

Measurement of Antibodies to Infectious Bronchitis Virus in Indigenous Chicken Flocks Around Maharlou Lake in Iran

M.M. Hadipour, F. Azad, A. Vosoughi, M. Fakhrabadipour and A. Olyaie
Department of Veterinary Clinical Sciences, Kazerun Branch, Islamic Azad University,
Kazerun, Iran

Abstract: To evaluate the seroprevalence of Infectious Bronchitis Virus (IBV) in indigenous chicken flocks, serum samples from 200 mature indigenous chickens in villages around Maharlou Lake in Southwest of Iran were tested for IBV antibodies using commercial IBV Enzyme-Linked Immunosorbent Assay (ELISA). The studied indigenous chickens had not been previously vaccinated and showed no clinical signs of disease. The overall ELISA titer and seroprevalence of IBV antibodies revealed in this study were 1427 and 68%, respectively. The results indicate a relatively high prevalence of IBV in indigenous chicken flocks in Southwest of Iran and necessitate the regular vaccination programme against IB in native flocks.

Key words: ELISA, indigenous chickens, infectious bronchitis, Maharlou lake, Iran

INTRODUCTION

Infectious Bronchitis Virus (IBV), a member of the family Coronaviridae, causes an acute highly contagious disease of chickens resulting in significant economic losses to poultry industry worldwide (El-Houadfi *et al.*, 1986; Mauermans *et al.*, 1987; Parsons *et al.*, 1992; Chen *et al.*, 1996; Gough *et al.*, 1996; Cavanagh and Naqi, 2003). It is an enveloped virus with a diameter of 120 nm (Davies and Macnaughton, 1979). There are more than 20 known serotypes within IBV recognized worldwide (Lee and Jackwood, 2000). It is characterized by respiratory signs which include rales, gasping and sneezing, sometimes accompanied by lacrimation, facial swelling and negligible mortality (Cavanagh and Naqi, 2003; Schalk and Hawn, 1931; Collisson *et al.*, 1992). Mortality may occur in young chicks due to respiratory or kidney manifestations of the infection. The respiratory infection is usually mild and self-limiting in chickens. However, the economic importance of the disease is often complicated by infectious bronchitis virus strains that cause kidney and oviduct damage as well as by secondary bacterial infection (Cavanagh and Naqi, 2003). The disease is of major economic importance because it causes poor weight gain and poor feed conversion efficiency. It is often a component of mixed infections that produce air sacculitis, which may result in meat condemnation during processing and/or losses from production and egg quality. The losses from production inefficiencies are usually of greater concern than losses from mortality (Cavanagh and Naqi, 2003; Emikpe *et al.*,

2010). Although effective vaccines are available and utilized routinely in commercial poultry production, the virus has a tendency of frequent mutations (Wang *et al.*, 1993). The first isolation of IBV in Iran was reported by Aghakhan *et al.* (1994). The isolate showed antigenic relationship to the Mass serotype. Momayez *et al.* (2002) were identified some IBV field strains suggested the presence of IBV variants in Iran. In spite of regular vaccination with Mass strains, IB is still a serious problem in Iran, causing mortality and adverse effects on quantity and quality of egg production as well as renal failure in broilers and layers (Seyfiabadshapouri *et al.*, 2002; Nouri *et al.*, 2003; Haghshenas *et al.*, 2005). An outbreak of severe respiratory disease in both unvaccinated and vaccinated chicken flocks was occurred in January 2000 in Iran (Marandi and Bozorgmehrfard, 2000; Momayez *et al.*, 2002). The disease rapidly spread to other flocks with cardinal signs of respiratory distress and swollen renal with urate deposition or death in broiler chickens and egg production drops and watery albumen in layer birds.

The ELISA assay is a convenient method for monitoring of both the immune status and virus infection in chicken flocks. Several commercial ELISA kits for IBV specific antibodies detection are already available, which used inactivated virions as coating antigen (Zhang *et al.*, 2005; Chen *et al.*, 2003). Poultry happen to be the most numerous species of livestock in Iran. The indigenous chickens constitute over 35% of the Iranian poultry population. Indigenous (village) chickens throughout the world, especially in Middle Eastern countries, play an

important role in people nutrition due to meat and egg production (Hadipour, 2010). Maharlou lake has been situated at the Southwest of Iran. It was believed that the free range chicken act as potential reservoir of infection to themselves and the commercial birds (Adene *et al.*, 1985, Emikpe *et al.*, 2003), but little is known about the disease status of backyard poultry. The aim of this study was to evaluate the IBV exposure of indigenous chickens around Maharlou Lake using the enzyme-linked immunosorbent assay.

