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A TYPICAL INFECTIOUS BURSAL DISEASE WITH MDR *ESCHERICHIA COLI* CO-INFECTION AMONG 11-WEEK-OLD PULLETS

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ABSTRACT

Infectious bursal disease (IBD) outbreak was reported in a flock of 11-week-old hyaline pullets on a commercial poultry farm located in Agbado town of Ogun State, Nigeria. The diagnosis of the disease was based on clinical signs and pathological lesions followed by assessment of IBD antibody sero-profile status by the Idexx ELISA technique. Mortality and morbidity were 45.6 and 93.7% respectively. Mortality pattern was a-typical of common IBD cases. There was secondary infection of multi-drug resistant *Escherichia coli*. Re-brooding and timely administration of appropriate antibiotic to control the secondary bacterial infection were important in reducing case fatality during the outbreak.

Key words: Antibiotic resistance, co-infection, *Escherichia coli,* Immunosuppression, infectious bursal disease, re-brooding

INTRODUCTION

Infectious bursal disease (IBD), otherwise known as Gumboro disease, has been reported throughout the world, and the socio-economic significance of the disease is considerable world-wide (van-den-Berg *et al.*, 2000). IBD is caused by infectious bursal disease virus (IBDV). IBDV is a member of the Birnaviridae family. The virus exhibits a wide range of immuno-suppressive potentials, pathogenicity and virulence for chickens (Brandt *et al.*, 2001).

Clinical and subclinical infections with IBDV often cause immuno-suppression. Both humoral and cellular immune responses are compromised (Sharma *et al.*, 2000). Numerous workers have long time documented the immunosuppresive effects of IBDV infection on either vaccination against or infection with other diseases such as newcastle disease, infectious bronchitis and *Mycoplasma synoviae* (Giambrone *et al.*, 1977*b*); coccidiosis (Giambrone *et al.*, 1977a); and *Salmonella typhimurium* and *Escherichia coli* infections (Wyeth, 1975). High mortality and morbidity have been reported worldwide in both IBDV – vaccinated and unvaccinated flocks (Zeirenberg *et al.*, 2000).

Escherichia coli is a normal inhabitant of the intestinal tracts of humans and animals. They adhere to the epithelial cells of the intestinal tract with the aid of pili or fimbrae. The organism is of both medical and

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veterinary importance. In poultry, colibacillosis is an important disease caused by *E. coli*. Colibacillosis is either acute generalised infection, which may be fatal, or a chronic debilitating condition (Ojo, 1993).

This article reports the outbreak of IBD with a secondary infection of *E. coli* in a flock of 11-week-old hyaline pullets.

MATERIALS AND METHODS

Epidemiological Study

Investigation for infectious bursal disease outbreak was conducted on a commercial poultry farm located in Agbado town of Ogun State, Nigeria. The affected flock had a stocking capacity of 2,757 hyaline pullets of 11 weeks of age prior to disease outbreak. The flock had received 3 doses of intermediate IBD vaccine and had a very good record of growth performance.

Early on 3rd October 2005, the farmer called on the consulting veterinarian (the leading author of this article) and complained that some of his birds were weak. After preliminary investigation, with about 2.18% morbidity observed without mortality and pathognomonic signs, the birds were placed immediately on oral conflox® (Concept Pharmaceutical Ltd., Mumbai, India), an enrofloxacin. The following day, 4th October 2005, morbidity has gone over 90% with heavy mortality and clinical signs typical of IBD. Re-brooding of the flock was commenced immediately and the birds continued on oral conflox®.

The diagnosis of IBD on the farm was based on the clinical signs and pathological lesions, which were pathognomonic of IBD as described by Cosgrove (1962). This was supported by assessment of the IBD antibody seroprofile status of the flock. The clinical signs observed were ruffled feathers, whitish, brownish and yellowish green diarrhoea, refusal to eat and drink, shaking, recumbency and a sitting posture with the beak into the litter. The postmortem lesions observed included dehydration, haemorrhagic lesions on the thigh and chest muscles, enlarged kidneys with ureters filled with urates and enlarged bursa of fabricious with some of the bursae having petechial haemorrhages on their surfaces.

