2 avian influenza virus. The mean antibody titers were 8.3, 5.7 \log_2 and the seroprevalences were found to be 78.4 and 62.9% in ducks and backyard chickens respectively ("data not shown"). The results were statistically analyzed by one-way analysis of variance. The mean HI titers and seroprevalence between ducks and backyard chickens in each farm were different significantly ($p < 0.05$). No significant variation ($p > 0.05$) in H9N2 avian influenza virus antibody titer or seroprevalence of H9N2 AIV were found among the 20 farms, although within each farms, significant variation ($p < 0.05$) was observed among individuals. The important role of waterbirds, especially waterfowl, as a reservoir for avian influenza viruses of all subtypes is well known from intensive investigations from many regions of the world (Alexander, 2003; De Marco *et al.*, 2003; Gaidet *et al.*, 2007). Avian influenza monitoring of wild birds in natural habitats and in areas at risk of transmission between domestic poultry and wild birds will increase the knowledge of epidemiology, ecology and genetic relationships of AIV infections. This knowledge will facilitate risk assessments concerning poultry and wild bird populations and provides information on currently circulating AIV which might also have the potential to become important for human health. However, little information is available about the circulation of influenza viruses in waterbirds in West and Central Asia and in the Middle East (Fereidouni *et al.*, 2010). Scavenging duck farming has been proposed as an important contributor to LPAI in poultry flocks in Southeast Asia. One explanation for the higher seroprevalence in ducks than in chickens is that LPAI (H9N2) virus circulated more successfully among ducks than among in-contact chickens; hence, ducks were more likely to harbor and transmit the virus. Another possible explanation for the difference in seroprevalences between poultry species is that duck flocks were exposed to LPAI more frequently than were chickens. In the study conducted by Fereidouni *et al.* (2010), 48.5% of serum samples of waterbirds were positive to LPAIV antibodies. Ducks including Mallard, Common Teal, Common Pochard, Northern Shoveler and Eurasian Wigeon revealed the highest antibody prevalence ranging from 44 to 75%. In the seromonitoring of H9N2 avian influenza virus in backyard chickens around the Caspian sea in Iran,

the seroprevalence of this virus was 72.98% (Hadipour, 2010). In the present study, 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) showed influenza A antibodies in sera of free-range village fowls. Cheng *et al.* (2002) found H9N2 avian influenza antibody titers in 26% of human sera and only in 7% of chicken sera, and concluded that human H9N2 virus infection would probably derived from chicken H9N2 virus. An investigation was undertaken by Naeem *et al.* (2003) in selected broiler-breeder, broiler and layer flocks, from which nine H9N2 AIV isolates were recovered. Serological data from this investigation indicated that both chickens in flocks with a previous history of respiratory tract infection and some without overt clinical respiratory signs had seroconverted. In another study conducted by Li *et al.* (2004), anti-H9N2 antibodies were found in 12.8% of the chickens and 5.1% of the poultry-farm workers. In Hong Kong during 2001-2003, the H9N2 avian influenza virus had the highest prevalence among live poultry markets (Choi *et al.*, 2004). 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 was correlated with flock size and to farms located within the migratory route of migratory wild fowl. In another study conducted by Nooruddin *et al.* (2006) in Bangladesh, an overall 9.82% seroprevalence of avian influenza was recorded. In our study, the backyard chickens in the studied areas were reared under semi-scavenging system and were allowed to scavenge with ducks in the yard, in the crop fields near water reservoirs, where there were domestic ducks, wild ducks, and migratory birds, and this may have contributed to the natural infection of the backyard chickens (Alexander, 2003; De Marco *et al.*, 2003; Senne *et al.*, 2003; Vander *et al.*, 2003; Capua and Alexander, 2004). In a survey during 1999-2000 in Northern Europe, 2.6% of wild ducks and 1.4% of wild geese were positive in rRT-PCR (Fouchier *et al.*, 2003). In more recent monitoring studies of wild birds during 2003-2005 in Italy, 5.1% of Anseriformes were positive in rRT-PCR (Cattoli *et al.*, 2007). Also, in an AIV screening in 2005 in Norway, 13.2% of Anseriformes were positive in rRT-PCR (Jonassen and Handeland, 2007). The prevalence of LPAIV in wild bird in Alaska and Canada seems to be more variable (Ip *et al.*, 2008; Parmley *et al.*, 2008).

CONCLUSION

According to the results of the present study, H9N2 avian influenza virus is endemic in smallholder backyard farm of Iran especially in scavenging ducks and backyard chickens, so these birds can be asymptomatic carriers of the influenza virus. In summary, Scavenging ducks are the natural reservoir of avian influenza viruses and play an important role in the epidemiology of H9N2 avian influenza virus infection. Close contact of these birds with backyard chickens may pose the risk of transmitting and distribution of avian influenza virus in the environment. The results of the current investigation provide important information about the prevalence of LPAIV in wild birds in Iran. Further studies are needed on the management of backyard chicken flocks and their contact with scavenging ducks and LPAI transmission pathways between different poultry species.

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