

## REPORT OF MIXED INFECTION OF INFECTIOUS BURSAL DISEASE AND CHICKEN INFECTIOUS ANAEMIA VIRUSES

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### ABSTRACT

Infectious bursa disease (IBD) was tentatively diagnosed from an experimental cockerel flock. Clinical signs observed included dullness, diarrhoea characterized by greenish yellow colour and spiking mortality lasting four days. Post-mortem examination results showed pale and slightly enlarged liver and kidney, enlarged and haemorrhagic bursa, petechia haemorrhages on the spleen, keel and thigh muscles and thymus atrophy. Pale liver, thymus atrophy and haemorrhages in the bursa could also be presented in Chicken Infectious Anaemia (CIA) infection. The organs (Bursa, kidney, thymus and liver) from where the infected birds were further screened for IBD and CIA viruses using the polymerase chain reaction technique and were positive for both. This confirms IBD and CIA are responsible for the losses in the flock. This implies that many CIA infections or its mixed infection may be mistaken for IBD alone if no confirmatory diagnosis is carried out, since most post-mortem lesions presenting extensive syndrome are considered to be caused by IBD.

### INTRODUCTION

Infectious bursal disease is a highly contagious immunosuppressive infection of young chickens (Lukert & Saif, 1997; van den Berg, 2000). The disease is caused by infectious bursal disease virus (IBDV), classified in the *Avibirnavirus* genus of the family *Birnaviridae* (Murphy *et al.*, 1995). Infectious bursal disease was first described by Cosgrove (1962) in the US, and subsequently from other countries including Nigeria (Ojo *et al.*, 1973; Onunkwo, 1975). The virus is important because of its potential for inducing immunosuppression alone or in combination with other infectious agents. Its economic importance manifests in two ways, first, some IBD virus strains may cause up to 20% mortality in chickens

of 3 weeks age and older, the second and more important manifestation is a severe, prolonged immunosuppression of chickens infected at an early age.

Chicken Infections anaemia is a disease in poultry characterized by aplastic anaemia and generalized lymphoid atrophy with concomitant immunosuppression and is usually complicated by secondary viral, bacterial or fungal infections. It was first recognized in young chicken by Yuasa *et al.* (1979). It has also been reported in Nigeria by Owoade *et al.* (2004) and Oluwayelu *et al.* (2005). Chicken Infections Anemia virus (CIAV) plays a major role in many of the multifactorial diseases associated with haemorrhagic syndrome and or aplastic anemia. Chicken

Infectious anaemia virus, the only member of the Gyrovirus Circovirus (family Circoviridae) is a non-enveloped, icosahedral virus of about 25nm in diameter with a negative sense single-stranded circular DNA genome.

The muscular haemorrhages and the immunosuppressive nature of IBD and CIA infections do not allow for proper distinguishing of the infections especially when there is dual infection of birds with both viruses. The pathognomonic enlarged cloacal bursa in IBD can result in diagnosing all mixed infections of both diseases as IBD infection only, and this will give a false impression on the endemicity or prevalence of CIA in the environment. Hence, this study involves using the polymerase chain reaction technique in detecting IBD and CIA viruses from bursa, liver, spleen, kidney and thymus obtained from chickens that were tentatively diagnosed with IBD based on post mortem examination.

## **MATERIALS AND METHODS**

Sixty day old cockerels were obtained from a local hatchery with the aim of performing chemo prophylactic experiment on them. Birds were raised in experimental cages from day old, chicks mash and water were given ad-libitum and were vaccinated against Newcastle disease orally using the Lasota strain of Newcastle disease vaccine virus at 17 days of age. B1 strain of Newcastle disease vaccine had previously been given to the chicks at the hatchery.

Clinical signs including dullness, anorexia, and greenish/yellow diarrhea were noticed when birds were 32 days old and mortalities began to occur before the commencement of the intended experiment. There were no preventive medication and no treatment

given to the birds before and during the outbreak.

Post mortem examination was carried out on all the birds that died. Samples of the bursa, kidney, thymus and liver were also collected and screened for IBD and CIA using the polymerase chain reaction technique.

### ***Detection of IBD and CIAV by PCR Isolation and purification of RNA from sample***

Samples were homogenized in PBS, the RNA and DNA were extracted from 140µl of the homogenized samples using the QIAamp viral RNA mini kit following the manufacturers' instructions.

### ***RT-PCR for IBD RNA***

Reverse transcription was done by mixing 5µl of extracted RNA with 7µl of mix 1 which consist of 1µl of double distilled H<sub>2</sub>O (ddH<sub>2</sub>O), 1µl of dNTPs (10mM), and 5µl of random primer (0.03 µg/ µl) and was incubated at 72°C for 10min. Eight microlitre (8µl) of mix 2 which contain 2µl of DDT, 0.5µl of RNAase inhibitor, 0.5 µl of double distilled H<sub>2</sub>O, 4µl of 5x first strand Buffer and 1 µl of superscript was added and then incubate at 50°C for 1 hour 20 minutes and 70°C for 15 minutes.

