Possible association of sulphonamide coccidiostats with enhanced susceptibility of chicken to Infectious Bursal Disease.

Okiki PA.

Department of Microbiology, University of Benin, Benin-City Nigeria.

Abstract

Infectious bursal disease (IBD) outbreak in 3 commercial poultry farms were reported. The diagnosis of the disease was based on clinical signs and pathological lesions, followed by laboratory confirmation by the Ouchterlony agar precipitation test. All the affected chickens were from vaccinated flocks. Very high mortality and morbidity were recorded; 61% mortality from flocks where coccidiostats were used compared with 31% and 32% mortalities from flocks where coccidiosis vaccines were used. A retrospective and prospective investigation of mortality patterns in the rearing flocks of these three farms, without IBD outbreak between 1999 and 2002 indicated 8.3% average mortality when sulphonamide coccidiostats were used as against 4.3% average mortality when coccidiosis vaccines were used. It is suggested that sulphonamide coccidiostats administration might be a precipitating factor of the heavy losses recorded during IBD outbreak.

Keywords: Chicken, immuno-suppression, Infectious Bursal Disease, Sulphonamide-coccidiostats, vaccine.

Introduction

Infectious bursal disease (IBD), otherwise known as Gumboro disease, is a contagious disease of *Gallus domesticus* (domestic fowl) caused by infectious bursal virus (IBDV). IBDV is a non-enveloped avian virus with a two-segmented double-stranded RNA genome enclosed in a icosahedral capsid of diameter approximately 60nm, belonging to the family Birnaviridae. IBDV exhibits a wide range of immunosuppressive potentials, pathogenicity and virulence (Brandt *et al* 2001; Chevalier *et al* 2002).

IBD is pandemic and the virus ubiquitous (Sharma *et al*, 2000; van-den-Berg *et al.*, 2000). The disease was first reported in Nigeria by Ojo *et al* (1973). Since the initial report by Ojo and his co-workers, there have been reports of outbreak of IBD year in year out in poultry farms in the country. IBD is primarily a disease of young

chickens. Chickens between the age of 2 and 7 weeks of age are most susceptible (Ojo *et al* 1973). The disease has however been reported in 20 week old chicken (Durojaiye *et al* 1984). Under natural condition chicken acquire infection by the oral route. IBDV replication takes place in the bursa of Fabricius and the IgM⁺ cells serve as target for the virus. The acute phase of the disease lasts for about 7-10 days. Within this phase bursal follicles are depleted of B cells and the bursa becomes atrophic. Abundant viral antigens can be detected in bursal follicles and other peripheral lymphoid organs such as caecal tonsils and the spleen. Chicken may die during the acute phase of the disease. Chicken that survive the acute phase clear the virus, recover from pathological effects and bursal follicles are repopulated with IgM⁺ B cells. Clinical and subclinical infection with IBDV may cause immunosuppression. Both humoral and cellular

immune responses are compromised (Sharma *et al.*, 2000).

Prior to the mid 1980s, IBD reported cases were of low mortalities but high morbidity. But since then cases of high mortalities have been reported worldwide in both vaccinated and unvaccinated flocks and the emergence of very virulent IBDV strain has been implicated (Zeirenbeg *et al.*, 2000) IBD causes heavy losses with a negative socio-economic impact on farmers, as starters may fold up the business if they encounter IBD in their flocks and discourage others of going into poultry business.

The article reports a field observation of outbreak of IBD in three poultry farms in Lagos in the year 2001. The possible precipitating factors and possible way out of this poultry business frustrating disease were discussed.

