

Short communication

SEROSURVEY OF H9N2 AVIAN INFLUENZA VIRUS
DURING RESPIRATORY DISEASE OUTBREAKS IN
BROILER FLOCKS IN DEZFUL, SOUTHERN IRAN

M. M. HADIPOUR & P. GOLCHIN

Department of Clinical Sciences, School of Veterinary Medicine,
Islamic Azad University, Kazerun Branch, Kazerun, Iran

Summary

Hadipour, M. M. & P. Golchin, 2011. Serosurvey of H9N2 avian influenza virus during respiratory disease outbreaks in broiler flocks in Dezful, southern Iran. *Bulg. J. Vet. Med.*, **14**, No 1, 62–65.

Since 1998, an epidemic of avian influenza occurred in the Iranian poultry industry. The identified agent was a low-pathogenicity H9N2 avian influenza virus that caused frequent episodes of high mortality in broiler chicken farms in Iran and some other Asian countries. This study was designed to investigate the prevalence of AIV H9N2 subtype in commercial chicken flocks in Dezful, southern Iran. Serum samples from 160 broilers (8 broiler flocks) with respiratory symptoms, were examined by hemagglutination inhibition (HI) test for specific antibodies against AIV H9N2 subtype. Overall HI titre and seroprevalence against H9N2 were 7.3 and 75.95%, respectively.

Key words: Avian influenza, broiler chickens, Dezful, H9N2 subtype, prevalence

Avian influenza virus (AIV) has been recognized as one of the most important pathogens in poultry. The H9N2 avian influenza virus (AIV) was reported to be of low pathogenicity in chickens (Alexander, 2003; Bano *et al.*, 2003). The H9N2 subtype was first reported in 1966 in the United States (Homme & Easterday, 1970). Since then, the virus has been isolated in various countries (Alexander, 2003; Senne, 2003). In Middle Eastern countries during 1998–2000, H9N2 viruses were responsible for widespread and serious disease in commercial chickens in Iran (Nili & Asasi, 2002; 2003) Pakistan (Naeem *et al.*, 1999; 2003; 2007), the United Arab Emirates (Man-

vell *et al.*, 2000; Aamir *et al.*, 2007) and Saudi Arabia (Banks *et al.*, 2000). In Iran, the incidence and severity of respiratory disease in commercial chicken flocks have been recently increased due to poultry industry intensification. AIV is believed to be one of the main causes of chicken respiratory diseases in the country as indicated by many field reports (Nili & Asasi, 2002; 2003). Phylogenetic analysis of H9N2 isolates in Pakistan, Iran and Saudi Arabia showed very close relationships, suggesting a common source (Banks *et al.*, 2000).

Numerous infections of poultry and other birds with the subtype H9 during the 1990s originated from separate intro-

ductions from feral birds (Banks *et al.*, 2000; Al-Natour & Abo-Shehada, 2005). Dezful is situated in southern Iran, near to Karoun lake and Persian Gulf and is on the route of migratory wild birds. Local wild and feral birds remain in the area for several months each year. In the area of this study, there are also several backyard chicken flocks. The presence of different bird species in this region and around the broiler farms may result in the risk of transmission of infectious agents, such as avian influenza virus (Hadipour, 2010).

This study was designed to investigate the prevalence of AIV H9 subtype in broiler flocks in the Dezful region, southern Iran, using a serological technique.

During the period from February 2010 to September 2010, 160 commercial broilers from 8 broiler flocks located in Dezful, South of Iran with respiratory disease outbreaks were examined. Each farm owned one flock, and the chickens were of various ages ranging from 20 to 43 days. The birds are usually kept for a production life of 41–45 days, and then sold for slaughter. None of flocks received any of AIV vaccines. The stocking density was 10 birds/m². Before birds were placed, the houses were cleaned, washed, disinfected and provided with new wood shavings. Informed consent was obtained from farms' owners to participate in the study. Blood samples were collected by venipuncture of the brachial vein from 20 birds from each farm in an acute phase of respiratory symptoms. Sera were separated and stored at –20 °C until tested for AIV H9 subtype antibodies.

