Research Article

Concurrent Infections of Chicken Infectious Anemia and Infectious Bursal Disease in 5 Weeks Old Pullets in Jos, Plateau State, Nigeria

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Abstract | In the field it is very difficult to differentiate between chicken infectious anemia (CIA) and infectious bursal disease (IBD) and co-infection of CIA and IBD. Infectious bursal disease was tentatively diagnosed in 5,000, 5 weeks old Hy-Line and ISA Brown pullets in a commercial poultry farm in Jos, Plateau State, Nigeria with mortality of up to 79.12%. The clinical signs observed were prostration, ruffled feathers, diarrhea, anorexia, trembling and mortality that lasted for 6 days. Postmortem findings include ecchymotic hemorrhages in the breast and thigh muscles, mucosa of the junction between the proventriculus and gizzard, severely enlarged and hemorrhagic bursa of Fabricius (BF) and splenomegaly. The bursa of Fabricius was harvested for laboratory investigation using AGID for infectious bursal disease virus (IBDV). As part of investigation to screen for co-infection of CIA and IBD in the flock, the BF was also screened for the presence of chicken anemia virus (CAV) using conventional polymerase chain reaction (PCR). IBDV antigen was detected by AGID while CAV by PCR. This confirms the diagnosis of concurrent infection of CIA and IBD in the affected flock. This is the first case report of a concurrent and natural field outbreak of CIA and IBD in a commercial poultry farm in Nigeria.

Editor | Muhammad Abubakar, National Veterinary Laboratories, Park Road, Islamabad, Pakistan.
Received | September 01, 2016; **Accepted** | October 10, 2016; **Published** | October 20, 2016
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Citation | Adedeji, A.J, N.M. Sati, S.B. Pewan, K.I. Ogbu, J.A. Adole, D.D. Lazarus, S.J. Ijiwo, A. Okpanachi, I.O. Nwagbo, T.M. Joannis and P.A. Abdu. 2016. Concurrent infections of chicken infectious anemia and infectious bursal disease in 5 weeks old pullets in Jos, Plateau State, Nigeria. *Veterinary Sciences: Research and Reviews*, 2(3): 60-65.
DOI | http://dx.doi.org/10.17582/journal.vsrr/2016.2.3.60.65

Introduction

Chicken infectious anemia (CIA) is a viral disease of chickens which causes aplastic anemia and atrophy of the thymus (McNulty, 1997; Smyth and Schat, 2013). Chicken anemia virus (CAV) the etiology of CIA was first detected while investigating contaminated Marek's disease (MD) vaccine in 1979 (Yuasa et al., 1979). Chicken anemia virus belongs to the genus *Circovirus* of the family *Circoviridae,* and is a small, non-enveloped, icosahedral and very stable DNA virus that is transmitted vertically and horizontally (McNulty and Todd, 2008; Smyth and Schat, 2013). Clinical signs due to CIA include depression, reluctance to move, ruffled feathers, drooping wings and pallor of combs, beaks and mucous membranes. In the last three decades, CIA has emerged as a new economically important disease that is associated with immunosuppression and subclinical illness, leading to enhanced infections by secondary viral, bacterial or fungal agents (Oluwayelu, 2010). Immunosuppression resulting from CAV infection could

also lead to vaccination failures due to poor response to vaccination as a result of damage to bone marrow and prevention of lymphoid organs from regenerating (Haridy et al., 2009; Oluwayelu, 2010). Economic losses due to CAV infections arise from poor growth, increased mortality and the cost of antibiotics used to control secondary bacterial infections (McNulty, 1991). It has been reported that the net income per 1,000 birds, feed conversion ratio and average weight per bird were lower in flocks with antibodies to CAV compared to those without it (Oluwayelu, 2010). In another report, a loss of net income of about 18.5% due to decreased weight at processing and increased mortality in CAV-infected birds has been observed (McIlroy et al. 1992).

Infectious bursal disease (IBD) is an acute highly contagious viral disease of mostly young chickens with high morbidity and mortality caused by IBD virus (IBDV). The IBDV is a double stranded RNA virus belonging to the genus *Avibirnavirus* within the family *Birnaviridae* (Rosenberger et al., 2008; Dolz and Majo, 2013). It was first reported in the early 1960s in the United States of America (Dolz and Majo, 2013). In recent years, there has been emergence of the acute forms of the disease with devastating effect on the poultry industry (Dolz and Majo, 2013). In fact, IBD is a major threat to the poultry industry worldwide (van den Berg, 2000; Dolz and Majo, 2013). The virus is transmitted horizontally by oral or respiratory routes through direct contact with infected chickens or by direct contact with contaminated fomites (Dolz and Majo, 2013). Clinical signs of IBD include anorexia, ruffled feathers, diarrhea, prostration and death (Dolz and Majo, 2013).