MATERIALS AND METHODS

Sample collection: A seroepidemiological investigation was conducted in July 2010 by taking 4 mL blood samples from 200 mature and healthy indigenous chickens from villages around Maharlou Lake at the Southwest of Iran. These chickens, had no history of vaccination against infectious bronchitis virus. All indigenous flocks were contacted through the appropriate veterinarians and all owners agreed to participate in this study. Blood samples were collected by wing venipuncture and transported to the shiraz veterinary research laboratory for serological assay.

Enzyme-Linked Immunosorbent Assay (ELISA): The harvested sera were heat inactivated at 56°C for 30 min. They were then screened with the Indirect Enzyme Linked Immunosorbent Assay (ELISA) for infectious bronchitis virus antibodies. The optimum working dilution for each of the analytes in the test procedure (antigen, serum and conjugate) was determined empirically by a checkerboard titration (Case *et al.*, 1983). The optimum dilutions obtained following the checkerboard titrations were: antigen 1/100, serum 1/50 and conjugate 1/10,000. The antigen used was freeze-dried live attenuated avian infectious bronchitis vaccine (Mass type, H120 strain, Merial Company). A commercial ELISA kit (Flockchek) from IDEXX Laboratories, Inc. USA, was used to determine IBV antibodies in sera of chickens, according to the manufacturer's instruction. Descriptive statistics was used to summarize the data generated from the study. The prevalence was expressed as a percentage in comparison to the total number of chickens screened.

RESULTS AND DISCUSSION

The difference between the positive control mean and the negative control mean was greater than 0.075. The negative control mean absorbance was less than or equal to 0.150. The presence or absence of antibody to IBV was determined by relating the A (650) value of the unknown to the positive control mean. The positive control was standardized and represents significant antibody levels to

IBV in chicken serum. The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. Serum samples with S/P ratios of less than or equal to 0.20 were considered negative. S/P ratios greater than 0.20 (titers greater than 396) were considered positive and indicate exposure to IBV. The studied indigenous chickens did not show any clinical signs of disease. Results revealed that all indigenous flocks had chickens that were positive for antibodies against infectious bronchitis virus. The overall ELISA titer and seroprevalence of IBV antibodies revealed in this study were 1427 and 68%, respectively. Most of the studied indigenous flocks showed high level of antibody titers to IBV by ELISA technique. The results obtained in this study showed that 68% of the indigenous chickens tested were positive for IBV antibodies that is agreement with reports from Jordan where infectious bronchitis virus nucleic acid was detected in 64% of broiler, 53% of layer and 54.54% of broiler breeder flocks affected with respiratory disease (Dergham *et al.*, 2009). In another study in different chicken flocks of Jordan that suffered from respiratory disease 70% of flocks were positive to IBV (Gharaibeh, 2007). Ahmed *et al.* (2007) in the study about seroprevalence of infectious bronchitis virus strains in commercial poultry of Pakistan showed that the 88% of the flocks were seropositive for M-41 antibodies. The high prevalence of IBV antibodies in indigenous chickens in our study may be a result of field infection with IB virus. (Cavanagh and Naqi, 2003). Emikpe *et al.* (2010) reported that the total seroprevalence of IBV in chickens in Southwestern Nigeria was 82.7%. Seroprevalence of IBV among the layers, breeders, growers and local chickens were 91.67, 90.91, 63 and 78.32%, respectively. The prevalence of IB in indigenous chickens of Kano was 91.3% (Oyejide *et al.*, 1988). The prevalence of IB in indigenous chickens in this study was 68%. This was not as high as the result of the study on indigenous chickens from Kano, because although the same serological technique has been used, this variance could be attributed to different climatic conditions that exist in two different regions. Our study area being in the Southwestern Iran with semi-humid climatic condition for a large part of the year while Kano is usually dry and dusty for most parts of the year. A dusty and dry weather will aid the transmission of IB virus, which is usually via aerosol. However, the prevalence in indigenous chickens in the current study was higher compared to the 53.3% reported by Emikpe *et al.* (2003) for IB prevalence in Ibadan. In infectious bronchitis virus surveillance in Ontario commercial layer flocks, was indicated that the farms with previous IB outbreaks could harbor the virus without clinical signs in the flocks. In addition, some farms, although they did not report having IB associated problems, were IBV positive by N-gene RT-PCR