The HA/HI test was performed as described in by the Office International des Epizooties (OIE, 2000), using a reference antigen for AIV H9 subtype (A/Chicken/Iran/772/99(H9N2)). The HA titre of AIV H9 subtype antigen was calcu-

lated as the average of two dilutions (1:2 and 1:3). The HI test was performed using the above-titrated reference antigen, positive control antisera against AIV H9 subtype and a negative control serum. The maximum dilution of each serum sample causing inhibition of haemagglutination was used as endpoint. The HI titre of each serum sample was expressed as reciprocal of the serum dilution (top to bottom). Samples were considered negative if titres were ≤ 8 . Positive flocks had at least one serum sample with titre > 8 or at least 3/15 with titre 8 (Nooruddin *et al.*, 2006). The results were submitted to Student's *t* test to determine the significance in the antibodies prevalence between broiler flocks.

In the majority of all broiler flocks, chickens suffered from gasping, coughing, conjunctivitis, nasal and ocular discharge, depression, inappetance, weakness and were reluctant to move. The mortality rate was 15–25%. All flocks had chickens positive for antibodies against H9N2 avian influenza virus. Mean antibody titres ranged between 8.3 and 6.4 log₂, and the seroprevalences between 84.3% and 68.2% (Table 1). The overall HI titre and seroprevalence of AIV H9N2 subtype antibodies in this study were 7.3 and 75.95%, respectively. No significant statistically variation in H9N2 AIV antibody titre or seroprevalence were found among the eight flocks.

In the present study, H9N2 AIV antibody titres between 0 to 10 log₂ were found in all flocks. This may be explained by the intensification of broiler flocks which may result in different stages of infection in these chickens. The presence of clinical signs of influenza in broiler flocks associated with high antibody titres in some of non-vaccinated birds, could be due to persistent exposure of broiler flocks to AIV. All commercial broiler farms examined were positive for H9 AIV

subtype. In the serological and molecular assays for detection of avian influenza in domestic pigeons in Kavar area of Iran, 34% of samples had antibody titres $\geq 2^{-5}$ against the H9N2 AI virus (Mohammadi *et al.*, 2010). In another survey of H9N2 avian influenza virus in backyard chickens around the Caspian Sea in Iran, the overall HI titre and seroprevalence against H9N2 were 6.52 and 72.98%, respectively (Hadipour, 2010).

Table 1. Mean HI antibody titres and seroprevalence in eight broiler flocks. Data are presented as mean \pm SEM (n=20)

Flock	Mean HI anti-body titres	Seroprevalence (%)
1	7.8 \pm 0.37	84.30
2	7.3 \pm 0.24	72.25
3	8.3 \pm 0.40	78.40
4	6.5 \pm 0.15	70.13
5	7.7 \pm 0.26	79.50
6	6.4 \pm 0.13	68.20
7	7.5 \pm 0.31	74.23
8	6.9 \pm 0.18	80.60

In virological, molecular and serological studies carried out to determine the status of infections with avian influenza viruses in different wild waterfowl species in Iran during 2003–2007, 48.5% of serum samples were positive by using a nucleoprotein-specific competitive ELISA (NP-C-ELISA). Duck species including mallards, common teals, common pochards, Northern shovelers and Eurasian wigeons revealed the highest antibody prevalence from 44 to 75% (Fereidouni *et al.*, 2010). 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. 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 to farms located within the migratory route of migratory wild fowl. In another study, 54.2% of broiler flocks and 78.3% of layer flocks in Jordan were positive for AIV H9 subtype antibodies (Roussan *et al.*, 2009). Woo *et al.* (2008) reported that the 26% of layers and 23% of broilers in Korea were seropositive against H9N2 AIV. The high seroprevalence of AIV H9 subtype antibodies observed in the current investigation and previously reported by Nili & Asasi (2002, 2003) and Hadipour (2010) suggests the endemic nature of the disease in Iran. The H9N2 virus has been isolated from various avian species in other countries in the region (Banks *et al.*, 2000; Manvell *et al.*, 2000; Aamir *et al.*, 2007; Naeem *et al.*, 2003; Kwon *et al.*, 2006).

In conclusion, it is essential that the biosecurity on poultry farms should be improved to prevent the introduction and dissemination of influenza and other viruses. Furthermore, farmers need to be educated about the signs, lesions, and the importance of this viral infection.