Materials and Methods

Case History

In the month of May, 2014, mortality was reported in 5,000, 5 week old pullets from a commercial poultry farm in Jos. The mortality was observed in one of the seven pens within the rearing section of the farm. The birds were off-feed and huddled together. The birds were Hy-line and ISA Brown. The birds had a history of vaccination against IBD at 9 and 21 days and against Newcastle disease (ND) at 16 days of age. History also revealed that the farm had recorded an outbreak of IBD in February 2014. The clinical signs observed following a farm visit were prostration, ruffled feathers, green-yellowish diarrhea, anorexia and morbidity was over 80%. Mortality was recorded between day 1 and 6 of onset of clinical signs and 79.12% (3,956) of the birds died of the disease (Figure 1, 2 and 3).

Figure 1: *High morbidity in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

Figure 2: *High morbidity and mortality in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

Figure 3: *High mortality in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

Postmortem Findings

Postmortem findings revealed ecchymotic hemorrhages in the breast and thigh muscles, hemorrhages at the proventriculus-gizzard junction, severely enlarged and hemorrhagic BF and enlarged spleen (Figure 4, 5 and 6). The bursa of Fabricius was harvested for laboratory diagnosis.

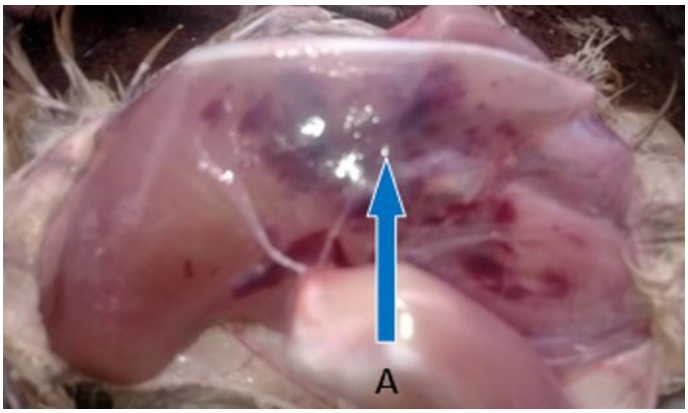

Figure 4: *Severe hemorrhages in the beast muscles* **(A)** *in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

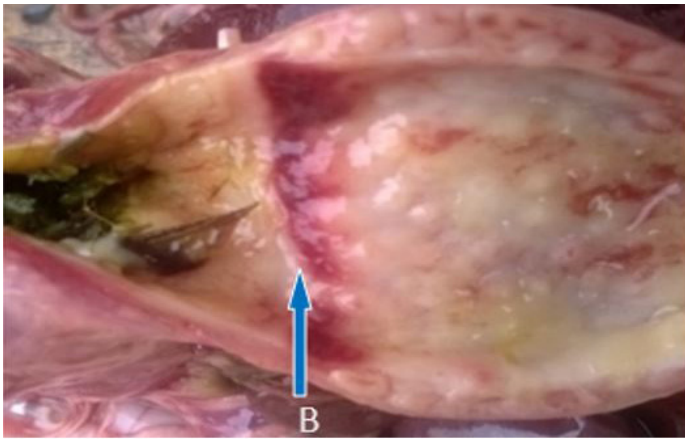

Figure 5: *Hemorrhages at the proventriculus-gizzard junction* **(B)** *in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

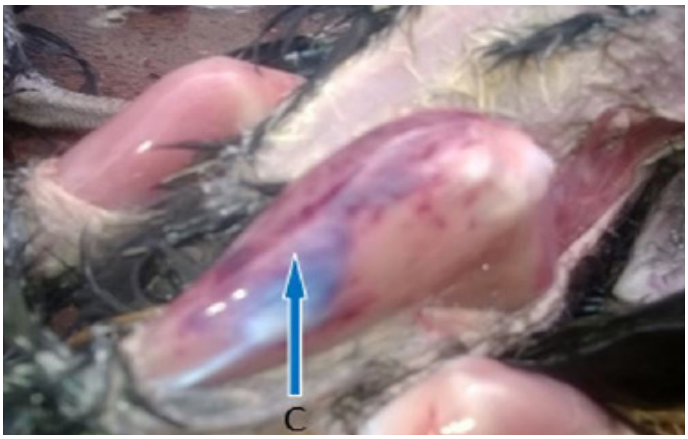

Figure 6: *Hemorrhages in the leg muscles (C) in 5 weeks old commercial pullets due to chicken infectious anemia and infectious bursal disease*

