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The Role of Avian Influenza, Newcastle Disease and Infectious Bronchitis Viruses During the Respiratory Disease Outbreak in Commercial Broiler Farms of Iran

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Abstract: Avian influenza, newcastle disease and infectious bronchitis viruses are pathogens with economical importance in poultry industry. To determine the role of three mentioned viruses in respiratory diseases outbreak in Iranian broiler farms, 160 serum samples from 8 broiler flocks with respiratory disease symptoms were examined by Hemagglutination Inhibition (HI) and ELISA tests. None of these flocks received any of influenza A, newcastle disease and infectious bronchitis viruses vaccines. The overall antibody titres and seroprevalence of AIV H9 subtype, newcastle disease and infectious bronchitis viruses in this study were 7.6, 5.34 $\log_2$, 1123.12 $\log_{10}$ and 31.2, 18.47, 82.43%, respectively. The results revealed the important role of infectious bronchitis virus in respiratory disease outbreak in commercial broiler farms of Iran. Therefore, for preventing the IBV infection in the broiler farms, serotype of the new IBV isolates must regularly be determined.

Key words: Broilers, Iran, respiratory disease, seroprevalence

INTRODUCTION

Newcastle disease and influenza A viruses have been responsible for serious losses in the poultry industry (Swayne, 1997; Yang *et al*., 1997). Dissemination of various strains of these viruses by migratory birds has been noted several times, while showing no detectable clinical signs of the disease (Sharp *et al*., 1997; Ito *et al*., 1995; Lancaster, 1964), some of which purported to have caused important human and animal pandemics (Claas *et al*., 1998). Newcastle disease is one of the most important viral diseases of poultry in Iran. It is an endemic and sometimes epizootic disease in chickens (Bouzari and Mousavi Morekani, 2006). These strains of the virus require several passages in chickens before becoming highly virulent for chickens (Alexander and Parsons, 1984). Recent findings suggest that virulent virus may emerge in poultry as a result of mutations in viruses of low virulence (Alexander, 2001). During 1998-2000, H9N2 viruses were reported in Middle Eastern countries and were responsible for widespread and serious disease in commercial chickens in Iran (Nili and Asasi, 2002, 2003), Pakistan (Naeem *et al*., 1999, 2003, 2007), the United Arab Emirates (Manvell *et al*., 2000; Aamir *et al*., 2007) and Saudi Arabia (Banks *et al*., 2000). The incidence and severity of respiratory disease in commercial chicken flocks have been increased recently in Iran due to intensification of the poultry industry. AIV is believed as one of the main causes of chicken respiratory diseases in Iran as indicated by many field reports (Nili and Asasi, 2002, 2003).

Infectious bronchitis (IB), as a very contagious respiratory disease of chickens, was firstly described in North Dakota, USA, in 1930. Nowadays, it has been spread all over the world. Prevention of the disease by immunization is worthwhile due to contagious nature of the disease and occurrence of numerous serotypes of IB virus (Cavanagh and Naqi, 2003). Nevertheless, prevention of the disease by vaccination has been associated with partial success. 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. An outbreak of severe respiratory disease in both unvaccinated and vaccinated chicken flocks was occurred in January 2000 in Iran (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. Very limited studies have addressed the seroprevalence of three mentioned viruses simultaneously during respiratory disease outbreak in broiler flocks, so the aim of this study

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was to investigate the serological status of commercial broiler flocks against avian influenza, newcastle disease and infectious bronchitis viruses in acute phase of respiratory disease.

MATERIALS AND METHODS

Chicken flocks: During the period of February 2010 to September 2010, we examined 160 commercial broilers from 8 broiler flocks in southwestern Iran, in which the respiratory disease outbreak were happened. Each farm had one flock, and these flocks were of various ages ranging from 20 to 43 days. None of these flocks received any of influenza A, newcastle disease and infectious bronchitis viruses vaccines. All flocks were contacted through the appropriate veterinarians and all owners agreed to participate in this study. A total of 20 birds/farm in an acute phase of respiratory symptoms were picked up and blood samples were collected by venipuncture of the wing vein. Sera were separated and stored at -20ºC until used. Twenty serum samples per flock were tested for AIV H9 subtype, newcastle disease virus and infectious bronchitis virus antibodies.

Hemagglutination and hemagglutination inhibition test (HA/HI): 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)) and B1 strain of NDV (Razi Institute, Karadj, Iran). The HA titer of AIV H9 subtype and B1 NDV 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 B1 NDV and negative control serum samples. 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).

Enzyme-Linked Immunosorbent assay (ELISA): The harvested sera were heat inactivated at 56ºC 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.

RESULTS AND DISCUSSION

In the majority of all broiler flocks, chickens suffered from respiratory distress include rales, gasping and sneezing, sometimes accompanied by lacrimation and facial swelling. In some flocks, renal failure, depression, inappetance, weakness and a mortality rate of 15-25% were seen. The cut-off point for seropositivity in HI test was determined as HI Ab titers >7 in all broiler flocks and titers $\leq$7 were considered as negative result. In the Elisa test, 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 overall antibody titres and seroprevalence of AIV H9 subtype, newcastle disease and infectious bronchitis viruses in this study were 7.6, 5.34 $\log_2$, 1123.12 $\log_{10}$ and 31.2, 18.47, 82.43%, respectively. The seroprevalence of simultaneous infection of (AI-ND), (AI-IB), (ND-IB) and (AI-ND-IB) viruses in broiler flocks were 68.2, 75, 43.2 and 62.5%, respectively. Respiratory diseases of broiler chickens have become a serious problem. All the flocks tested in this study were suffering from respiratory distress. The results of this study revealed that the IB, AI and ND viruses have important role in the respiratory disease outbreak in broiler flocks of Iran, respectively. Most of the examined flocks showed high level of antibody titers to IBV by ELISA technique. The findings of the current study indicate a high infectious bronchitis virus activity among commercial broilers of Iran. Even in broiler flocks vaccinated against these viruses according to veterinary organization programme, IB virus is responsible for the majority of respiratory disease outbreaks, because in Iran, like many other parts of the world, Massachusetts type vaccines are the only live attenuated IBV vaccines in use (Seyfi Abad Shapouri *et al*., 2002). As demonstrated by Parson *et al*. (1992), Massachusetts type vaccines such as H120 are only partially effective against other serotypes of IBV such as 4/91. If 4/91 type of the virus which can break through immunity induced by Massachusetts vaccines persist in the field, appropriate vaccines and vaccination programs may be necessary (Seyfi Abad Shapouri *et al*., 2002, 2004). The results obtained in this study showed that 82.43% of the commercial chickens tested were positive for IBV antibodies. This is quite high when compared 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). The results of current study is comparable to that obtained from Pakistan using the hemagglutination inhibition (HI) test where 88% of the flocks were seropositive for M-41 antibodies

(Ahmed *et al*., 2007). 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). 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 (Seyfi Abad Shapouri *et al*., 2004).

CONCLUSION

These findings indicate a high infectious bronchitis virus activity among commercial broilers of Iran, hence for preventing the IBV induced losses to the Iranian poultry industry, disease surveillance efforts must increase and serotype of the new IBV isolates must regularly be determined. The final results of this investigation will help us to decide for the vaccination strategy against IBV in Iran.

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