



## Seroprevalence of Newcastle Disease, Chicken Infectious Anemia and Avian Influenza in Indigenous Chickens in Grenada, West Indies

Ravindra Nath Sharma<sup>1</sup>, Alma Bréjeon<sup>2</sup>, Salomé Bruyant<sup>2</sup>, Keshaw Tiwari<sup>1</sup>, Alfred Chikweto<sup>1</sup> and Muhammad Iqbal Bhaiyat<sup>1</sup>

<sup>1</sup>Department of Pathobiology, School of Veterinary Medicine, St. George's University, Grenada, WEST INDIES

<sup>2</sup>Ecole Nationale Vétérinaire De Toulouse, Toulouse, FRANCE

Corresponding author: Ravindra Nath Sharma; Email: rsharma@sgu.edu

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### ABSTRACT

This study was conducted to determine the seroprevalence of antibodies against Newcastle disease virus (NDV), Chicken infectious anemia virus (CIAV) and Avian influenza virus (AIV) in indigenous chickens in Grenada, West Indies. Indigenous chickens are kept for eggs and meat for either domestic consumption or local sale. These birds are usually kept in the backyard of the house with little or no shelter. The mean size of the flock per household was 14 birds (range 5-40 birds). Blood was collected from 368 birds from all the six parishes of Grenada and serum samples were tested for antibodies against NDV, CIAV and AIV using commercial enzyme-linked immunosorbent assay (ELISA) kits. The seroprevalence of antibodies against NDV, CIA and AI was 66.3% (95% CI; 61.5% to 71.1%), 59.5% (95% CI; 54.4% to 64.5%) and 10.3% (95% CI; 7.2% to 13.4%), respectively. Since indigenous chickens in Grenada are not vaccinated against poultry pathogens, these results indicate exposure of chickens to NDV, AIV and CIAV. Indigenous chickens are thus among the risk factors acting as vectors of pathogens that can threaten commercial poultry and other avian species in Grenada.

**Keywords:** Grenada, Indigenous chickens, Serology, NDV, CIAV, AIV.

Generally, the income of Grenadians is very marginal and so, to raise family income, many households (approximately 30%) rear indigenous chickens in their backyard with little or no financial investment. Chickens are fed on kitchen waste and harvest residues. They are given shelter during the night and set free during the day. Flocks comprise cocks, hens, and chicks with flock sizes ranging between 5 and 40: In 2012, the total backyard chicken population in Grenada was estimated to be approximately 25, 688 birds (Dr. Louison, Chief Veterinary Officer, Ministry of Agriculture, Lands, Forestry, Fisheries and Environment, Grenada: Personal communication).

Because of the role of backyard chickens in the epidemiology of many bacterial and viral diseases, there

are numerous reports on diseases affecting backyard chickens in many developing countries. However, there is paucity of literature on diseases of backyard chickens in the Caribbean region including Grenada. The first serological survey of various avian pathogens on 91 backyard chickens in Grenada (Sharma *et al.* 2006) revealed seropositivity for Newcastle disease virus (99%), Infectious bronchitis virus (77%), *Mycoplasma gallisepticum* (86%), *Pastuerella multocida* (35%), and *Salmonella enteritidis* (45%). Arathy *et al.* (2011) reported that out of 143 serum samples from backyard chickens in Grenada, tested by ELISA, 27 (18.8%) were positive for antibodies against AIV. In the present study, we investigated the seroprevalence of three important viral diseases (ND, AI and CIA) in backyard chickens of Grenada.



## MATERIALS AND METHODS

### Ethical approval

The project was approved by the Institutional Animal Care and Use Committee (IACUC) of St. George's University.

### Birds, sampling and ELISA test

Grenada is the southernmost island country in the southeastern Caribbean Sea with an area of approximately 348.5 Km<sup>2</sup> and a population estimated at 100,000. The Island is divided into six parishes. Blood from 368 randomly selected backyard chickens between the age of 1 year and 2 year, from all the six parishes, were tested for antibodies against NDV, AIV and CIAV. Blood from five birds from flocks consisting of up to 10 birds and from 20% of birds from flock size greater than 10 birds was collected. Sera were separated by blood centrifugation at 3000xg for 5 min and frozen at -20°C until use. Commercial ELISA kits (Pourquier Laboratories, ID Vet, France) for each pathogen (NDV, CIAV, AIV) were used according to the manufacturer's instructions.

