

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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Investigation on Infectious Bursal Disease Outbreak in Debre Zeit, Ethiopia

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Abstract: An outbreak of infectious bursal disease affecting 20-45 days old broiler and layer chickens was investigated for the first time in Ethiopia in the months of March and April 2002. Death of chickens started at the 30th day of age and continues to the 55th day. The mortality rate of the disease in different poultry houses ranges from 45-50 %. The over all mortality rate was 49.89%. Broiler mortality was 56.09% while 25.08% for layer chickens. The major clinical symptoms were sudden drop in feed and water consumption, severe depression, white watery droppings and mass death. Grossly, hemorrhages in leg muscles, degeneration of the pectoral muscle, white mass (Urate deposit) in kidneys and in Cloaca were frequently observed during post mortem examination. In addition, haemorrhagic & swollen bursas filled with straw colored fluid were identified in few cases. Histopathology revealed hyperplasia of the reticulo-endothelia cells and interfollicular tissue of affected bursa of fabricius. The Agar Gel Immuno Diffusion (AGID) Test detected precipitating antibodies against Infectious Bursal disease virus in sera collected from convalescent chicken. Virus cytopathic effect was observed in chicken fibroblast cells (CFC) inoculated with bursa and spleen tissue suspension of sick chicken. Vaccination failures were encountered to Newcastle disease vaccine (Clone 30). This paper probably is the first to report the occurrence of infectious bursal disease in Ethiopia as the country has been known to be free from Infectious Bursal Disease (IBD).

Key words: AGID, CFC, IBD, pathology, symptoms, vaccination failure

Introduction

Small and large-scale chicken farms are rapidly growing in Ethiopia. The chicken strains imported are temperate breeds that are less adapted to the heat stress and disease challenges in the country. Accompanying intensification of poultry farming, there is occurrence of epidemics of newly introduced diseases and/or epidemics of endemic diseases. One of the diseases that is of growing concern in poultry is Infectious Bursal disease (Gumboro disease). As in this report a large-scale occurrence of Infectious Bursal disease in the central part of Ethiopia with intensive and high-density juvenile farms.

Infectious Bursal disease (IBD) is an acute highly contagious viral infection of young chickens (three to five weeks age) with morbidity approaching 100% and mortality up to 50% depending on virus strain and flock history. IBDV (the virus) is an RNA virus belonging to *Birnaviridae* family. The disease is characterized mainly by the swollen and hyperemic bursa of fabricius during acute stage (3 to 4 days post infection) and then severe atrophy of the organ. Accompanying symptoms include white watery droppings, accumulation of urate in the urinary structures, and severe depression (Saif and Barnes, 2003). The disease is transmitted through water, feed, droppings and through fomites (Sun Ming *et*

al., 2001).

The virus has predilection for lymphoid tissue special target organ being the bursa, and also can be isolated from the thymus, spleen, and bone marrow. Besides the loss due to mortality and morbidity, immunosuppression is a very important problem associated with IBD infection (Saif, 1998). The IBD virus destroys lymphocytes and macrophages as a result cripples the immune system with marked immunosuppressive effect leading to vaccination failures and concurrent infections (FAO, 1991). The clinical form of the disease, of less importance nowadays, occurs in chickens over weeks of age when the bursae are well developed. The greatest economic losses are due to sub clinical disease in chicks from one to twenty one days of age. At this stage the virus impairs the immune response and render the chicks susceptible to various infections. The effects of late infection from three to ten or more weeks of age result in the clinical disease. (Dwight *et al.*, 1988).

The disease has been described worldwide (Buxton and Fraser, 1987; Van den Berg *et al.*, 2000). In Ethiopia, so far there is no recorded occurrence of IBD case. (OIE, 2003). This paper states the recent introduction of infectious bursal disease to Ethiopia and discusses the

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consequential occurrence of Newcastle disease vaccine failure.

Materials and Methods

Study area and chickens: Outbreak of suspected IBD case was reported to the National Veterinary Institute from a commercial poultry farm in Debre-Zeit town located 45 Kms to the South east of the capital city, Addis Ababa. Two near by poultry farms were also affected by the outbreak. The farms share a common feed mill and vehicles. At the onset of the outbreak there were about 40,000 broilers and 10,000 layers. Both farms import day old chicks from The Netherlands and Egypt. Other farms in the same area import day old chicks from Kenya, South Africa and Germany. All chicken were vaccinated against Newcastle disease (Clone 30 ND vaccine, Intervet®, Boxmeer, Netherlands), Fowl pox (NVI, Debre-Zeit, Ethiopia). But all the chickens were not vaccinated against Gumboro.

Clinical assessment and postmortem examination: A case of outbreak with high mortality (22,437 Broiler chicken and 2508 layer chicken in 4 weeks time) was reported to the National Veterinary Institute. Affected chickens were 20-45 day old broiler and layer chicks. 40,000 Hubbard broiler chickens and 10 000 Lohman Brown layer chicks were at risk. Repeated farm visits were made and analysis was made on the clinical episodes of the disease, farm records including vaccination, age, breed, origin of the chicks, major symptoms observed and the mortality rate. Sick and dying birds were collected from five broiler and three layer houses. Autopsy was performed on two hundred sick, moribund and dead chickens and the lesions were closely examined.

