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Serological Evaluation for Supporting the Potential Role of House Sparrows in LPAIV (H9N2) Transmission

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Abstract: House sparrows (Passer domesticus) are abundant and widespread species of birds within the order Passeriformes and may contribute to AIV transmission and maintenance in nature. This study was carried out to find the role of house sparrows in the spread of low-pathogenicity avian influenza viruses as carrier birds. For this reason seroprevalence survey was carried out in two-hundred house sparrows, using the hemagglutination-inhibition (HI) test. The studied sparrows did not show any clinical signs of disease. The Overall HI titer and seroprevalence against H9N2 were 7.14 and 76%, respectively. Results of this study revealed that house sparrows showed ability to be infected with H9N2 avian influenza virus and play an important role in spreading of AIV as natural carriers.

Key words: H9N2, Influenza, Serology, House sparrows

INTRODUCTION

Avian influenza viruses (AIV) (family Orthomyxoviridae, genus Influenzavirus A) are an important cause of large scale economic losses in the poultry industry, as well as disease in humans (Spickler *et al*., 2008). Many species of domesticated or wild birds can be infected with AI virus (Astorga *et al*., 1994). Both Low Pathogenicity Avian Influenza Viruses (LPAIV) and High Pathogenicity Avian Influenza Viruses (HPAIV) are shed in the excrement of infected birds, and some AIV remain viable in water for relatively long periods of time (Stallknecht *et al*., 1990; Brown *et al*., 2007). In particular, members of the order Anseriformes (i.e., ducks, swans, geese) and Charadriiformes (i.e., shore birds, gulls, terns) represent a significant reservoir of influenza A viruses in nature and are often the focus of research and surveillance (Webster *et al*., 1992; Olsen *et al*., 2006; Munster *et al*., 2007). However, additional free-ranging bird species may contribute to AIV transmission and maintenance in nature (Stallknecht and Shane, 1988).

Some bird species within the order Passeriformes are ubiquitous and abundant throughout the world and often occupy a variety of habitats that overlap with both rural and residential areas. AIV, including some HPAIV, have been isolated or detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in samples originating from free-living passerines of at least 24 species, although these detections are relatively rare (Lipkind *et al*., 1979; Boudreault *et al*., 1980; Roy *et al*., 1983; Nestorowicz *et al*., 1987; Mase *et al*., 2005; Peterson *et al*., 2008). Surveys in the USA, Canada, Egypt, Hungary, and Slovakia suggest that numerous free ranging passerines become infected with LPAIV, and therefore could potentially play a role in transmission and spread (Romvary *et al*., 1976, Stallknecht and Shane, 1988; Johnson *et al*., 1977; Amin *et al*., 1980; Boudreault *et al*., 1980; Al-Attar *et al*., 2008; Fuller *et al*., 2010).

Numerous passerine species, such as the house sparrow (Passer domesticus) and European starling (Sturnus vulgaris), commonly intermingle with domestic game birds and poultry. For proper risk assessments to be made, a better understanding of the interface between wild and domestic birds and potential AIV transmission between these groups is needed (Olsen *et al*., 2006). Passerines especially sparrows are abundant, widespread, and commonly come into contact with free-ranging birds as well as captive game birds and poultry (Webster *et al*., 1992; Olsen *et al*., 2006; Munster *et al*., 2007). Close contact of sparrows with migratory birds, backyard chickes and neighboring poultry farms, may pose the risk of transmitting avian influenza virus, however, little is known about the role of sparrows in avian influenza virus ecology. Furthermore, knowledge of shedding and seroconversion among passerine birds is necessary to understand the potential role of passerines for AIV transmission to domestic bird flocks and/or free-ranging birds. The major objective of the present study was to delineate the potential role of passerines in LPAIV transmission ecology based on the measurement of antibody titers using the haemagglutination-inhibition assay.

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MATERIALS AND METHODS

Sample collection: Two-hundred house sparrows (Passer domesticus) were captured from Shiraz city, Southwestern Iran in August 2010. The birds were transported by vehicle to the animal house in the razi veterinary research laboratory in Shiraz. No clinical illness was observed among sparrows. Following arrival, birds were bled via jugular venipuncture (1 to 2 mL). Blood samples were maintained at room temperature and allowed to clot, then centrifuged for serum separation. Sera were separated and stored at -20ºC until used (Khawaja *et al*., 2005).

Serology: Haemagglutination-Inhibition (HI) test was performed as described in the Office International des Epizooties (OIE, 2000), using reference antigen for AIV H9 subtype (A/Chicken/Iran/772/99(H9N2)). 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 (Hadipour, 2010).