Bacteriological Investigations

Dead birds were taken to the laboratory for investigation of secondary bacterial infection. Organs such as liver, spleen, bursa of fabricius were harvested and blood collected from hearts of dead fowls. A loopful of fluid from these organs were spread on freshly prepared MacConkey agar. The agar plates were incubated aerobically at $37^{O}C$ for 18 – 24 hours. Pure cultures were subjected to biochemical assays. The bacterial isolates were characterised according to Cowan (1993) on the basis of their cultural, morphological and biochemical properties.

Antibiotic susceptibility tests (AST) on the isolated bacteria were determined by the disk diffusion techniques. The antibiotics and their concentrations in microgrammes were: Augmentin (30), Nitrofurantoin (20), Ofloxacin (10), Nalixidic acid (30), Gentamycin (10), Contrimoxazole (20), Amoxicillin (20) and Tetracycline (25). The results obtained were classified as highly sensitive, intermediate sensitivity and resistant.

Early on the third day of mortality, follow-

ing the outcome of the AST, conflox was withdrawn and the birds were placed on oral administration of furaltadone (a nitrofurantoin) for a period of 5 days.

IBD Antibody seroprofile assay

The IBD antibody seroprofile of the infected flock was determined during the IBD outbreak and 2 weeks post infection. IBD antibody seroprofile of a healthy flock of 2500 hyaline pullets of 7 weeks of age (2 weeks after administration of 3rd IBD vaccine), in an adjacent farm, was determined to ascertain that the flock was protected against IBD and to serve as control.

IBD antibody assay was carried out using the Idexx ELISA technique and kits (Idexx Laboratories, USA). The technique was based on indirect ELISA assay. Blood samples (0.5ml) were collected from birds via the wing veins. Blood samples were allowed to clot and sera separated by centrifugation. Fifty micro-litres of 1:500 dilutions of sera were placed in wells of antigen coated polystrene plate. Following incubation for 60 minutes at room temperature, the wells were washed to remove any unbound antibody. Conjugate was then added to the wells and allowed to incubate for 30 minutes at room temperature. The wells were washed again and an enzyme substrate-chromogen added. The plates were incubated for 10 minutes, followed by addition of stop solution. The colour changes were read photometrically at optical density of 650nm with the aid of a fully automated ELISA plate reader.

RESULTS

Infectious bursal disease (IBD) was reported in a flock of 11-week-old hyaline pullets. Morbidity and mortality were 93.7 and 45.6% respectively. The mortality pattern, illustrated in Figure 1, was found to be atypical of that commonly reported for IBD infections.

There was secondary *Escherichia coli* infection. The isolated bacterium was Gram-negative, lactose fermenter, catalase positive, oxidase negative, indole positive, produced yellow colour with gas in triple sugar iron agar and motile. The bacterium was isolated from the liver, bursal of Fabricious, spleen and heart blood of dead birds. The antibiotic susceptivity test revealed that the isolated *E. coli* was very sensitive to augmentin and nitrofurantoin. There was intermediate susceptibility to ofloxacin and total resistance to nalixidic acid, tetracycline, gentamycin, amoxicillin and contrimoxazole.

IBD antibody seroprofile during outbreak using the Idexx ELISA kits, gave an infective titre as illustrated in Figure 2. The antibody titre range was 981 – 6780 and mean ±SD was 3149 ± 1767. It was observed that 54.55% of the flock were having IBD antibody titre below the protective level of 2500 (Idexx ELISA). Two weeks post infection and barely one week after the birds recovered clinically from the infection, IBD antibody seroprofile still gave an unprotective/infective titre with a range of 1924 – 6720 and mean ±SD of 3529 ±1459. It was observed that 27.27% of the flock still had antibody titre below the protective level of 2500, Figure 3. The IBD antibody seroprofile of the uninfected 7-week-old flock, which served as control, gave a good protection with IBD antibody titre range of 5470 – 8468 and mean ±SD value of 7289 ±1024 (Fig. 4).

