

## Surveillance of Scavenging Ducks for Low-Pathogenicity (H9N2) Avian Influenza Virus

M.M. Hadipour, M.R. Hadipourfard, N. Shayanpour, M. Fakhrabadipour and F. Azad  
Department of Veterinary Clinical Sciences,  
Kazerun Branch, Islamic Azad University, Kazerun, Iran

---

**Abstract:** Scavenging ducks play an important role in the epidemiology of H9N2 avian influenza virus infection but little is known about the serological status of infection in these birds in Iran. Serological studies were carried out to determine the status of infection with H9N2 avian influenza virus in scavenging ducks. Samples were collected from 400 birds from 4 different locations (North, South, East and West) in Iran and evaluated by using the Hemagglutination-Inhibition (HI) test. The studied ducks had not been previously vaccinated and showed no clinical signs of disease. The overall HI titer and seroprevalence of H9N2 were 7.9 and 80.92%, respectively. Results of this investigation provide important information about the prevalence of LPAIV in scavenging ducks in Iran especially wetlands which represent an important wintering site for migratory water birds.

**Key words:** Scavenging ducks, surveillance, H9N2, influenza, birds, Iran

---

### INTRODUCTION

Wild waterbirds are considered the main reservoir of all subtypes of Avian Influenza Viruses (AIV). 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). These viruses replicate in epithelial cells of the respiratory and intestinal tracts of birds and are excreted in high concentrations in their faeces (Alexander, 2000; Ellstrom *et al.*, 2008; Fereidouni *et al.*, 2010). When surveillance of commercial ducks has been undertaken, enormous pools of virus and many subtype combinations have been detected especially from meat birds which are usually fattened on open fields (Fereidouni *et al.*, 2010). For example, Alexander (2000) reported the isolation of 32 viruses from 60 pools of cloacal swabs taken from ducks at slaughter. Studies in Hong Kong in the late 1970s and early 1980s isolating virus from carcasses at duck dressing plants or on duck farms indicated about 6% of the ducks were infected with influenza viruses of various subtypes (Shortridge, 1982). Most of the evidence obtained on the prevalence of influenza in different types of poultry and different geographical locations supports the view that the primary introduction is from feral birds (Fouchier *et al.*, 2003; Jonassen and Handeland, 2007). So

that influenza viruses are most likely to infect poultry reared in a way that allows contact with feral birds such as fattening ducks reared on fields or ponds or turkeys and ostriches reared on range especially when these are also situated on migratory waterfowl routes and far less likely to occur in poultry reared in bird-proof confinement. This understanding also allows strategies for the prevention of introduction to poultry to be developed (Keawcharoen *et al.*, 2008). Scavenging ducks throughout the world especially in Middle Eastern countries play an important role in people nutrition due to meat and egg production. However, in many countries practices likely to encourage wild birds to poultry farms such as surface storage of drinking water, rearing mixed species on the same farm, failure to bird-proof food stores and even the construction of artificial ponds to attract waterfowl are still pursued. When influenza viruses do move from feral birds to poultry, they may spread from flock to flock and farm to farm by a number of methods. Primarily, these consist of the mechanical transfer of infective faeces from infected to susceptible birds and inevitably there is human involvement in this transfer. Prevention of secondary spread after an initial outbreak can be achieved by good biosecurity procedures especially control of movements of personnel and equipment to and from the premises. Where such practices are not enforced widespread distribution of the virus may occur with associated disease and economic losses (Alexander, 2000;

Henning *et al.*, 2010). Recent demand for increased understanding of avian influenza virus in its natural hosts together with the development of high-throughput diagnostics has heralded a new era in wildlife disease surveillance. The influenza status of commercial ducks in most countries is poorly understood or has not been investigated so the aim of this study was to serological evaluation of LPAIV H9N2 in scavenging ducks in Iran using the hemagglutination inhibition test.

## MATERIALS AND METHODS

**Sample collection and HI assay:** A total of 400 blood samples were randomly collected from the brachial vein of scavenging ducks (unvaccinated, mature and healthy) belonging to 4 different locations of Iran. Samples were centrifuged and frozen at -20°C before being submitted to the laboratory. HA/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 HA titer of AIV H9 subtype antigen was calculated as the average of two dilutions 1:2 and 1:3. The HI test was performed using the above-titrated reference antigen and positive control antisera against AIV H9 subtype and a negative control serum. The maximum dilution of each serum sample causing inhibition of hemagglutination was used as endpoint. The HI titer of each serum sample was expressed as reciprocal of the serum dilution (top to bottom).

## RESULTS

In all studied regions, ducks had not been previously vaccinated and showed no clinical signs of disease. HI titers  $\geq 8$  were considered positive (Nooruddin *et al.*, 2006). Results revealed that all regions had birds that were positive for antibodies against H9N2 avian influenza virus. The mean antibody titers in 4 locations were 9.1, 8.6, 6.7, 7.2 and seroprevalences were 87.3, 84.2, 73.8, 78.4, respectively. The overall HI titer and seroprevalence of AIV H9 subtype antibodies revealed in this study were 7.9 and 80.92%, respectively.

