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Seroprevalence of Infectious Bronchitis Virus in Birds of Grenada

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Abstract: Infectious Bronchitis (IB) is one of the most important viral diseases of poultry and it causes major economic losses in the poultry industry. The present study was conducted to investigate the prevalence of antibodies for Infectious Bronchitis Virus (IBV) in birds of Grenada. Serum samples from 474 birds including free range chickens (210), broilers (172), Muscovy ducks (50), turkeys (10), rock pigeons (31) and guinea fowl (1) were collected and screened for the presence of antibodies by a flock check ELISA kit. Overall 147 sera were positive for IBV (31.01%). This study demonstrates for the first time, the prevalence of IBV in the island and the Caribbean indicating natural exposure to the virus.

Key words: IBV ELISA, corona virus, Grenada

INTRODUCTION

Infectious bronchitis is an acute highly contagious viral respiratory disease of chickens of all ages characterized by tracheal rales, coughing and sneezing. In addition it may affect the kidneys and causes adverse effect on egg quality, egg production and marked depression in growth. The etiologic agent of IB, Infectious Bronchitis Virus is placed in Group 3 of Genus Corona virus of the family Coronaviridae (Cavanagh and Naqi, 2003). The disease is common worldwide and has significant economic consequences. Although effective vaccines are available and utilized routinely in the commercial poultry production; the virus has the tendency to mutate frequently. This tendency of the virus to undergo continuous genomic drift and shift has lead to emergence of new serotypes especially in the areas of intensive poultry farming (Zanella *et al*., 2003). More than 20 serotypes within the IBV are recognized worldwide.

Grenada is an island country of 344 square kilometers in size. It is located at the southern end of Caribbean Sea. Respiratory diseases of unknown etiology affecting chickens have already been detected in the poultry farms of Grenada (Personal communication with Ministry of Agriculture, Grenada). However, published information regarding the detection of respiratory disease agents in the birds of Grenada is not available. In the surrounding Latin American region there are reports of outbreaks of Infectious Bronchitis in poultry. In Brazil, Argentina, Chile and Honduras the outbreaks are reported and the existing vaccine strains namely the Massachusetts and the Connecticut strains are not protective enough to the circulating strains (Witt *et al*., 2010). This current situation is of concern for the poultry farming in the Caribbean region. So far there are no published reports of IBV infection in the Caribbean region on the native birds. In this context the present study was conducted to investigate the incidence of IBV in the broilers and free range chicken and other types of birds in Grenada.

MATERIALS AND METHODS

Birds: source and sampling: A total of four hundred and seventy four sera samples were collected from free range chicken (210), broilers (172), muscovy ducks (50), turkeys (10), rock pigeons (31) and guinea fowl (1) from different parts of Grenada. Birds were apparently free from any clinical signs of disease. All flocks were contacted through appropriate veterinarians and all owners agreed to participate in the study. Approximately 2 ml blood was collected from each bird from wing vein. Sera was separated and stored at -70°C.

ELISA: Infectious Bronchitis Virus Antibody Test Kit, FlockCheck IBV (IDEXX, Maine, USA) was used for the detection of antibody to IBV in the sera samples according to manufacturer's instruction. Briefly, 100 µl of diluted samples were added to the pre-coated plate and incubated at room temperature for 30 min. Appropriate positive and negative control was also included. After aspirating the liquid content of all wells, the wells were washed with deionized water. 100 µl of (Goat) Anti-Chicken:HRPO conjugate was added into each well and the plates were incubated at room temperature for 30 min. After washing procedure, 100 µl the substrate reagent was added into the appropriate wells and incubated at room temperature for 15 min. To stop the reaction, 100 µl of Stop solution was added into each

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well. The ELISA plates were read by ELx 800 Universal Microtitre plate reader (BIO-TEK Instruments, Inc.US.) at 650 nm.

The relative level of antibody in the sample was determined by calculating the Sample to Positive (S/P) ratio. The endpoint titers were calculated using the equation described by the manufacturer. Serum samples with S/P ratio of less than or equal to 0.2 were considered negative and those samples with S/P ratio greater than 0.20 (titer >396) were considered positive.

Table 1: Prevalence of IBV antibodies in species of birds in Grenada

Species	No. of samples	No. (%) of positive samples
Chicken (Broilers)	172	31 (18.02)
Chicken (Free range)	210	114 (54.28)
Ducks	50	1 (2.00)
Turkey	10	1 (10.00)
Guinea fowl	1	0 (0.00)
Pigeon	31	0 (0.00)
Total	474	147 (31.01)

RESULTS AND DISCUSSION

Seroprevalence of the IBV in various species of birds in Grenada is given in Table 1. The overall seroprevalence of IBV in all species of birds in the present study was 31.01%. Vaccination against IBV is not practiced in Grenada. Therefore, the results of the present study indicate that all types of chicken (broilers and free range) were exposed to IBV at some point. The prevalence rate in chicken is quite low when compared with previous reports from other countries. In Jordan, Overall, 92.9% of the flocks free from respiratory disease were seropositive for antibodies to the M-41 strain, whereas 90% and 61.4% of the flocks were seropositive for antibodies to the 4/91 and D274 strains, respectively (Roussan *et al*., 2009). Similarly, in Pakistan, a survey conducted in the commercial poultry revealed, 88% of the flocks were seropositive for M-41 antibodies, whereas 40, 52 and 8% of the flocks were positive for D-274, D-1466 and 4-91 IBV strains, respectively (Ahmad *et al*., 2007). A high seroprevalence of 82.7% was reported in chickens in southwestern Nigeria (Emikpe *et al*., 2010). However, a 56.5% seroprevalence of IBV was reported in the backyard poultry of Mexico in the year 2000 (Guitirrez-Ruiz *et al*., 2000) which is comparable to the present study (54.28%).

In addition to chicken, 50 Muscovy ducks (*Cairina moschata*), 31 rock pigeons (*Columba livia*), 10 turkeys (*Meleagris gallopavo*) and I guinea fowl (*Numida meleagris*) were also screened for IBV. A single positive was obtained in each duck and turkey samples. Infectious Bronchitis Virus (IBV) together with genetically related coronaviruses of turkey and pheasant, belongs to the group 3 coronaviruses (Vijgen *et al*., 2006). IBV has also been isolated from a peafowl and a teal, in a recent coronavirus screening of domestic birds (Liu *et al*., 2005), as well as from a flock of racing pigeons (Barr *et al*., 1988). Genetically similar corona viruses were also identified from graylag geese (*Anser anser*), feral pigeons (*Columbia livia*) and mallards (*Anas platyrhynchos*) (Jonassen *et al*., 2005) and also from guinea fowl (Ito *et al*., 1991). The poultry coronaviruses are antigenically similar and phylogenetically related (Breslin *et al*., 1999; Cavanagh *et al*., 2002). Although it has been shown that an avian coronavirus from one species can replicate in other avian species, no clinical signs are observed in most instances (Lister *et al*., 1985; Guy, 2000; Ismail *et al*., 2003).

In this study seropositive turkey and duck were identified indicating these species may act as a reservoir for IBV or related viruses. Such an alternative reservoir would have major implications for vaccination and control programs for IBV prevention. Future work should include isolation and serotyping of IBV from Grenada and other Caribbean islands also so that a suitable vaccination program using common field serotype as vaccines can be adopted to protect against IBV caused disease in the region. Furthermore farmers need to be educated regarding the importance of IBV.

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