Medication of the infected birds with appropriate drug (furaltadone), re-brooding of birds and adequate biosecurity improved the health of the birds considerably.

DISCUSSION

Infectious bursal disease (IBD) is primarily a disease of young chicken and birds within the age of 2 and 7 weeks are most susceptible (Ojo *et al.,* 1973; Okiki, 2007). The IBD outbreak in this report occurred in a flock of 11-week-old pullets. However, the disease had earlier been reported in 20 week old chicken (Durojaiye *et al.,* 1984).

The mortality pattern (Figure 1) observed in this report was a-typical of IBD. The typical mortality pattern in IBD infection is that mortality increases from day 1 to day 3, then declines to day 5 or 7 when mortality stops completely. Re-brooding of the flock from the first day of mortality could have been responsible for the positive change in mortality pattern, otherwise mortality may have approached 100%. High mortalities in IBD infections have been associated with (a) dehydration resulting from profuse diarrhoea and inability of the birds to drink water, and (b) drop in body temperature below normal before death (Cho and Edgar, 1972; Cosgrove, 1962). Elevating the ambient temperature of the pen improved the consumption of the medicated water (enriched with vitamin) that was provided.

Infectious bursal disease virus (IBDV) causes severe immunodeficiency in young chicken by destroying the precursor of antibody producing B cells in the bursal of fabricious (Yao and Vakharia, 2001). It has been reported that chickens infected with infectious bronchitis virus (IBV) and infectious bursal disease virus (IBDV) commonly developed secondary infection of the respiratory tract with *Escherichia coli,* resulting in significant economic loses (Naqi *et al.,* 2001). Naqi *et al.* (2001) equally observed that IBDV had the potential to markedly reduce opsonizing ability of antibody, an effect that was found to be significantly exacerbated by co-infection with IBV, thereby mimicking macrophage-mediated *E coli* phagocytosis.

Eschericha coli isolated in this study was highly susceptible to Augmentin and Nitrofurantoin with intermediate susceptibility to ofloxacin. A day preceding onset of mortality the birds were placed on oral conflox, an enrofloxacin, but was replaced by oral furaltadone (a nitrofurantoin) following the outcome of the AST. A sharp drop in mortality, from the 3rd day of mortality, following medication with very sensitive drug, is an indication that the heavy mortalities observed was due to the secondary multidrug resistant *E. coli* infection. The emerging of multidrug resistant gram-negative bacteria has been reported worldwide (Yamane *et al.,* 2005).

RECOMMENDATION

1. Whenever there is IBD outbreak:
(a) The chickens should be rebrooded.
(b) (b) Bacterial isolation and AST should be carried out and the birds treated accordingly
2. IBD antibody seroprofile should be carried out on a flock 2 weeks after the last IBD vaccine is administered. If the flock is protected the result will be similar to the one presented in Figure 4. If unprotected the result will be similar to those presented in Figures 2&3 with

some IBD antibody titres falling below the protective level of 2500 (Idexx ELISA). If found unprotected it is advisable to revaccinate the flock preferably with oil based inactivated IBD vaccine.

REFERENCES

Brandt M, Yao K, Liu M, Heckert R. Vakharia V. N. 2001**.** Molecular determinants of virulence, cell tropism and pathogenic phenotype of infectious bursal disease virus. *Journal of Virology* 75 (24): 11974 – 11982.

Cho Y., Edgar S.A. 1972. Characterisation of infectious bursal disease. *Poultry Science* 5:60-69.

Cosgrove A. S. 1962. An apparently new disease of chickens – avian nephrosis. *Avian Disease* 6: 385 – 389.

Cowan S.T. 1993. *Cowan and Steel's Manual of Identification of Medical Bacteria*. Cambridge University Press, U.K.

Durojaiye O.A. Ajibade H.A., Olafimihan G.D. 1984. An outbreak of infectious bursal disease in 20 weeks-old birds. *Tropical Veterinarian* 2:175 – 176.

