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Shekaro A.


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Infectious Bursal Disease Outbreak in Fifteen Weeks Old Pullets in Kaduna, Nigeria

*1Shekaro A. and 2Josiah I. E.

1 National Veterinary Research Institute, Vom Nigeria.
2 Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.

Abstract

A case of infectious bursal disease outbreak in a flock of 100 Dominant black pullets, age 15-weeks, raise under intensive management system on a deep litter at a backyard farm. History revealed that the birds were vaccinated against Infectious bursal disease at one and three weeks of age using unidentified foreign Infectious bursal disease vaccine. An unidentified dose of Virucine (R) solution was added to the vaccine mixture. Infectious bursal disease was diagnosed base on clinical signs, postmortem findings, histopathological studies of the bursa of Fabricius and agar gel immunodiffusion test. The clinical signs observed on the sick birds in the farm include diarrhea, somnolence and anorexia. The disease ran a three day course with a mortality pattern of 2, on the first day; then 17 on the second day, and 5 on the third day. Necropsy findings showed echymotic to diffuse hemorrhages on the pectoral muscles, the bursa of Fabricius was enlarged, edematous and turgid, the kidneys were enlarged while the cloaca was swollen and filled with whitish to yellowish feces. Histopathological lesions in the bursae of Fabricius showed areas of cystic formation, interfollicular fibrosis and degeneration of the follicles. The bursae of Fabricius of the dead chickens were confirmed positive for Infectious bursal disease viral antigens by Agar gel immunodiffusion test. It was concluded that Infectious bursal disease, commonly observed in young birds of age between 3-6 weeks old was reported in growers of 15 weeks old in this study.

Keywords: Infectious bursal disease, pullets, vaccination, virucine (R) solution.
Introduction

Infectious bursal disease (IBD), otherwise known as Gumboro disease, is an acute and highly contagious viral disease of mostly young chickens caused by a non-envelope double stranded RNA virus belonging to a family Birnaviridae (Parkhurst, 1964; Lukert and Saif, 1997; Hair-Bejo et al., 2004). Infectious bursal disease is extremely contagious, in infected flocks, morbidity is high with up to 100% seroconversion after infection, whilst mortality is variable (van den Berg et al., 2000). The disease is responsible for great economic losses in the poultry industry worldwide (Lukert and Saif, 1997; van den Berg et al., 2000; OIE, 2008). In Nigeria IBD ranks second to Newcastle disease (ND) of highest prevalence (Obi et al., 2008).

The enormous economic losses associated with the disease is due to high mortality, decreased performance, immunosuppression that leads to increase susceptibility to other diseases and decrease response to vaccination (Abdu et al., 2001; Khan et al., 2007; OIE, 2008). Other names of the disease entity based on the lesions produced includes; avian nephrosis-nephritis and avian infectious bursitis.

There are two distinct serotypes (1 and 2) of infectious bursal disease virus (IBDV) that have been reported (Lukert and Saif, 1997). However, only serotypes 1 has been associated with clinical disease, thereby all commercial vaccines available have been prepared against serotype 1 (OIE, 2008). Out of the four existing pathotypes of serotype 1, very virulent IBD (vvIBDV) have been incriminated for most vaccination failures (Lukert and Saif, 1997).

Infectious bursal disease has a tropism to actively dividing pre-B lymphocytes, primarily in the bursa of Fabricius, but also in the other organs of the immune system (Anru et al., 2011). The most susceptible age groups to IBDV are chickens between the ages of 3 and 6 weeks, at which age the bursa of Fabricius is at its peak of development where follicles are filled with immature lymphocytes (Lukert and Saif, 1997; Baxandale, 1981; Khan et al., 2007). Outbreaks of IBD in vaccinated chickens of between 14 and 20 weeks of age have been reported in Nigeria (Okoye and Uzoukwu, 1981; Owoade and Durojaiye, 1995; Igbokwe et al., 1996 and Musa et al., 2010). Factors that have been reported to affect the susceptibility of chickens to IBD infection include; the type of chickens, sex, virus strain, presence or absence of maternally derived antibodies and presence or absence of certain antioxidant supplements in feeds (David, 2000; Okoye and Uzoukwu, 2000; Abdu et al., 2001; deWitt et al., 2001; Khan et al., 2007; Shekaro et al., 2012). In Nigeria IBD has continued to be a serious challenge to commercial poultry industry with the occurrence of outbreaks in both vaccinated and non-vaccinated flocks (Abdu, 1997; Ezeokonkwo, 1997; Oyekunle and Adeniji, 2008).