RESULTS AND DISCUSSION

Samples were considered negative if titers were ≤8. Positive samples had titers > 8 (Hadipour, 2010). Results revealed that all birds were positive for antibodies against H9N2 avian influenza virus. H9N2 AIV antibody titers between 3 to 10 $\log_2$ HI were found in examined samples. The overall antibody titer and seroprevalence of H9N2 avian influenza virus recorded were 7.14 and 76%, respectively. In spite of presence of high antibody titers in sparrows, no clinical symptoms were observed. The range of H9N2 AIV antibody titers in examined samples may be explained to the free-ranging properties of house sparrows which are ubiquitous throughout the world and often occupy a variety of habitats or due to its long period contact with other wild birds, so these properties may contribute to AIV transmission and maintenance in nature (Stallknecht and Shane, 1988; Al-Attar *et al*., 2008). The absence of clinical signs of influenza in sparrows, 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 and play an important role in spreading of AIV as natural carriers. Al-Attar *et al*. (2008) in the study about serological status of wild pigeons and starlings to avian influenza virus, reported that the percentage of positive serum antibody titers in pigeons against H9N2 AIV was 81.82 and 50% with ELISA and HI tests, respectively, but all serum samples of starlings showed negative results by ELISA and HI tests. In another study, after inoculation of house sparrows and european starlings with low-pathogenicity avian influenza virus, cloacal shedding was rare in both species. Infectious LPAIV was cultured from oropharyngeal and cloacal swabs and gastrointestinal and respiratory tissues from both species. Seroconversion was detected as early as 3 days post inoculation (16.7% of sparrows and 0% of starlings); 50% of these individuals seroconverted by 5 d.p.i., and nearly all birds (97%) seroconverted by 28 d.p.i. (Nemeth *et al*., 2010). The susceptibility of both house sparrows and european starlings to intranasal, oropharyngeal and ocular inoculation with wild-bird-origin LPAIV in the study conducted by Nemeth *et al*. (2010) suggests that these birds could become infected through contact with actively shedding waterfowl. These results also suggest that both species may be capable of transmitting LPAIV through respiratory and oropharyngeal secretions. In contrast to oropharyngeal shedding, cloacal shedding of LPAIV H3N8 among sparrows and starlings was minimal. This could be due to differing pathogenesis in passerines versus ducks or chickens, in which cloacal shedding appears to be more common than oropharyngeal shedding (Lu and Castro, 2004; Mundt *et al*., 2009; Jourdain *et al*., 2010). Serologic responses following LPAIV infection vary by host species and depend upon the origin, dose and subtype of the infecting virus (Kida *et al*., 1980; Lu and Castro, 2004; Mundt *et al*., 2009). Antibodies to AIV have been detected in free-ranging passerines, including house sparrows in the vicinity of an AIV outbreak in poultry (Nestorowicz *et al*., 1987). Nemeth *et al*. (2010) in their study reported that nearly all birds seroconverted within 7 days of inoculation and antibody levels remained relatively constant over 4 weeks.

These results suggest that passerines could be useful in serosurveillance for recent AIV activity. In Seroprevalence Survey of H9N2 avian influenza virus in backyard chickens around the Caspian sea in Iran, The overall HI antibody titer and seroprevalence against H9N2 were 6.52 and 72.98%, respectively (Hadipour, 2010). Nooruddin *et al*. (2006) reported that the overall seroprevalence of avian influenza in native chicken in Bangladesh was 9.82%. 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 surveys listed by Stallknecht and Shane (1988) a total of 21,318 samples from all species resulted in the isolation of 2317 (10.9%) viruses. Of these samples 14,303 were from birds of the Order Anseriformes and yielded 2173 (15.2%) isolates. The next highest isolation rates were 2.9 and 2.2% from the Passeriformes and Charadriiformes, respectively and the overall isolation rate from all birds other than ducks and geese was 2.1%. Data from the 3-year study by Hinshaw *et al*. (1980) on ducks congregating on lakes in Alberta, Canada prior to their southern migration showed that influenza virus isolation rates from juvenile ducks may exceed 60%. The complex ecology of AIV has direct impacts on both

human and animal health, and these impacts are dynamic and often unpredictable. Therefore, it is necessary to evaluate a wide spectrum of species for potential involvement in virus transmission and maintenance, as well as for susceptibility to disease. Further, it is important to evaluate the consequences of infection with AIV strains originating from both poultry and wild birds (Nemeth *et al*., 2010). While the majority of LPAIV isolates from free ranging birds have originated from waterfowl (Stallknecht and Shane, 1988), both LPAIV and HPAIV have been isolated from passerine birds throughout a wide geographic area encompassing portions of Europe, Australia, North America, Africa, Asia, and the Middle East (Romvary *et al*., 1976, in Stallknecht and Shane, 1988; Lipkind *et al*., 1979; Amin *et al*., 1980; Boudreault *et al*., 1980; Boudreault and LeComte, 1981; Nestorowicz *et al*., 1987; Kwon *et al*., 2005; Gronesova *et al*., 2008; Desvaux *et al*., 2009). In addition, cloacal swabs collected from 22 passerine species throughout the USA from 2005 to 2008 were AIV-positive by RT-PCR, with an approximate prevalence of 1% among passerines (Fuller *et al*., 2010). These peridomestic, free-living passerines have frequent contact with other wild birds as well as domestic poultry and game birds (Stallknecht and Shane, 1988), thereby creating opportunities for pathogen transmission. Shared food, substrate, and water sources probably involve cross-species virus exposure originating from faeces, oropharyngeal secretions and possibly aerosolized droplets. Epidemiological studies have implicated poultry in the transmission of LPAIV to free-ranging birds, and vice versa (Nestorowicz *et al*., 1987). Repeated LPAIV infections of multiple host species as well as same-host co-infections that may result from this close relationship between domestic and free-ranging birds could increase the exchange of viral genetic material, and therefore the likelihood of increasingly virulent reassortant viruses (Campitelli *et al*., 2008; Moon *et al*., 2010). Future studies of passerines with LPAIV, including strains of poultry-origin and poultry-adapted viruses, would further expand the present knowledge on their role in AIV ecology, while passerines in nature should be monitored to better understand their involvement in natural transmission dynamics and potential contribution to genetic recombination events (Roy *et al*., 1983).

CONCLUSION

House sparrows readily and consistently seroconverted to LPAIV, and therefore inclusion of these birds in epidemiological studies of influenza outbreaks in wildlife and domestic animals may provide further insight into the potential involvement of passerines in avian influenza virus transmission ecology. The results of the present study indicates that the house sparrows may play an important role in spreading of AIV as natural carriers like other wild birds.

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