Giambrone J.J., Anderson W.I., Reid W. M., Eidson C.S 1977a. effect of infectious bursal disease on the severity of *Eimeria tenella* infections in broilers. *Poultry Science* 56:243 - 245.

Giambrone J. ., Kleven S.H., Eidson C. S. 1977b. Effect of infectious bursal disease on the response of chickens to *Mycoplasma synoviae*, Newcastle disease virus and infectious bronchitis virus. *American Journal of Veterinary Research* 38:251 – 253.

Naqi S. Thompson G, Bauman B., Mohammed, H. 2001. The exacerbating effect of infectious bronchitis virus infection on the infectious bursal disease virus-induced suppression of opsonisation by *Escherichia coli* antibody in chickens. *Avian Disease* 45 (1): 52 – 60.

Ojo M.O. 1993. *Manual of Pathogenic Bacteria.* Shaneson C. I. Ltd. Ibadan, Nigeria. 412 pp.

Ojo M. O., Oduye O. O., Noibi L. M., Idowu A. L. 1973. Gumboro – like disease in Nigeria. *Tropical Animal Health and Production*. 5:52 – 56.

Okiki P. A. 2007. Possible association of sulphonamide occidiostats with enhanced susceptibility of chickens to IBD. *Nigerian Journal of Animal Production* Vol. 34. (1): 156-162).

Sharma J. M., Kim I. J., Rautenschleins A. 2000. Infectious bursal disease of chickens: pathogenesis and immunosupression. *Developments in Comparative Immunology* 24 (2-3) 223-235.

van den – Berg T. P. Eterradessi N., Joquin D, Meulemans G. 2000. Infectious bursal disease (Gumboro disease). *Reviews in Science and Technology* 19(2): 509 – 543.

Wyeth P. J. 1975. Effect of infectious bursal disease on the response of chickens to *S typhimurium* and *E. coli* infections. *Veterinary Record* 96: 238 – 243.

Yamane K, Wachino J. Doi Y Kurokawa H. Arakawa Y. 2005. Global spread of multiple amynogycoside resistant genes. *Emerging Infectious Disease* 11(6): 951-953.

Yao K., Vakharia V. N. 2001. Induction of apoptosis *in vitro* by the 17 – KDA nonstructunal protein of infectious bursal disease virus: possible role in viral pathogenesis. *Virology* 285 (1) 50 – 58.

Zierenberg k., Nieper H, van ven Berg T. P, Ezeokoli C. D, Voss M. Muller H. 2000. The VP2 variable region of African and German isolates of infectious bursal disease virus; comparison with very virulent 'classical' virulent and attenuated tissue culture – adapted strains. *Archives of virology* 145 (1): 113-125.