This paper report an outbreak of IBD in 15-weeks old Dominant black pullets in a backyard farm in Kaduna Nigeria, vaccinated twice with IBD vaccine in mixture with Virucine (R) solution.

Case Report

The case involved a flock of 100 Dominant black pullets, age 15-weeks, raise under intensive management system on a deep litter at a backyard farm. The birds were said to have been vaccinated against IBD at one and three weeks of age using unidentified foreign IBD vaccine purchased from local vaccine vendor and administered by the farmer herself. History also revealed that an unidentified dose of Virucine (R) solution (a locally produced anti-viral drug, with a composition of active iodine, boric acid, ascorbic acid and vitamin K) was added to the vaccine mixture. The birds were on enrofloxacin and water soluble multivitamins medication at the time of presentation. Furthermore they were debeaked on the day the first symptom of illness was noticed. The birds had earlier received Newcastle disease vaccine (La Sota), fowl pox vaccine and Newcastle disease vaccine (Komarov) at second and sixth weeks of age respectively.

Materials and Methods

Clinical Signs

The clinical signs were observed through visual evaluation of the birds in their pen without physical contact (Hassan and Egege, 2004). The temperament, condition, gait, discharges and the
characteristics of fecal droppings on the liter were observed and noted.

**Necropsy Examination**
Post mortem examination of the dead carcasses was carried out using the method described by Carol and Peter, 2009.

**Histopathology**
Tissues were collected from the bursae of Fabricius and fixed in 10% buffered neutral formalin solution. The tissues were cut into blocks and identified and were then dehydrated through a series of graded alcohols (70%, 80%, 90%, 95% and 100%). The blocks were cleared in xylene and then infiltrated with molten paraffin wax. Sections of 5 microns (μm) were cut from embedded tissue using Jung Rotary Microtome (model 42339). The tissues were mounted on grease free clean glass slides and kept at room temperature then stained alternatively with Hematoxylin and Eosin (H & E). The prepared slides were studied using light microscope (Olympus binocular microscope) and examined at x 40 and x 100 magnifications. Photomicrographs of the tissues were taken using a digital microscopic objective camera. The pictures were transferred to a computer and detail studies carried out to ascertain the histopathological lesions (Babiker and Tawfeeg, 2008).

**Identification of Infectious Bursal Disease Viral Antigen**
The identification of IBD viral antigen from the bursa of Fabricius was done using Agar Gel Immunodiffusion test protocol, as described by OIE, 2008.

**Results**

**Clinical Signs**
On farm visit, the poultry house was observed to be poorly ventilated with the birds overcrowded in the pen (plate I). Clinical history revealed sudden death of two birds from the flock without prior signs of illness. The disease ran a three day course with a mortality pattern of 2, on the first day; then 17 on the second day and 5 on the third day. The clinical signs observed on the sick birds in the farm include whitish yellowish diarrhea, somnolence and anorexia.

![Plate I: Cross section of 15 weeks old pullets with IBD outbreak, (note the overcrowdings).](image-url)
Necropsy Findings

Five carcasses from the birds that died on the second day were presented for necropsy. At necropsy all the carcasses were observed to be well fleshted, with their crops moderately filled with ingested feeds. Ecchymotic diffuse hemorrhages were observed on the pectoral muscles (plate II).