Materials and Methods

Epidemiological Investigation

Three poultry farms designated A, B and C recorded outbreak of IBD in the year 2001. The IBD infected flocks had initial stock of 2000, 1300 and 6000 Bovan Nera day old pullet chicken for farm A, B and C respectively. The flock in farm A received 3 doses of intermediate plus IBD vaccines, while those of farms B and C received 2 doses. All vaccines were administered by the oral route and within the first 28 days of life. In farm A, sulphonamide coccidiostats namely Triple sulfa (Agrar, Holland) and Superhipracox-P (Laboratorios Hipra, Spain) were used for the control of coccidiosis while farms B and C coccidiosis vaccine, Immucox® (Vetec Laboratories Inc. Ontario, Canada) was used.

The diagnoses of IBD in the 3 farms was based on the age of the chickens, mortality and morbidity patterns, the clinical signs and pathological lesions, which are pathognomonic. The clinical

signs observed included: feathers, whitish and yellowish green diarrhoea, refusal to eat and drink, recumbency and a sitting posture with the break into the litter. The postmortem lesions included: dehydration, paint brush like and ecchymotic haemorrhages in legs and thigh muscles, hepatic infarction enlarged kidneys, ureters filled with urates and enlarged bursa. The intestines were tugged containing mucus with few muscosal petechiation. The proventriculus was oedematous containing mucus with a band of haemorrhagic lesions at the posterior border of the proventriculus giving a ring shape appearance.

Laboratory Investigation

Laboratory investigations carried out, which included IBD viral antigen preparation from bursae and the Ouchterlony agar precipitation test (AGPT), were as described by Durojaiye *et al.* (1985) but with some modifications. Bursae were harvested from freshly dead chickens, frozen and kept for laboratory analysis. Virus antigen was prepared by homogenizing a one in three (weight/volume) suspension of the bursal of Fabricius in phosphate buffered saline (PBS) in a manual tissue homogenizer. The homogenate was frozen and thawed twice and clarified by centrifugal force generated by 2000 rev.min⁻¹ for 10min. The supernatant was then used as the test antigen. Each bursal sample was treated and tested separately.

Known positive serum was derived from chicken, which had received three successive intramuscular doses of IBD vaccine over a period of 17 days. The chicken reared in isolation, which was negative for precipitating antibodies, were employed as negative control.

One percent Noble agar (Oxoid, England) was prepared in 0.025M barbitone acetate buffer. The agar was layered on each glass slide 2-3mm thick.

Pairs of wells 2.5mm in diameter and separated by 6.5mm interspace were punched into each agar slide such that there were two columns of 3 pairs of wells on the longitudinal of the agar slide. Each pair of wells was filled with serial dilutions of the supernatant of the tested bursa. Each clinical sample was tested side by side with a positive control (IBD vaccine antigen and IBD positive serum) and a negative control (IBD vaccine antigen and a known IBD negative serum). Incubation was carried out at room temperature in a humid chamber. After 18-24hrs, the slides were read against an illuminated background and the appearance of a precipitin line between the antigen and a serum well was taken as an indicator of a positive reaction. A total of 63 clinical bursa specimens were tested. The set-up was as illustrated in figure 1.

Retrospective and Prospective Mortality Investigation

The mortality patterns in rearing flocks, without IBD outbreak, of chicken reared in farms A, B and C were investigated between the years 1999 and 2002. Records of 4 flocks of chickens of best performance were considered in each farm; 2 flocks reared with coccidiostats and 2 flocks reared with coccidiosis vaccines were considered for each farm. The investigation was to ascertain

the possible role of coccidiostats (sulphonamies) in chicken mortalities and its likely role in precipitating heavy losses during IBD outbreaks.

Results

Heavy losses were recorded in farms A, B and C following IBD outbreak. The severity of IBD infection was more in farm A, where the birds were on sulphonamide coccidiostats than farms B and C where coccidiosis vaccines were administered. The mortality and morbidity patterns in the three farms following IBD outbreak are contained in Table 1.

A total of 63 bursal specimens from the 3 IBD outbreaks were screened by AGPT for IBD virus antigen. Forty-nine (77.8%) of the bursal specimens produced precipitin lines with the control positive serum (Table II). Titres as high as 1 in 64 were obtained in some cases.