### Statistical analysis

Confidence intervals were calculated using a statistical package from the following website: <http://www.Mccallumlayton.co.UK/States/confidenceintervalCalcProportion.aspx>.

## RESULTS AND DISCUSSION

The seroprevalence of the viral diseases under investigation are presented in Table 1. Concurrent presence of antibodies for more than one pathogen in a chicken is presented in Table 2.

Newcastle disease (ND) is a worldwide serious disease of domestic poultry and wild birds. It has been reported in at least 241 species of birds (Kaleta and Baldouf, 1988); however, the susceptibility to infection with NDV varies with the species of birds. Generally, NDV strains can be divided into five pathotypes depending on the spectrum of disease produced in chickens. Viscerotropic velogenic NDVs cause peracute systemic disease and a mortality of 90% or higher with hemorrhagic and inflammatory lesions in the intestinal tract (Doyle's form). Neurotropic

velogenic NDVs cause high mortality (50-90%) following respiratory and nervous signs, encephalitis being the dominant microscopic lesion (Beach's form). The mesogenic NDVs cause respiratory disease followed by nervous signs (Beaudette's form) with mortality ranging from 5-50%. Lentogenic NDVs cause mild or inapparent respiratory infection with mild tracheitis to pneumonia (Hitchner's form) and asymptomatic NDVs cause an inapparent enteric infection in which the lesions are usually negligible

Reports on exposure of backyard chickens to NDV are available from many countries of the world. All researchers emphasized the importance of NDV in backyard chickens as a serious threat for commercial chickens and other avian species. Most of the studies on diseases of backyard chickens are reported from the African continent and countries in South America including the Caribbean region. Outbreaks of NDV have been reported in the Dominican Republic (Sean *et al.*, 2013), Belize (Caribbean 360), Brazil (Orsi *et al.*, 2010), Venezuela (Flu-Trackers, 2006), Chile, Peru (Poultry World, 2007) and Argentina (Berenstein *et al.*, 1999). These outbreaks involved both commercial and local chickens. Studies specific to backyard chickens are reported from Costa Rica and Mexico. Sonia *et al.* (2008) reported high prevalence of NDV in backyard chickens in Costa Rica. They found a number of birds with titers >4000 and suggested that backyard chickens in the region were in contact with pathogenic strain of NDV. In a serological survey in Mexico (Gutierrez-Ruiz, 2000), 2.2% of backyard chickens had antibodies for NDV. In Zimbabwe, Patrick *et al.* (1994), while investigating 52 flocks of backyard chickens, found 27% of birds to be seropositive for NDV. In Nigeria, NDV is endemic and with varied incidence in different parts of the country. Abdu *et al.* (2002) reporting the incidence of various diseases in local Nigerian poultry found 21.8% of the diseases to be outbreaks of NDV.

In the present study, the seroprevalence of NDV in backyard chickens in Grenada was 66.3% which is comparable to an antibody level at 57.3% in indigenous chickens in Botswana (Mushi *et al.* 2001). In a previous study conducted in Grenada in 2004 (before Hurricane Ivan), the seroprevalence of NDV was 99% (Sharma *et al.* 2006). During the hurricane all indigenous chickens on the island were destroyed This fall in seroprevalence observed in the present study may be due to a new crop of

**Table 1.** Seroprevalence of antibodies for Newcastle disease (ND), Chicken Infectious Anemia (CIA) and avian influenza (AI) in indigenous chickens in Grenada, West Indies.