REFERENCES

- Aamir, U. B., U. Wernery & N. Ilyushina, 2007. Characterization of avian H9N2 influenza viruses from United Arab Emirates 2000 to 2003. *Virology*, **361**, 45–55.
- Alexander, D. J., 2003. Report on avian influenza in the Eastern Hemisphere during 1997–2002. *Avian Diseases*, **47**, 792–797.
- Al-Natour, M. Q. & M. N. Abo-Shehada, 2005. Sero-prevalence of avian influenza among broiler-breeder flocks in Jordan. *Preventive Veterinary Medicine*, **70**, 45–50.
- Banks, J., E. C. Specidel & P. A. Harris, 2000. Phylogenetic analysis of influenza A vi-

- ruses of H9 hemagglutinin subtype. *Avian Pathology*, **29**, 353–360.
- Bano, S., K. Naeem & S. A. Malik, 2003. Evaluation of pathogenic potential of avian influenza virus serotype H9N2 in chickens. *Avian Diseases*, **47**, 817–822.
- Fereidouni, S. R., O. Werener, E. Starick, M. Beer & T. C. Harder, 2010. Avian influenza virus monitoring in wintering waterbirds in Iran, 2003–2007. *Virology Journal*, **7**, 43.
- Hadipour, M. M., 2010. Seroprevalence survey of H9N2 avian influenza virus in backyard chickens around the caspian sea in Iran. *Brazilian Journal of Poultry Science*, **12**, 53–55.
- Homme, P. J. & B. C. Easterday, 1970. Avian influenza virus infections. I. Characteristics of influenza A-Turkey-Wisconsin-1966 virus. *Avian Diseases*, **14**, 66–74.
- Kwon, H. J., S. H. Cho, M. C. Kim, Y. J. Ahn & S. J. Kim, 2006. Molecular epizootiology of recurrent low pathogenic avian influenza by H9N2 subtype virus in Korea. *Avian Pathology*, **35**, 309–315.
- Manvell, R. J., P. McKinney, U. Wernery & K. Frost, 2000. Isolation of highly pathogenic influenza A virus of subtype H7N3 from a peregrine falcon (*Falco peregrinus*). *Avian Pathology*, **29**, 635–637.
- Mohammadi, A., M. Masoudian, Y. Nemati & S. Seifi, 2010. Serological and RT-PCR assays for detection of avian influenza of domestic pigeons in Kavar area (Fars province, Iran). *Bulgarian Journal of Veterinary Medicine*, **13**, 117–121.
- Naeem, K., A. Ulah, R. J. Manvell & D. J. Alexander, 1999. Avian influenza subtype H9N2 in poultry in Pakistan. *Veterinary Record*, **145**, 560.
- Naeem, K., M. Naurin & S. Rashid, 2003. Seroprevalence of avian influenza virus and its relationship with increased mortality and decreased egg production. *Avian Pathology*, **32**, 285–289.
- Naeem, K., N. Siddique & M. Ayaz, 2007. Avian influenza in Pakistan: Outbreaks of low- and high-pathogenicity avian influenza in Pakistan during 2003–2006. *Avian Diseases*, **51**, 189–193.
- Nili, H. & K. Asasi, 2002. Natural cases and an experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. *Avian Pathology*, **31**, 247–252.
- Nili, H. & K. Asasi, 2003. Avian influenza (H9N2) outbreak in Iran. *Avian Diseases*, **47**, 828–831.
- Nooruddin, G. M., M. T. Hossain, M. Mohammad & M. M. Rahman, 2006. Seroprevalence of avian influenza virus in native chicken in Bangladesh. *International Journal of Poultry Science*, **5**, 1029–1033.
- Office International des Epizooties, 2000. Manual of Standards for Diagnostic Tests and Vaccines, 4th edn, OIE, Paris.
- Roussan, D. A., Y. Ghassan, G. Y. Khawaldeh, R. H. Al Rifai, W. S. Totanji & I. A. Shaheen, 2009. Avian influenza virus H9 subtype in poultry flocks in Jordan. *Preventive Veterinary Medicine*, **88**, 77–81.
- Senne, D. A., 2003. Avian influenza in western hemisphere including the Pacific Islands and Australia. *Avian Diseases*, **47**, 798–805.
- Woo, J. T. & B. K. Park, 2008. Seroprevalence of low pathogenic avian influenza (H9N2) and associated risk factors in the Gyeonggi-do of Korea during 2005–2006. *Journal of Veterinary Science*, **9**, 161–168.

Paper received 27.10.2010; accepted for publication 03.12.2010

Correspondence

Dr. Mohammad Mehdi Hadipour
Department of Clinical Sciences,
School of Veterinary Medicine,
Islamic Azad University, Kazerun Branch,
Kazerun, Iran
P.O.Box 73135-168; phone: 00989177189086
e-mail: hadipourmm@yahoo.com