The bursa of Fabricius was enlarged, edematous and turgid (plates III), the kidneys were also enlarged (plate IV). The cloacae was swollen and filled with whitish to yellowish feces.–There were no significant pathological findings in the upper and lower respiratory tracts but hydro pericardium was observed in one of the carcasses. No specific pathological findings were observed on the Proventriculus and gizzards.
Histopathology

Histopathological lesions in the bursae of Fabricius showed areas of cystic formation, interfollicular fibrosis and degeneration of the follicles (Plate V).
Agar Gel Immunodiffusion Test

The bursae of Fabricius of the dead chickens were confirmed positive for IBDV antigens by AGID test.

Diagnosis

In the present report our differential diagnosis based on clinical signs and postmortem lesions was Infectious bursal disease, Coccidiosis, Infectious bronchitis and Marek’s disease (Lukert and Saif, 2003; Musa et al., 2010). We however arrived at tentative diagnosis of IBD. The disease was confirmed by Agar gel immunodiffusion (AGID) test and Histopathological findings observed in the bursa of Fabricius (van den Berg et al., 2000; OIE, 2008; Anru et al., 2011).

To manage the flock, we advised the farmer to administer multivitamins/electrolyte (Vitalyte®) in drinking water for five days and improve ventilation in the pen. She was advised to decontaminate the poultry pen and allow the house to rest for at least three weeks whenever the surviving birds were disposed of, before she re-instock. We also, discourage the farmer from using Virucine (R) solution mix with IBD vaccine before administering to birds.

Discussion

The clinical signs and postmortem lesions observed in this report was confused with coccidiosis because of the spiky mortality and whitish diarrhea but muscular hemorrhages, bursal enlargement and edema and kidney enlargement differentiated it from coccidiosis. Also cecal and intestinal hemorrhages, a common feature in coccidian infection was absent in the present report. The case was also confused for Infectious bronchitis (IB) because of the age of the chickens involved and the enlargement of the kidneys, but the absent of respiratory rales, catarrhal exudates in nasal cavities and caseous plugs in the bronchi which are characteristic features in IB infection (Lukert and Saif, 2003) was absent in the present case. Moreover muscular hemorrhages and bursal enlargement observed in this case distinguished it from IB infection. Another disease entity that was confused with the present case is Marek’s disease (MD), this was because of the age of the birds and
bursal enlargement that was observed, but the absence of visceral tumors, unilateral or binocular tumors which is a characteristic feature of MD made it to be ruled out, likewise the spiky mortality curve, short course of the disease and muscular hemorrhages (Lukert and Saif, 2003) noticed in this case, ruled out MD in favor of IBD.

The bursae of Fabricius of the dead chickens in the present report were confirmed positive for IBDV antigens by AGID test. Agar gel immunodiffusion test is one of the alternative tests recommended for IBD diagnosis by OIE in its list of tests for international trade (OIE, 2012). The detection of the IBDV antigen in the bursae of Fabricius by AGID test suggests that we were dealing with acute form of the disease (van den Berg, 2000).

The Histopathological findings base on the detection of modifications occurring in the bursa of Fabricius has been reported in IBD by Van den Berg (2000). In the present case, the histopathological lesions in the bursae of Fabricius showed areas of cystic formation, interfollicular fibrosis and degeneration of the follicles similar to the report of Van den Berg (2000).

Like most poultry producing industry of the world, IBD infection in Nigeria has mostly been reported in young chicks of between 3-8 weeks of age, with peak occurrence at 6 weeks old (David, 2000; Abdu et al., 2001; Khan et al., 2007). Nevertheless, there have been reported cases of IBD in older birds of between 11-20 weeks old in both vaccinated and non-vaccinated birds in some parts of north western, north central and north eastern Nigeria and other parts of the world, in the breeds of Shika brown, Harco brown, Black harco, Isa brown and local Nigerian breeds (Ley et al., 1979; Okoye and Uzoukwu, 1981; Owoade and Durojaiye, 1995; Igbokwe et al., 1996 and Musa et al., 2010). Improper vaccine handling, storage, poor biosecurity and failure to identify the best time of age to vaccinate chicks against IBD have been identified as reasons for some of these vaccine failures. In the present case in addition to some of the factors mentioned above the use of virucine (R) solution in mixture with the live IBDV vaccine could have contributed to the vaccine failure and hence the outbreak of IBD in the flock despite vaccination.

