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Concurrent Infections of Poultry with Chicken Infectious Anaemia and Infectious Bursal Disease Viruses in Maiduguri, Nigeria: A Seroprevalence Study

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ABSTRACT

Chicken infectious anaemia virus (CAV) and infectious bursal disease virus (IBDV) are significant pathogens causing severe economic losses in the global poultry industry. In this study, serum samples from village chickens, broilers, layers, ducks, turkeys and geese in Maiduguri were tested for CAV and IBDV antibodies using Enzyme linked immunosorbent assay (ELISA). Among the 944 serum samples tested, 309 (32.7%) were seropositive for concurrent CAV and IBDV antibodies. The species distribution showed 29.9% (120/401) village chickens, 23.1% (12/52) layers, 46% (144/313) broilers, 21.3% (27/127) turkeys, 3.4% (1/29) ducks, and 22.7% (5/22) geese were positive for both CAV and IBDV antibodies. The sex distribution of the mixed CAV and IBDV seropositive samples showed an overall seroprevalence rates of 36.6% and 25.7% among males and females respectively. This study highlights the widespread presence of CAV and IBDV infections among poultry species in Maiduguri. Further research is needed to evaluate the economic impact and the cost-effectiveness of control measures.

Keywords: Chicken Anaemia Virus; Infectious Bursal Disease Virus; Poultry; Seroprevalence

INTRODUCTION

Chicken anaemia virus (CAV) and infectious bursal disease virus (IBDV) are economically significant poultry pathogens in developing countries, often linked to immunosuppression, anaemia, and high mortality (McIlroy *et al*., 1992). One of the most outstanding features of these two disease agents is the ability to cause immunosuppression by themselves directly or by participating indirectly with other viruses in chickens (Toro *et al*., 2009).

The growing demand for poultry products has intensified production practices, reduced genetic diversity and created conditions that allow CAV to persist in hosts and the environment (Witter, 2001). In Nigeria, CAV was first isolated and reported by Oluwayelu *et al.,* (*2005*) and Shettima *et al.,* (2017). The biological properties of these isolates were studied and found to be relatively similar to the Cux-1 isolate (Oluwayelu *et al.,* 2010). Chicken infectious anaemia otherwise known as anaemia-dermatitis syndrome or blue wing disease (Rozypal *et al.,* 1997) that affects mostly young birds 2-4 weeks old is characterized by anaemia, subcutaneous haemorrhage, immunosuppression, cachexia and high mortality (Farkas *et al.,* 1992; Rozypal *et al*., 1997). At postmortem, the

disease is characterized by severe anaemia, lymphoid depletion, and yellowish to whitish bone marrow, atrophy of bursa of fabricius, thymus and haemorrhage (Yuasa *et al.,* 1979). This disease is caused by a single stranded DNA virus with icosahedral symmetry belonging to the family *Circoviridae* (Fenner *et al.,* 1993). The virus is highly resistant to most disinfectants and is ubiquitous, in nature (Miller *et al.,* 2003).

Infectious bursal disease virus, is a bisegmented double stranded RNA, belonging to the family *Birnaviridae* of the genus *Avibirnavirus* with a diameter of 60 nm, a density of 1.336 g/ml in cecium chloride (Oluwayelu *et al.,* 2010) and about 3261 bp in serotype 1 and 3264 bp in serotype II (Van den Berge, 2000). This virus is the causative agent of infectious bursal disease (IBD) otherwise known as Gumboro, avian infectious bursitis and avian nephrosis-nephritis. It causes an acute contagious disease of young birds 3-6 weeks and even 8 weeks of age characterized by destruction of lymphoid cells in the bursa of fabricius (El-Yuguda *et al*., 2004; Oluwayelu *et al.,* 2010; Shettima *et al.,* 2018). In Nigeria, the disease was recognized in 1969 (Oluwayelu, 2010). Infectious bursal disease first named by Edgar in 1961 (Lukert and Saif, 2003) and its etiologic agent was first isolated by Winterfield in 1962 (Panigraph *et al.,* 1986). The disease was initially confused with a variant of infectious bronchitis virus (gray strain) due to lesions in the kidney (Winterfield *et al.,* 1962). Hitchner studied clinical manifestations of infectious bursal agent and differentiated infectious bursal disease from IB (Hitchner, 1963). The virus is relatively resistant to

extreme temperature and pH and a wide range of chemical agents (Benton *et al.,* 1967; Cho and Edgar, 1969). The immunosuppressive effects of IBDV infection at an early age was reported by Allan *et al.* (1972). By studying the concurrent infection of CAV and IBDV, we can gain valuable insight into the dynamics of co-infections and develop effective strategies for the control and prevention of the diseases.