(Stachowiak *et al.*, 2005). In a serological survey using paired sera collected during the period from 1980-1981 in Japan, the roles of IBV and *Mycoplasma gallisepticum* were studied in outbreaks of respiratory diseases of broiler flocks, and 59.2% of IB, 8.3% of Mg and 10.9% of mixed infection with IB and Mg were found in the diseased flocks not vaccinated with IB vaccine, while 36.6% of IB, 13.4% of Mg and 8.9% of mixed infection were diagnosed in vaccinated flocks (Sawaguchi *et al.*, 1983). In serological survey of infectious bronchitis virus in Iran by RT-PCR and type-specific nested PCR, the viral RNA was detected in 42.8% of broiler flocks from different provinces (Seyfiabadshapouri *et al.*, 2004).

CONCLUSION

These findings indicate a high infectious bronchitis virus activity among indigenous chickens of Iran, hence there is an urgent need for the development of prevention and control policies against IB in Iranian poultry farms and a national control programme for infectious bronchitis virus infection should be planned.

ACKNOWLEDGMENT

We thank the villagers around Maharlou lake who allowed us access to their indigenous chickens and we are grateful for laboratory assistance provided by veterinary research laboratory staff.

REFERENCES

Adene, D.F., A. Oyejide and A.A. Owoade, 1985. Studies on the possible roles of naturally infected Nigerian local chickens and vaccine virus in the epidemiology of infectious bursal disease. *Rev. Elevage Med. Vet. Pays Trop.*, 38: 122-126.

Aghakhan, S.M., N. Abshar, S. Rasoulnejadferaidouni, C. Marunesi, and M. Khodashenas, 1994. Studies on avian viral infections in Iran. *Arch. Razi Inst.*, 44/45: 1-10.

Ahmed, Z., K. Naeem and A. Hameed, 2007. Detection and seroprevalence of infectious bronchitis virus strains in commercial poultry in Pakistan. *Poul. Sci.*, 86: 1329-1335.

Case, J.T., A.A. Ardans, D.C. Bolton and B.J. Reynolds, 1983. Optimization of parameters for detecting antibodies against infectious bronchitis virus using an enzyme-linked immunosorbent assay: Temporal response to vaccination and challenge with live virus. *Avian Dis.*, 27: 196-210.

Cavanagh, D. and S.A. Naqi, 2003. *Diseases of Poultry*. 11th Edn., Iowa State University Press, Iowa.

Chen, B.Y. and C. Itakura, 1996. Cytopathology of

chick's renal epithelial cells experimentally infected with avian infectious bronchitis virus. *Avian Pathol.*, 25: 675-690.

Chen, H., B. Coote, S. Attree and J.A. Hiscox, 2003. Evaluation of a nucleoprotein-based enzyme-linked immunosorbent assay for the detection of antibodies against infectious bronchitis virus. *Avian Pathol.*, 32: 519-526.

Collisson, E.W., A. Williams, R. Parr and L. Wang, 1992. An overview of the molecular biology of infectious bronchitis virus. *Poul. Sci. Rev.*, 4: 41-55.

Davies, H.A. and M.R. Macnaughton, 1979. Comparison of the morphology of three coronaviruses. *Arch. Virol.*, 59: 25-33.

Dergham, A.R., Y.K. Ghassan and A.S. Ibrahim, 2009. Infectious bronchitis virus in Jordanian chickens: Seroprevalence and detection. *Can. Vet. J.*, 50: 77-80.

El-Houadfi, M.D., R.C. Jones, J.K.A. Cook and A.G. Ambali, 1986. The isolation and characterization of six avian infectious bronchitis virus isolated in Morocco. *Avian Pathol.*, 15: 93-105.

Emikpe, B.O., O.G. Ohore, M. Olujonwo and S.O. Akpavie, 2010. Prevalence of antibodies to infectious bronchitis virus (IBV) in chickens in Southwestern Nigeria. *Afr. J. Microbiol. Res.*, 4: 092-095.

Emikpe, B.O., O.G. Ohore, D.O. Oluwayelu, O.A. Oladele, M.A. Ockiya and S.O. Eniola, 2003. Sero-prevalence of antibodies to infectious bronchitis virus in Nigerian indigenous chickens in Ibadan. *Nig. Vet. J.*, 24: 9-12.