Histopathology: Grossly affected bursa were histopathologically examined at the Pathology Unit of the National Animal Health Research Center (NAHRC). Haematoxyline Eosine stains were used.

Serological tests: Haemagglutination Inhibition test (Beard and Wilkes, 1985) was carried out to differentiate the disease from Newcastle disease and to verify the presence of immuno-suppression and Agar Gel Immuno- diffusion test (Hirai *et al.*, 1972) was conducted to detect the presence of precipitating antibodies to IBD virus.

Virus isolation: Virus isolation was performed by inoculating bursa and spleen tissue suspensions of the affected birds on chicken embryo fibroblast cells following a standard operating procedure as described in Cuningham (1973).

Results and Discussion

IBD has a worldwide distribution; however, there has been no reported case of infectious bursal disease in Ethiopia. This could be attributed to the resistance of the

indigenous chickens to IBDV and other viruses. With the raising commercial scale chicken farming there are growing concerns on emergence of diseases such as Marek's that recently been described both in exotic and indigenous breeds of the country (Ashenafi, 2000).

The case report described here was observed in 20-45 day old broiler and layer chicks. The owners complained that the chicks rapidly dropped feed and water consumption and diarrhea was common. The most frequent clinical symptoms were; marked drop in feed and water intake, mass death, white creamy diarrhea and soiling of the vent, loss of body weight and recovered broilers were stunted and remain unthrifty. High mortality started on the 3rd day after the onset of the observable symptoms and continued up to the 15th day. The overall mortality for both the broilers and layers by the end of 8th week was 49.89%. While mortality in broiler chicks was 56.09% and 25.1 in layer chicks. The onset of the disease in the third week of age and the overt clinical symptoms were compatible with the clinical form of the disease (Radostitis *et al.*, 1994).

Pathological lesions include hemorrhages in leg muscles usually of pinpoint type but in few cases ecchymosed; swollen kidneys filled with white 'urate' deposits; uraters filled with the white substance and further accumulation of white stony substance in cloaca. The expected lesions of swollen and hemorrhagic or atrophied bursas were less frequent. In some cases bursa was found filled with serous fluid and in some cases it was hemorrhagic. Histological studies of the affected bursa revealed congestion of the vascular layer, lymphoid cell necrosis, hyperplasia of the reticulo-endothelia cells and interfollicular tissue. The autopsy findings particularly the hemorrhages in the muscles and the pathognomonic bursa lesions were in agreement with those described by (Sun Ming *et al.*, 2001).

Serological results on Agar Gel Immuno-diffusion test, showed a strong and specific precipitating antibodies against IBDV (Table 2). AGID is known to be highly specific (OIE, 2000). Knowing that the chicks were vaccinated with clone 30 Newcastle disease vaccine, haemagglutination inhibition test was performed to differentiate the disease in question from Newcastle and to indirectly verify immunosuppression, if occurs. The test result showed that vaccinated chicken have a lower antibody titers $\log 2^0 - \log 2^3$, than what is expected ($\log 2^{5-9}$) at this period after vaccination (Table 1). Records of sero monitoring and data from the National Veterinary Institute laboratory after vaccination indicated that vaccinated birds usually possess antibody titer ranging $\log 2^5 - \log 2^9$. This indicates an immunosuppression (vaccine failure), which should be related to depletion of lymphocytes and macrophage by IBDV infection Marquardt *et al.* (1980). The HI results showed that the disease in question is not ND, however concurrent infection can not be ruled out. To further elaborate the

Table 1: Sera screened for Newcastle disease antibodies by haemagglutination inhibition test

Type of chicken	No. Sera tested	Range of Antibody titer				No.+Ve	% -Ve	%+Ve
		None	Log 2 ¹	Log 2 ²	Log 2 ³			
Layer	20	10	5	-	5	5	15	25
Broiler	20	16		4	-	0	20	0

Antibody titer $\geq \log 2^3$ was taken as positive

Table 2: Total number of sera screened for precipitating antibodies of infectious bursal disease virus by Agar gel immuno-diffusion test

Sera taken at early stage of the disease		Sera taken from recovered chickens	
No. of sera tested	No. and % positive	No. of sera tested	No. and % positive
12	5 (41.6)*	15	14 (93.3)

*Numbers in parentheses indicate percentages

disease, virus isolation was also made possible by inoculating a tissue suspension (homogen) prepared from spleen and bursa of infected chicken into a chicken embryo fibroblast cells (CEF). Clear viral cytopathic changes (CPE) were observed at the 2nd day of incubation. The infected CEF cells showed rounding of the cells in 48 hours post infection. Clumping, syncytial formation, vacuolation and finally the cells started detaching from the surface of the cell culture bottles in 96 hours of post infection.

In conclusion, based on the clinico-pathological pictures, histopathology and the serological tests performed, the presently investigated poultry disease outbreak was identified as infectious bursal disease. To our knowledge this is the first report of IBD in Ethiopia and can be considered as an emerging threat to the growing chicken farming in the country.

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