Processing of the Bursa of Fabricius

Bursa of Fabricius (BF) of dead chickens from the farm were removed and stored at -20°C in the laboratory for four months until they were processed. The BF was homogenized using a mortar and pestle. Phosphate buffered saline (PBS) (pH 7.4) was added to the homogenized BF at the ratio of 1 ml PBS to 1gram BF and centrifuged at 3,000 rpm for 10 minutes. The supernatant was decanted into a sample bottle and stored in a deep freezer. The sediment was mixed with 10% formalin and discarded in an incinerator. Agar gel immunodiffusion (AGID) test was used for detection of IBDV antigens and PCR for CAV.

Agar Gel Immunodiffusion (AGID) Test

Antigens to IBD were detected using AGID test. The samples were placed in wells against IBDV antigen. 25 µL of sera were placed in the peripheral wells against 25 µl of IBD antigen in the central wells and incubated in a humid chamber for 3 days at room temperature (22-30°C). Wells positive for IBD antibody were those with precipitin lines between the central wells containing the IBD antigen and the peripheral wells containing the sera (OIE, 2008).

DNA Extraction for PCR

DNA was extracted from the tissue homogenate using QIAamp® DNA mini extraction (QIAGEN, Hilden, Germany) following the manufacturer's instruction. The DNA was kept at -20 ^{0}C until used.

Polymerase Chain Reaction

Polymerase Chain Reaction was carried out to amplify the 186-bp region on the highly conserved VP2 coding gene of CAV. The primers used were as follows: 5' GCA GTA GGT ATA CGC AAG GC 3' (forward) and 5' CTG AAC GTT GAT GGT C 3' (reverse) and were synthesized as published by Eltahir et al. (2011). The reaction was carried out in a 50 µL reaction volume consisting of 2 µL of DNA, 5 µL of 10X reaction buffer (Tris-hydrochloric acid), 2mM dNTPs, 1.8 mM $MgCl_2$, 1 U of *Taq* DNA polymerase, 20 pmol of each primer, and nuclease- free water. The PCR thermal cycler (GeneAmp, Applied Biosystem, USA) reactions consisted of denaturation for 2 minutes at 94 °C followed by 35 cycles of denaturation for 30 seconds at 94 °C, annealing for 30 seconds at 60 °C and extension for 1 minute at 72 °C. After the final extension at 72 °C for 7 min, the samples were held at 4 °C.

Results

Agar Gel Immunodiffusion (AGID) Test

The antigen against IBDV was detected by AGID (Figure 7). The precipitin line were seen on the positive control (+ve) and the samples labeled A. There was no line around the negative control (-ve). The bursal sample screened for IBDV antigen was positive as indicated by the presence of a precipitin line (Figure 7).

Figure 7: *Agar gel immunodiffusion test positive wells with a precipitin between positive control (+ve) and wells A. There was no precipitin around the negative control (-ve)*

Detection of Chicken Anemia Virus DNA in Bursa of Fabricius by PCR

Analysis by agar gel electrophoresis showed a single DNA fragment of 186bp produced from the DNA extracted from the bursa subjected to PCR amplification (Figure 8). The size of the PCR product in lane 1 and lane 2 which are the DNA samples was the same as the product of the positive control of CAV in lane 3. The ladder was 50bp.

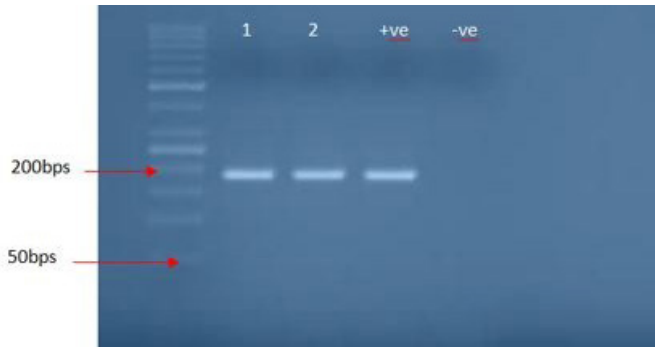

Figure 8: *Visualization of polymerase reaction product of chicken anemia virus from bursa of chicken,* **Lanes 1 and 2** *are the sample; +ve is the positive control and –ve ois the negative control; Positive samples were amplified at 186 bps and the ladder is 50 bps*