MATERIALS AND METHODS

Study Sites

This study was conducted in Maiduguri, the capital city of Borno State, Nigeria. The city lies between latitude 10.20^{O}N and 13.40^{O}N to the north, longitude 9.80^{O}E and 14.40^{O}E to the east and occupies an area of 69.436 sq/km. Borno State shares international border with Niger to the north, Chad to the north east and Cameroon to the east (Musa and Pindar, 2005). The state has an estimated population of 4.2 million people with an average temperature ranging between 34ºC - 40ºC (Ishaku and Majid, 2010). Maiduguri, the capital of Borno State, is historically rooted in agriculture, livestock farming, and trade, with many residents engaged in subsistence farming, livestock rearing, and fishing and traditional crafts like leatherwork and weaving are common occupations (Blench, 1997). Traditional ceremonies, storytelling, and distinctive clothing styles are key aspects of the region's culture, reflecting a blend of Islamic and indigenous customs (Mustapha, 2014).

Study population

In this study, a non-probability convenience sampling method was employed. A total of 944 blood samples were gathered from various apparently healthy poultry species, presenting no signs of illness or abnormality, including village chickens, broilers, layers, turkeys, ducks, and geese. A total of 714 blood samples from village chickens and broilers were obtained from the poultry slaughter section at Maiduguri's Monday market. Additionally, 230 blood samples were collected from live birds at poultry farms and individual households within Maiduguri. Birds of all age regardless of sex were sampled.

Blood Sampling and Storage

Blood samples were collected using sterile 2 mL syringes from the wing vein of live birds and the jugular vein of slaughtered birds. Samples were transferred into labelled plain vacutainer tubes and allowed to clot at room temperature. Each blood was centrifuged at 1,500 rpm for 10 minutes and serum was harvested and transferred into labelled cryotubes and stored at -20ºC until tested.

Serology

The sera from the different poultry species were tested for the presence of CAV and IBDV antibodies using commercial ELISA kits (X-Ovo Flockscreen^{TM}). The ELISA tests were carried out following the procedures outlined by the manufacturers for CAV and IBDV. Each ELISA plate was supplied pre-coated with purified viral (CAV or IBDV) antigens. Briefly, the test sera were diluted using the sample diluent supplied by the kit and 50 µl of each diluted serum was added to a corresponding well using multichannel automated pipette and incubated at 37ºC for 30 minutes. This is to allow antibody specific to CAV or IBDV antigen to bind and form a complex. Excess unbound antibodies were manually washed from the wells using 500 ul of washing buffer and an enzyme (alkaline phosphatase) conjugated antispecies or secondary or detector (rabbit anti-chicken) antibody was added to all the wells and incubated at 37ºC for 30 minutes. This will allow the enzyme conjugated secondary antibody to locate and bind to the primary anti CAV or IBDV antibodies in the wells. Again, excess unbound enzyme conjugated antispecies antibodies was washed away four times with wash buffer (300ul per well). A substrate [phenophthalene monophosphate (PMP)] was then added to the wells and incubated at 37ºC for 30 minutes. The reaction was stopped using 50 ul of stop solution (sodium hydroxide). The plate was immediately read using a microtiter plate reader at 550 nm filter. The degree of colour development (optical density) is directly related to the amount of antibody to CAV or IBDV present in the sample.