The rearing performance in farms A, B and C when there was no IBD outbreak were investigated. The mortalities recorded from day old to 20 weeks of age are contained in Table III. An average mortality of 8.3% when coccidiostats were used as against an average mortality of 4.8% when coccidiosis vaccines were used. This difference in mortalities between when drugs were

Figure 1: An Illustration of the Ouchterlony AGPT test on slide showing pairs of wells containing IBD antigens and sera.

Sulphonamides administration and chicken susceptibility to IBD

Table 1 IBD infected flocks in farms A, B and C

	Farm A	Farm B	Farm C
Initial stock (No. of chicks)	2000	1300	6,000
Age of chicks (days) When IBD struck	40	29	35
Duration of disease (days) 10		8	10
Mortality (%)	61	32	33
Morbidity	95	80	85
Post IBD Mortality (%) &	6	5	3
Causes	Coccidiosis	ND & CRD	ND and CRD
Infectious	Coryza		
Total Mortality (%) at 20 Weeks of age	67	37	36

ND = Newcastle disease; CRD = Chronic respiratory disease

Table II Detection of IBD virus in bursa of Fabricius Using the Ouchterlony AGPT

Farm	No. of bursae tested	No. positive	Percentage Positive
A	25	20	80.0
B	16	12	75.0
C	22	17	77.3
Total	63	49	77.8

Table III Mortality records of birds reared in farms A, B and C without IBD outbreak

Farm	Coccidiostats Stock	Administered % Mortality	Coccidiosis Stock	Vaccine Administered % Mortality
A	2500	10.0	3000	3.0
	3000	5.5	2000	4.1
B	2000	8.5	2000	5.0
	2000	12.0	2000	3.6
C	3000	9.0	3500	4.5
	6000	4.9	6000	5.6
Average Mortality		8.3	4.3	

Wilcoxin rank sum test: $P < 0.01$

used and when vaccines were used, was found to be statistically significant ($P < 0.01$) (Wilcoxin Rank Sum test).

Discussion

The outbreaks of IBD in 3 commercial poultry farms in the year 2001 were reported. The epidemiological evidence and identification by AGPT of IBDV in the harvested bursae ascertained the disease. The heavy mortalities and high in morbidity observed during the IBD outbreak in this report despite administration of IBD vaccines is food for thought. Although the very virulent IBDV strain (vvIBDV) could be implicated as a cause of outbreak because antibodies produced by IBD intermediate plus vaccine may not be able to completely neutralize vvIBD viral antigens challenge. There is thus the need for a re-examination of the IBD vaccination programme in this country.

The primary role of the avian bursa of fabricius is to provide an essential microenvironment for B-lymphocytes to diversify their immunoglobulin genes by gene hyperconversion. IBD vaccination using the intermediate plus vaccines is known to cause temporary depletion of the bursal follicles, also it interrupts normal B-cell development. But this is generally followed by

repopulation and histological regeneration (Ivan *et al* 2001). Presently in Nigeria, IBD vaccination using IBD intermediate plus vaccines is always in multiple doses post hatch and within the first 4 weeks of life. There is variation in number of doses and routes of administration from one farm to the other. The multiple dose approach subjects the bursa of Fabricius to a series of depletion and regeneration of follicles, a great disadvantage to a challenge by a virulent IBDV.

In United States of America, *in-ovo* vaccination against Marek's disease virus and IBDV is common. The safety and efficacy of *in-ovo* administration of IBD viral vaccines has been reported by Giambrone *et al* (2001). Jungmann *et al* (2001), in their investigation of the kinetics of IBDV replication and induction of apoptosis, observed that UV-inactivated IBDV particles did not induce apoptosis. We may even need to look beyond attenuated live vaccines so that at no instance is apoptosis induced in chicks.