### ***Polymerase Chain Reaction (PCR) for IBDV and CIAV***

The polymerase chain reaction for IBDV and CIAV was done separately by adding 2.5µl of cDNA (DNA for CIAV) to 22.5µl of PCR mix containing 17.99µl (16.4µl for CIAV) of ddH<sub>2</sub>O, 2.5µl of 10X PCR Buffer, 1.0µl (2µl for CIAV) of MgCl<sub>2</sub> (50mM), 0.5µl of dNTP (10mM), 0.25µl of forward primer F4' (5' AGTGACAGGCCCA-GAGTCTACAC3'), reverse primer R' (ACCAGGTTCTTTGCTAGTTCAG3')

which amplifies 450bp on the VP2 strand of IBDV, 0.5µl of forward primer 03F (5'CAAGTAATTTCAAATGAACG3'), 0.5µl of reverse primer 03R (5'TTGCCATCTTACAGTCTTAT3'), which amplifies 386bp on from the DNA of CIAV and 0.1µl of taq polymerase (5µ/µl). The PCR reactions were carried out using the following cycling conditions; initial denaturation at 94°C for 5min, 35 cycles of amplification at 94°C for 30 sec, 58°C (54°C for CIAV) for 30 sec and 72°C for 1min and final extension at 72°C for 5min. The PCR product sizes were visualized by UV illumination in 2% agarose gel stained with ethidium bromide as compared to the 1kb+ size market (Invitrogen).

## RESULTS AND DISCUSSION

Following the occurrence of clinical signs in birds, mortalities began to occur among the birds. Mortality rate had a spiking occurrence which is characteristic of IBD (Table 1). Post mortem examination carried out on dead birds revealed the presence of a pale and enlarged liver, enlarged kidney, enlarged and hemorrhagic bursa, petechial hemorrhages on the spleen, hydropericardium with extensive hemorrhages on the keel and thigh muscle (Table 2). The clinical signs and post mortem lesions were suggestive of IBD and a tentative diagnosis of IBD was made. However further examination was carried out using the PCR technique by screening samples from the dead birds for IBDV and CIAV due to presence of extensive hemorrhage. The result of the PCR indicated all organs including Bursa of Fabri-

cius, kidney, liver, spleen and thymus were positive for both IBDV and CIAV. The PCR technique result further confirmed the tentative diagnosis and also detected the involvement of another viral infection which would not have been diagnosed ordinarily. Chicken infectious anemia virus is not commonly diagnosed probably not due to the absence of the virus or low incidence but most likely because it does not have a pathognomonic lesion and laboratory test are not carried out most times. The detection of CIAV further confirms the presence of the virus in Nigerian poultry flocks as reported by Owoade *et al.* (2004) were 6 out of 7 flocks tested for CIAV antibody tested positive. Chicken infectious anemia seroprevalence in chickens has been reported to be high in many countries (Dergham, 2006). Prior to the detection of CIAV antibody in Nigerian chicken, post mortem examinations presenting haemorrhagic syndrome in young chicken were considered to be caused by IBDV. None was reported to be a mixed infection of IBD and CIA.

As a result it is recommended that diseases that present extensive hemorrhage at post mortem should undergo further screening for the presence of CIAV. Further studies are also necessary to assess the economic losses due to a mixed infection of IBD and CIA.

**Table 1: Mortality and morbidity pattern**

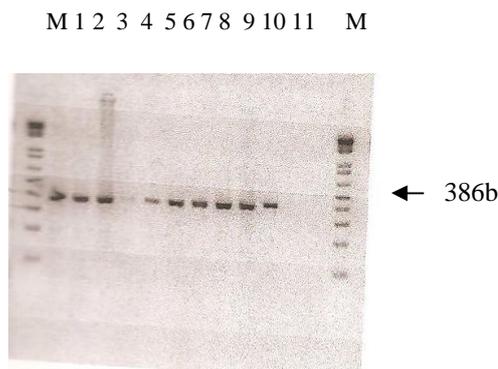
Age (days)	Clinical signs	Mortality
8	-	1
26	-	1
32	Dullness (35)*	1
33	Dullness and anorexia (35)*	4
34	Dullness. Anorexia and greenish diarrhoea. (30)*	8
35	Dullness. Anorexia and greenish diarrhoea. (22)*	1
36	Dullness. Anorexia and greenish diarrhoea. (12)*	0
37	None	0

\*Number involved

**Table 2: Post Mortem lesions**

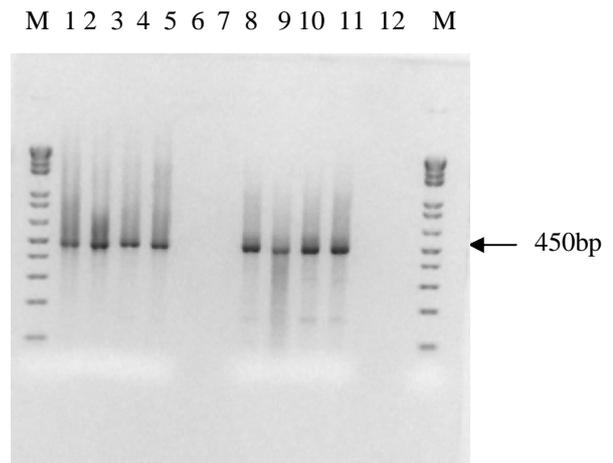
Organ	Lesions
Kidney (14)*	Slightly enlarged
Liver (16)*	Pale and slightly enlarged
Bursa (26)*	Hemorrhagic and enlarged
Spleen (13)*	Hemorrhagic
Keel and thigh muscle (19)*	Extensive hemorrhage
Thymus atrophy (22)*	Hydropericardium:

\*Number involved



M=Marker lane, lane 10 = Positive control  
Lane 11 = Negative control.  
Lanes 1 to 9 tissue samples

**Fig 1: Electrophoresis bands of CAV detection**



**Fig 2: Electrophoresis band for IBDV detection**

## ACKNOWLEDGEMENT

The authors are grateful to Prof. C.P. Muller of LNS, Institute of Immunology, Luxembourg for the supply of equipment and reagents used in this study.

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(Manuscript received: 15th January, 2010; accepted: 18th June, 2010).