Discussion

Concurrent infections of CAV and other poultry diseases are common in commercial poultry flock, and birds under intensive production are vulnerable to immunosuppression (Hoerr, 2010; Smyth and Schat, 2013). Epidemiological data shows that CAV and IBDV are causing problems in commercial poultry farms despite maternal immunity and vaccination (Toro et al., 2009). Chicken anemia virus and IBDV are resistant to chemical and physical agents, the viruses persist in poultry farm premises and hence rendering control difficult (Schat and van Santen, 2008; Toro et al., 2009). In this report CIA was not initially a differential diagnosis, because the clinical signs and gross lesions were highly suggestive of IBD which had diverted the attention of the clinician from possible concurrent outbreak of CIA and IBD. Severely inflamed and hemorrhagic BF and hemorrhages at the proventriculus-gizzard junction are usually observed in very virulent IBD (vvIBD) (Smyth and Schat, 2013; Abdu, 2014). The similarities of clinical presentations of CIA and IBD based on age of birds affected, clinical signs and gross lesions makes diagnosis of CIA in the field difficult in case of co-infection with IBD without laboratory screening as initially experienced in this report (Adair, 2000). In addition, field outbreaks involving young chickens showing signs of ruffled feathers, depression, gross lesions of muscular haemorrhages and high mortality are associated with IBD alone, therefore leading to possible misdiagnosis of CIA alone or in co-infection with IBD (Oluwayelu et al., 2005; Smyth and Schat, 2013). Hemorrhages seen in chickens with IBD may, in some instances, be a consequence of CAV rather than IBDV infection alone (Schat and van Santen 2008).

Detection of CAV in this case report was conducted retrospectively using PCR because of history of reoccurring and severe outbreaks of IBD on the farm despite all the necessary vaccinations and biosecurity measures taken by the farmer to forestall such outbreaks. From previous reports in Nigeria, CAV was retrospectively detected using PCR and isolated from cases initially diagnosed only as IBD (Oluwayelu et al., 2005; Oluwayelu, 2010; Owoade et al., 2010). Similarly, a study carried out in Zaria (Nigeria) and environs, revealed that 57.4% of the chicken flocks tested for antibodies to CIA were positive and 38.3% were seropositive for both CIA and IBD antibodies (Okpanachi, 2015). The reports above further elucidate the

fact that CAV is circulating in Nigerian poultry flocks but CIA is not being routinely diagnosed in the field.

High morbidity and mortality experienced in the flock in this report maybe as a result of immunosuppression caused by subclinical CAV infection which exacerbated IBDV infection in the flock, although the birds were vaccinated against IBD, they were not tested after vaccination to determine if the vaccination conferred immunity against IBD. Enhanced morbidity and mortality have been reported in concurrent infections of CAV with IBDV as a result of virus-induced immunosuppression (Schat and van Santen 2008; Hoerr, 2010). Chicken anemia virus has experimentally been demonstrated to enhance the pathogenicity of IBD, MD and ND viruses (Adair, 2000; Smyth and Schat, 2013). Studies have also shown that there is a synergistic effect between CAV and IBDV and both viruses enhance the effect of each other by preventing immune response of the birds (Toro et al., 2009; Hoerr, 2010).

Conclusion and Recommendations

This is a confirmed case of CIA and IBD in a flock and the first case report of a natural co-infection of CAV and IBDV in commercial pullets in Nigeria. Improved biosecurity practices are recommended on poultry farms in other to prevent co-infection of CAV and IBDV. Breeders should be vaccinated against CIA to prevent transmission of CAV but concurrently permitting the transfer of maternal antibodies to their offspring. Clinicians in the field should also and always include CIA as a differential diagnosis for IBD. It is recommended that a national surveillance be conducted to determine the correct and current status of CIA infection in Nigeria and strains of IBDV involved disease in outcome.

Acknowledgement

We acknowledge the National Veterinary Research Institute, Vom, Nigeria where the laboratory analysis were done. We also like to acknowledge Mr. Ismail Shittu who donate the primers used for the PCR, Miss Nneka Chima, Dyek Yohanna Dyek and Adrian Maguda who assisted with laboratory analysis.

Conflict of Interest

There is no conflict of interest in this study.

Authors' Contribution

APA, AAJ, PSB, ISJ and JTM designed the work, conducted the field work and collected the samples, AJA, NIO,OA conducted the laboratory investigations, APA, AAJ, SNM, OKI, LDD and NIO wrote the manuscript and all read and approved the manuscript.

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