Gharaibeh, S.M., 2007. Infectious bronchitis virus serotypes in poultry flocks in Jordan. *Prev. Vet. Med.*, 78: 317-324.

Gough, R.E., W.J. Cox, C.E. Winkler, M.W. Sharp and D. Spackman, 1996. Isolation and identification of infectious bronchitis virus from pheasants. *Vet. Rec.*, 138: 208-209.

Hadipour, M.M., 2010. Seroprevalence survey of H9N2 avian influenza virus in backyard chickens around the caspian sea in Iran. *Brazilian. J. Poul. Sci.*, 12: 53-55.

Haghshenas, G., K. Asasi and H. Akrami, 2005. Isolation and molecular characterization of infectious bronchitis virus (IBV) Shiraz 3 isolate by RT-PCR and restriction enzyme analysis. *Iranian. J. Vet. Res.*, 6: 9-15.

Lee, C.W. and M.W. Jackwood, 2000. Evidence of genetic diversity generated by recombination among avian coronavirus IBV. *Arch. Virol.*, 145: 2135-2148.

Marandi, M. and M.H. Bozorgmehrifard, 2000. Isolation

- and identification of infectious bronchitis viruses in chickens in Iran. Proceedings of the World's Poultry Congress 2000, Montreal, Canada, August 20-25.
- Mauermans, G., M.C. Carlier, M. Gonze, P. Petit and M. Vandebroek, 1987. Incidence, characterization and prophylaxis of nephropathogenic avian infectious bronchitis viruses. *Vet. Rec.*, 120: 205-206.
- Momayez, R., S.A. Pourbakhsh, M. Khodashenas and M. Banani, 2002. Isolation and identification of infectious bronchitis virus from commercial chickens. *Arch. Razi Inst.*, 53: 1-9.
- Nouri, A., K. Asasi and M.R. Seyfiabadshapouri, 2003. Field Study of infectious bronchitis virus in broiler using type-specific RT-PCR. *Arch. Razi Ins.*, 55: 1-10.
- Oyejide, A., V.L. Demangam and J.O. Akinyemi, 1988. Serological survey of antibodies to infectious bronchitis in commercial and indigenous Nigerian chickens using ELISA. *Bull. Anim. Health. Prod. Afr.*, 3: 259-262.
- Parsons, D., M.M. Ellis, D. Cavanagh and J.K.A. Cook, 1992. Characterization of an infectious bronchitis virus isolated from vaccinated broilers breeders flocks. *Vet. Rec.*, 131: 408-411.
- Sawaguchi, K., S. Iwaki, K. Mikazuki, S. Yachida, S. Aoyama, N. Takahashi, Y. Iritani, T. Kitabatake and K. Kawasaki, 1983. Survey of respiratory diseases among chicken flocks in Japan for avian infectious bronchitis by the neutralization test with embryo-adapted Beaudette strain. *Japanese. J. Vet. Sci.*, 45: 767-774.
- Schalk, A.F. and M.C. Hawn, 1931. An apparently new respiratory disease of baby chicks. *J. Am. Vet. Med. Assoc.*, 78: 413-422.
- Seyfiabadshapouri, M.R., M. Mayahi, S. Charkhkar and K. Asasi, 2002. Serotype identification of recent iranian isolates of infectious bronchitis virus by type-specific multiplex RT-PCR. *Arch. Razi Ins.*, 53: 79-85.
- Seyfiabadshapouri, M.R., M. Mayahi, K. Asasi and S. Charkhkar, 2004. A survey of the prevalence of infectious bronchitis virus type 4/91 in Iran. *Acta. Vet. Hung.*, 52: 163-166.
- Stachowiak, B., D. W. Key, P. Hunton, S. Gillingham and E. Nagy, 2005. Infectious bronchitis virus surveillance in Ontario commercial layer flocks. *J. Appl. Poult. Res.*, 14: 141-146.
- Wang, L., D. Junker and E.W. Collisson, 1993. Evidence of natural recombination within the S1 gene of infectious bronchitis virus. *Virology*, 192: 710-716.
- Zhang, D.Y., J.Y. Zhou, J. Fang, J.Q. Hu, J.X. Wu and A.X. Mu, 2005. An ELISA for antibodies to infectious bronchitis virus based on nucleocapsid protein produced in *Escherichia coli*. *Vet. Med.*, 8: 336-344.