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

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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 airsacculitis, 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 Bozorgmehrifard, 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 chequerboard titration (Case et al., 1983). The optimum dilutions obtained following the chequerboard 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.

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REFERENCES