Number of samples	Positive for ND	% Positive	Positive for CIA	% Positive	Positive for AI	% Positive
368	244	66.3	38	10.3	219	59.5

**Table 2.** Concurrent infection with NDV, AIV, and CIAV in indigenous chickens in Grenada, West Indies.

Presence of antibodies for	NDV and AIV	NDV and CIAV	AIV and CIAV	NDV, AIV and CIAV
Number of chickens	139 (37.8%)	11 (2.99%)	5 (1.36%)	24 (6.52%)

birds after the hurricane and awareness among farmers regarding better management systems. Backyard chickens in Grenada are not vaccinated for NDV and, in the absence of clinical signs, this study indicates the circulation of the lentogenic strain of NDV. Virus isolation and characterization of NDV isolates are warranted to assess virus pathogenicity and the pathotypes prevalent in Grenada.

Chicken infectious anemia virus (CIAV) was first reported in Japan by Yuasa *et al.* (1999). Subsequently, it has been serologically demonstrated in commercial chickens world wide including Grenada (Sharma *et al.*, 2014). There are very few reports of CIAV in backyard chickens. To best of our knowledge, this is the first report of the seroprevalence of CIAV in backyard chickens in the Caribbean region including Grenada.

CIAV is transmitted vertically and horizontally. Clinical signs of anemia and dermatitis are present in chicks of 1-2 week, infected vertically. Birds infected horizontally remain subclinical affecting their performance and profitability in production. CIAV also causes immunosuppression especially in concurrent infection with Marek's disease and infectious bursal disease. In this study, the seroprevalence of CIAV in backyard chickens in Grenada, was low (10.3%) compared to 92.2% in commercial layers and 58.3% in broilers (Sharma *et al.*, 2014). The difference in seropositivity in commercial and backyard chickens in Grenada may be related to types of husbandry practices. Commercial birds are more prone to horizontal infection because of their close confinement as compared to free range backyard chickens.

The role of backyard chickens and wild birds as reservoirs for the spread of AIV to commercial birds is well documented (Dennis, 2010; Douglas *et al.*, 2007). A high risk of AIV is suspected in the Caribbean region because of an extensive backyard system of poultry production and the influence of migratory birds in the region. There is a paucity of published reports on AIV in the Caribbean region. The first report of AIV was from the Dominican Republic and Haiti in 2007 from birds examined from the live bird market, village poultry, and fighting cocks (Dennis 2010). A survey conducted by Lefrancois *et al.* (2010) on wild and domestic birds from Guadeloupe, Martinique, St. Lucia and the Dominican Republic reported that all samples were negative for AIV. Douglas *et al.* (2007) reported isolation and characterization of AIV from wild water fowl in Barbados. In Grenada, Arathy *et al.* (2011), reported a seroprevalence of 18.8% for antibodies against AIV in backyard chickens. AIV ribonucleic acid (RNA) was not detected in the seropositive chickens. Ducks, turkeys, pigeons, and guinea fowl were found to be seronegative for AIV. In a recent study on commercial birds in Grenada, Sharma *et al.* (2014) did not detect antibodies for AIV. Further studies on the isolation and characterization of AIV from backyard chickens in Grenada are suggested.

The present survey revealed the presence of NDV, AIV and CIAV simultaneously in backyard chickens in Grenada. Similar reports are available from many countries. Simultaneous presence of AIV, NDV, egg drop syndrome virus, infectious bronchitis virus (IBV) and reovirus in chickens on small holdings in Bangladesh has been reported by Biswas *et al.* (2009). Owoade *et al.* (2006) reported on



the simultaneous presence of IBV, Avian pneumovirus, infectious laryngotracheitis (ILT) and avian leukosis virus (ALV) on the same premises in Nigerian poultry. Shadmanesh and Mokhtari (2013) detected antibodies for NDV, AIV, Salmonella, *Mycoplasma gallisepticum* and *M. synoviae* in native hens in Iran. These diseases cause serious loss in production and/or loss in profitability through mortality. Further research to assess the effect of these diseases on the health and performance of backyard chickens in Grenada is proposed.

Farmers in Grenada need to be educated on the effect of NDV, AIV and CIAV on their chickens and application of preventive measures for these diseases.

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