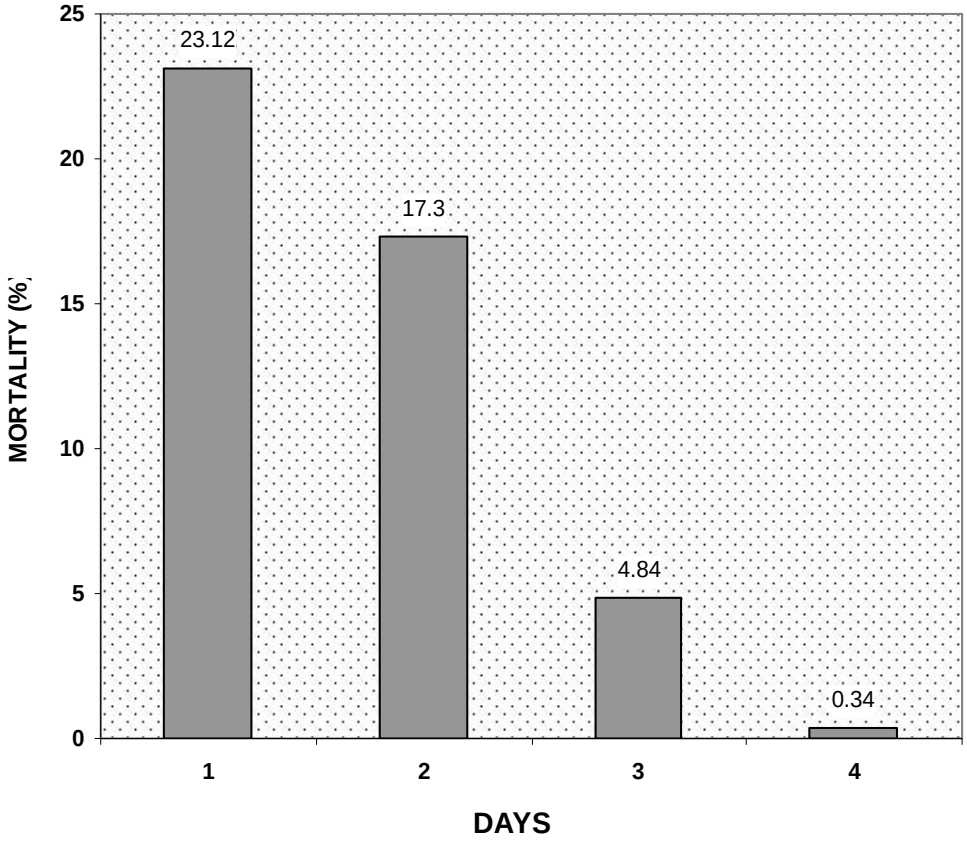

Fig. 1: Mortality pattern during IBD outbreak

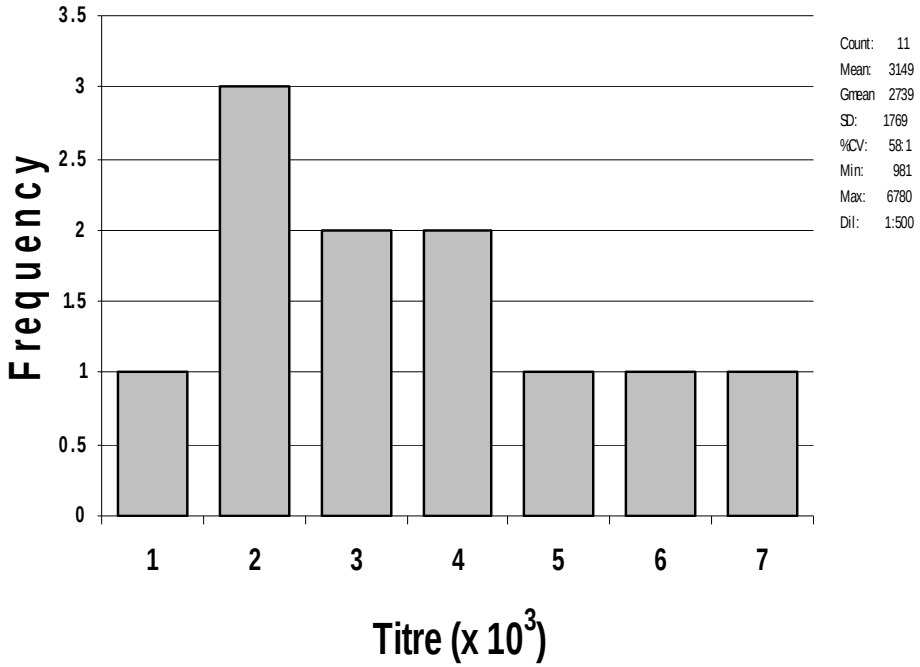

Fig. 2: IBD Antibody Sero-profile during IBD outbreak in a flock of 11 week old hyaline pullets