In flock that experienced IBD outbreak, as reported above, coccidiostats were used for the control of coccidiosis in farm A while coccidiosis vaccine (Immucox) was used in farms B and C. The very high (61%) mortality in farm A during the outbreak as compared to 32% and 33% for

farms B and C respectively inspired the retrospective and prospective investigation of mortality records in flocks of chicken reared without IBD outbreak in the 3 farms between 1999 and 2002. Table III shows that an average mortality of 8.3% was recorded when sulphonamide coccidiostats were used as against 4.3% when coccidiosis vaccines were used. This difference which was found to be statistically significant ($P < 0.01$) is an indication that the coccidiostats used when there was outbreak of IBD in farm A might have enhanced the severity of the disease resulting in the very high mortality observed. Sulphonamides which are the major components of coccidiostats are known for their adverse effects in causing gastrointestinal disturbances, hypersensitivity reactions, the Stevens – Johnson syndrome, blood dyscrasias, neurological disorders, nephritic reactions among others. Sulphonamides exert their toxic effects on almost every system of the body (The Royal Pharmaceutical Society of Great Britain, 1996). Farmers are thus advised to make a shift from the traditional use of coccidiostats to the use of coccidiosis vaccines. The coccidiosis vaccines are relatively cheaper, less laborious and give more peace of mind.

References

- Brandt, M.; Yao, K; Liu, A; Heckert, R.A and Vakharia, V.N 2001. Molecular determinant of virulence, cell tropism, and pathogenic phenotype of infectious bursal disease virus. *Journal of Virology* 75 (24): 11974-82.
- Chevalier, C; Lepault, J; Erk, I; Dacosta, B and Delmas, B 2000. The maturation process of pVP2 requires assembly of infectious bursal disease virus capsid *Journal of Virology* 76 (5): 2384-92.
- Durojaiye, O.A.; Adene, D.F. and Owoade, A.A. 1985 Counter immunoelectrophoresis in diagnosis of infectious bursal disease of poultry. *Tropical Animal Health and Production* 17: 225-229.
- Dorujaiye O.A.; Ajibade, H.A and Olafimihaan, G.D. 1984. An outbreak of infectious bursal disease in 20 week-old birds. *Tropical Veterinarian* 2:175-176.
- Giambrone, J.J.; Dormitorio, T. and Brown, T. 2001. Safety and efficacy of *in-ovo* administration of infectious bursal disease viral vaccines. *Avian Disease* 45 (1): 144-8.
- Ivan, J. Nagy, N, Magyar, A; Kacs Kovics, I and Meszacros, J. 2001. Functional restoration of the bursa of Fabricius following *in-ovo* infectious bursal disease vaccination. *Veterinary Immunology and Immunopathology* 79 (3-4): 235-48.
- Jungmann, A.; Nieper, H. and Muller, H. 2001. Apoptosis is induced by infectious bursal disease virus replication in productively infected cells as well as antigen-negative cells in their vicinity. *Journal of General Virology* 82: 1107-15
- Ojo, M.O.; Oduye, O.O.; Noibi, L.M. and Idowu 1973. Gumboro-like disease in Nigeria. *Tropical Animal Health and Production* 5:52-56.
- Sharma, J.M.; Kim, I.J; Ravtenschlein, S. and Yeh, H.Y. 2000. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. *Developments*

in Comparative Immunology 24(2-3): 223-35.

The Royal Pharmaceutical Society of Great Britain 1996. Martindale – *The Extra Pharmacopoeia* James E.F. Reynolds (Editor). Williams Glows Ltd, Beccles, Suffolk.

Van-Den Berg, T.P.; Eterradosi, N.; Toquin, D. and Mevlemans, G 2000. Infectious bursal disease (Gumboro disease). *Reviews in Science and Technology* 19(2): 509-43.

Zierenberg, K; Nieper, H; Van-Den-Berge, T.P; Ezeokoli, C.D; Voss, M. and 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 tissues culture-adapted strains *Archives of Virology* 145 (1): 113-25.

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