In Nigeria, vaccination has remained the major means of preventing and controlling the IBD infections in poultry, though this has not achieve the total control of the disease, due to factors like; vaccine handling, vaccine administration, type of vaccine, potency, schedule of use and poor biosecurity, have been identified as some of the causes of vaccine failures in Nigeria (Abdu et al., 2001). In the present study, poor biosecurity measures and improper vaccine handling and administration were observed as the probable cause of the IBD vaccine failure, hence the outbreak in the farm.

It has been observed that some poultry farmers routinely add Virucine (R) solution to already constituted IBD vaccine before administering same to their birds; this was similarly observed in the present report. Their reason is that addition of Virucine (R) solution into IBD vaccine mixture ameliorates post vaccination reactions. An Iodinated derivative (one of the active ingredients of Virucine (R) solution) has been reported to be active against IBDV (Landgraf et al., 1967; Meulemans and Halen, 1982 and Shirai et al., 1994). This could suggest why the IBD vaccine given in this case report could not protect the birds against IBDV challenge, even after receiving the live vaccine twice the iodinated component of the virucine solution could have inactivated the vaccine virus.

It is good practice to evaluate the immune status of chicks before vaccination to ascertain the levels of maternally derived antibody to IBDV, this will help in determining the right time of vaccination (Tsukamoto et al., 1995 and Haddad et al., 1997), it is equally important to evaluate the immune status of birds after vaccination with IBD vaccine to be sure of the potency of the vaccine and induction of enough antibody titer to the IBDV. This procedure was not followed in the present report.

The tropism of IBDV to B-lymphocytes makes the bursa of Fabricius an important organ in the pathogenesis of IBDV, since it serves as a reservoir where B-lymphocytes develops and differentiates in birds (Weis and Kaufer, 1994). To further underscore the importance of bursa of Fabricius in
IBDV infection, bursectomised birds at young age have been found to be refractory to IBDV infection (Baxendale, 1981). The bursa of Fabricius begin to develop on the fourth day of embryonic development until about 6 weeks of age when it attains maximum rate of development, there after it starts to regress in size (Auli et al., 1981), thus making birds between the age of 3-6 weeks most susceptible to IBDV infection. However certain breeds of birds such as Rhodes Island, Red and Bared crosses; their maximum weight of bursa of Fabricius occurs between the ages of 8-11 weeks and 10-12 weeks respectively (Baxendale, 1981), the maximum bursal weight could occur at more older ages in other breeds of birds than the one mentioned above thus could make such breeds susceptible to IBDV infection at later age in life as observed in the present case.

**Conclusion**

Infectious bursal disease commonly observed in the young birds of age between 3-6 weeks was reported in growers of 15 weeks old in this study. Improper vaccine administration (addition of Virucine (R) solution in mixture with IBD vaccine), poor vaccine handling and poor biosecurity condition of the farm, suggests why the IBD vaccine failed and subsequent outbreak of IBD in the face of the field challenge.

**Recommendations**

Farmers should be discourage on the use of Virucine (R) solution mix with constituted IBD vaccine meant to be administer to birds. Producers of IBD vaccines should as a policy include in their user manual stating that use of substances such as Virucine (R) solution in mixture with constituted IBD vaccine could inactivate the vaccine virus. Routine evaluation of the immune status of vaccinated birds against IBDV should be encouraged to ascertain the levels of antibody titer. It should be noted that biosecurity is indispensable in the control of IBD, in addition to vaccination against the disease. Farmers in Nigeria should be educated on proper IBD vaccine handling and administration, or better still be advice to engage the services of trained veterinarians in vaccinating their birds rather than self-vaccination.

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