## DISCUSSION

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). After  $\approx 50$  years of research in wild birds, a wide range of Low-Pathogenicity AIV (LPAIV) subtypes is

known to circulate in numerous species (Easterday *et al.*, 1968; Slemons *et al.*, 1974; Suss *et al.*, 1994) and LPAIVs are believed to perpetuate in aquatic bird populations (Webster *et al.*, 1992). 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 (Alexander, 2000). 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). In the current study, North and South regions have highest prevalence of H9N2 influenza virus because in these regions there are seas (Caspian sea and Persian Gulf) and several lakes so these regions are the best places for migratory waterfowl. Scavenging duck farming has been proposed as an important contributor to LPAI in poultry flocks in Southeast Asia. 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). One explanation for the higher seroprevalence in ducks than in chickens is that LPAI (H9N2) virus circulated more successfully among ducks than 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 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 44-75%. 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%. Each year waterfowl congregate in huge flocks usually on lakes before migratory flights are undertaken. Data from the 3 years 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%.

## CONCLUSION

In the present study, the absence of clinical signs of influenza in scavenging ducks in spite of high antibody titers 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.

## REFERENCES

- Alexander, D.J., 2000. A review of avian influenza in different bird species. *Vet. Microbiol.*, 74: 3-13.
- Alexander, D.J., 2003. Report on avian influenza in Eastern hemisphere during 1997-2002. *Avian Dis.*, 47: 792-797.
- De Marco, M.A., G.E. Foni, L. Campitelli, E. Raffini and L. di Trani *et al.*, 2003. Circulation of influenza viruses in wild waterfowl wintering in Italy during the 1993-99 period: Evidence of virus shedding and seroconversion in wild ducks. *Avian Dis.*, 47: 861-866.
- Easterday, B.C., D.O. Trainer, B. Tumova and H.G. Pereira, 1968. Evidence of infection with influenza viruses in migratory waterfowl. *Nature*, 219: 523-524.
- Ellstrom, P., N. Latorre-Margalef, P. Griekspoor, J. Waldenstrom, J. Olofsson, J. Wahlgren and B. Olsen, 2008. Sampling for low-pathogenic avian influenza A virus in wild Mallard ducks: Oropharyngeal versus cloacal swabbing. *Vaccine*, 26: 4414-4416.
- Fereidouni, S.R., O. Werner, E. Starick, M. Beer and T.C. Harder *et al.*, 2010. Avian influenza virus monitoring in wintering waterbirds in Iran, 2003-2007. *Virology*, 7: 43-43.
- Fouchier, R.A., B. Olsen, T.M. Bestebroer, S. Herfst, L. van der Kemp, G.F. Rimmelzwaan and A.D. Osterhaus, 2003. Influenza A virus surveillance in wild birds in Northern Europe in 1999 and 2000. *Avian Dis.*, 47: 857-860.
- Hadipour, M.M., 2010. Seroprevalence survey of H9N2 avian influenza virus in backyard chickens around the Caspian Sea in Iran. *Rev. Bras. Cienc. Avic.*, 12: 53-55.
- Henning, J., H. Wibawa, J. Morton, T.B. Usman, A. Junaidi and J. Meers, 2010. Scavenging ducks and transmission of highly pathogenic avian influenza, Java, Indonesia. *Emerg. Infect. Dis.*, 16: 1244-1250.
- Hinshaw, V.S., R.G. Webster and B. Turner, 1980. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. *Can. J. Microbiol.*, 26: 622-629.
- Jonassen, C.M. and K. Handeland, 2007. Avian influenza virus screening in wild waterfowl in Norway, 2005. *Avian Dis.*, 51: 425-428.
- Keawcharoen, J., D. van Riel, G. van Amerongen, T. Bestebroer and W.E. Beyer *et al.*, 2008. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). *Emerg. Infect. Dis.*, 14: 600-607.
- Nooruddin, G.M., M.T. Hossain, M. Mohammad and M.M. Rahman, 2006. Sero-epidemiology of avian influenza virus in native chicken in Bangladesh. *Int. J. Poult. Sci.*, 5: 1029-1033.
- OIE, 2000. Manual of Standards for Diagnostic Tests and Vaccines. 4th Edn., Office International des Epizooties, Paris.
- Shortridge, K.F., 1982. Avian influenza A viruses of Southern China and Hong Kong: Ecological aspects and implications for man. *Bull. World Health Organ.*, 60: 129-135.
- Slemons, R.D., D.C. Johnson, J.S. Osborn and F. Hayes, 1974. Type-A influenza viruses isolated from wild free-flying ducks in California. *Avian Dis.*, 18: 119-124.
- Stallknecht, D.E. and S.M. Shane, 1988. Host range of avian influenza virus in free-living birds. *Vet. Res. Commun.*, 12: 125-141.
- Suss, J., J. Schafer, H. Sinnecker and R.G. Webster, 1994. Influenza virus subtypes in aquatic birds of eastern Germany. *Arch. Virol.*, 135: 101-114.
- Webster, R.G., W.J. Bean, O.T. Gorman, T.M. Chambers and Y. Kawaoka, 1992. Evolution and ecology of influenza A viruses. *Microbiol. Rev.*, 56: 152-179.