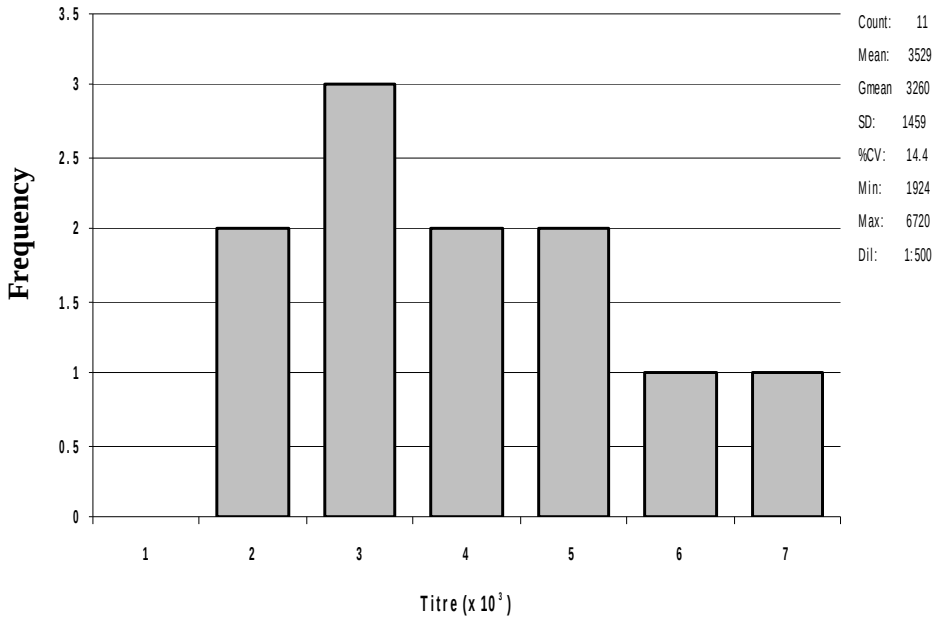

Fig. 3: IBD Antibody Sero-profile 2 weeks post IBD infection

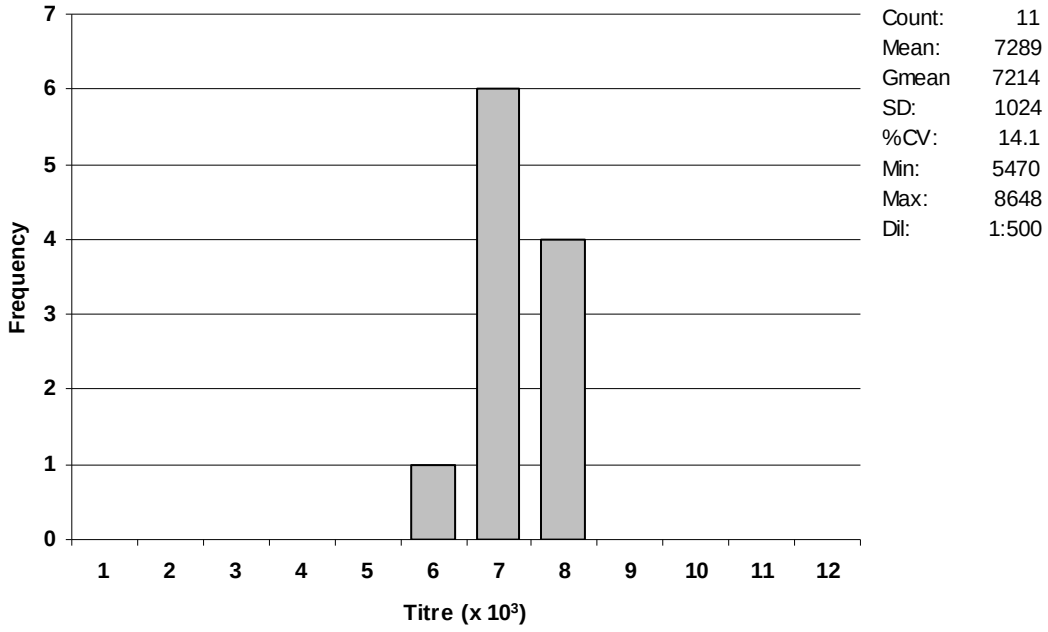

Fig. 4: IBD Antibody Sero-profile of a protected 7 week old hyaline pullets