Data Analysis

Data obtained from the study was presented in simple percentages and analyzed using (SPSS) Version 16 software, Chi-square test was also used to perform categorical comparison and determine significance at 95% confidence interval. P-value less than or equal to 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

ELISA testing revealed an overall seroprevalence of 32.7% (309/944) for concurrent IBDV and CAV antibodies among poultry in Maiduguri (Table 1). The species distribution of the seropositive samples showed 120/401 (29.9%) village chickens, 12/52(23.1%) layers, 144/313(46%) broilers, 27/127(21.3%) turkeys, 1/29(3.4%) ducks and 5/22 (22.7%) geese were positive for both CAV and IBDV antibodies (Table1). The sex distribution of the mixed CAV and IBDV seropositive samples showed and overall prevalence of 36.6% and 25.7% among males and females' species of poultry respectively (Table 2).

This high concurrent seroprevalence rate of CAV and IBDV of 32.7% observed in the present study could result from vaccination among the broiler and layer chicken populations, but the seroprevalence rates observed among other species (village chickens, turkeys, ducks and geese) might have been acquired through natural infection rather than from vaccination and/or maternally derived antibodies since these group of birds are not routinely vaccinated against IBDV or CAV and were all adult birds. Overall, vaccination practices in the poultry industry have significant implications for animal health, human health, the environment and the economy. These include reduced mortality rates, decreased antibiotic usage, improved feed efficiency, disease prevention, reduced zoonotic disease transmission, improved welfare, reduced environmental impact, food safety and consumer confidence among others (Wlaźlak *et al*., 2023).

Table 1: Distribution of concurrent infections of chicken anaemia virus and infectious bursal disease virus enzyme linked immunosorbent assay positive antibodies among different poultry species in Maiduguri, Nigeria.

Poultry Type	Total No. Tested	No. of positive IBDV + CAV (%)
Village chicken	401	120 (29.9)
Layers	52	12 (23.1)
Broilers	313	144 (46.0)
Turkeys	127	27 (8.6)
Ducks	29	1 (3.5)
Geese	22	5 (22.7)
Total	944	309 (32.7)

P≤0.05

Table 2: Sex distribution of concurrent infections of CAV + IBDV ELISA positive antibodies among.

Poultry Type	Males		Females	
	Total No. Tested	No. Positive (%)	Total No. Tested	No. Positive (%)
Village chicken	300	98 (32.7)	101	22 (27.8)
Layers	NA*	NA*	52	12 (23.1)
Broilers	180	98 (54.4)	133	46 (34.6)
Turkeys	100	20 (20)	27	7 (25.9)
Ducks	19	1 (5.3)	10	0 (0)
Geese	7	5 (71.4)	15	0 (0)
Total	606	222 (36.6)	338	87 (25.7)

P≤0.05

Key: NA*= Not Applicable

Furthermore, the result of this study showed that CAV and IBDV infections are widespread; all the poultry species sampled in this study were found to be seropositive for both viruses, which is in agreement with what was reported worldwide in all major poultry producing countries (Cardona *et al.,* 2000). The high seroprevalence rate observed in this study agrees with the report that there exists a synergistic interaction between IBDV and CAV (Imai *et al.,* 1999); and indicates that CAV should be considered as a differential diagnosis in cases that present with IBD-like clinical signs and pathology. Thus, which infection is triggered by which infection is not clear (Hadimli *et al.,* 2008). The use of embryonated chicken eggs in IBDV vaccine production could be the source of contamination with CAV (Amer *et al*., 2011). There is need for frequent screening of the eggs used for vaccine production and strict quality control of the IBDV vaccines. Though these monitoring process may affect the cost of producing the vaccine from eggs especially in CAV endemic area, therefore an alternative egg-free methods of vaccine production could be explored.

The overall prevalence (concurrent infection) based on sex of poultry was observed to be high in males than in female birds in the present study. The higher seroprevalence rate observed in males in the present study could be due to the fact that male birds were sampled more than the female birds, this is because more male birds are sold out and slaughtered at live bird markets and houses while the female birds are kept for breeding purposes (Lawal *et al*., 2014). And it is important to note that both viruses are not sex dependent. This study demonstrates the widespread occurrence of CAV and IBDV co-infections among poultry species in Maiduguri, underscoring the need for targeted control strategies.

Conflict of Interests

The authors have no conflict of interest to declare.

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Author's Contributions.

YMS- Research design, sample collection and literature search. THM and MUS-Data analysis and critical review of manuscript. HIG, MBA and ADEY-Supervised the work and provided critical review of manuscript. All authors have read and accepted the final manuscript for publication.

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