

## Concurrent outbreak of infectious bursal disease (IBD), aflatoxicosis and secondary microbial infection in broiler chicks

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### ABSTRACT

A deadly outbreak of aflatoxicosis, as well as infectious bursal disease (IBD), in two adjoining organized poultry farms, further complicated by secondary microbial invasion, was investigated. Morbidity and mortality was 80-90% among broiler chicks in the 4 to 6 week age group. Samples of vital organs comprising liver, spleen, kidney, bursa of Fabricius, lungs, heart blood, bile and intestines were processed microbiologically. Morbid materials yielded *Escherichia coli*, *Streptococcus faecalis* and *Proteus* spp. *Aspergillus flavus* was isolated from feed samples collected randomly from feed stocks. Representative specimens of liver, spleen, bursa and kidney collected at 10% formal saline were subjected to histopathological investigations. Agar gel precipitation test conducted on the bursal tissue homogenates of 22 birds discerned strong precipitation lines with known positive IBD hyperimmune serum. The aflatoxin level in feed offered to the affected birds varied from 100 ppb to 500 ppb. Conspicuous histomorphological alterations corresponding to aflatoxicosis, infectious bursal disease, as well as other secondary bacterial infections, were evident in these visceral organs.

**Key words:** aflatoxicosis, broiler chick, infectious bursal disease

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## **Introduction**

Infectious Bursal Disease (IBD) is an acute, highly contagious viral infection of growing chickens. It is caused by a double stranded, bisegmented RNA virus belonging to the genus *Avibirna* virus of the family *Birnaviridae* (MULLER et al., 1979; DOBOS et al., 1979). Of the two serotypes, only serotype 1 viruses have been incriminated as pathogenic to chicken (ISMAL et al., 1988). IBD virus (IBDV) primarily attacks the lymphoid tissue with a special predilection for the bursa of Fabricius. Replicating IBDV results in depletion of B cells by inducing apoptosis resulting in severely immunodepressed chickens (HIRAI et al., 1979). The decline in resistance leads to single or concurrent bacterial and viral infections, as well as frequent vaccination failures (SAIF, 1991).

IBD is a serious menace in the development of poultry enterprise and has resulted in major worldwide economic losses (CHETTLE et al., 1989; BERG et al., 1991). Such outbreaks have caused colossal losses to poultry farmers in India (VERMA et al., 1981; SAH et al., 1995) and may be due to the occurrence of antigenic variant strains, interference by maternal antibodies or to other immunosuppressive afflictions such as aflatoxicosis/mycotoxicosis. Any flaw in vaccine preparation, its transport and administration can also lead to vaccination failure. In India, virulent types of variant strains of serotype 1 caused catastrophic losses to the poultry industry during the period 1993-95 (SAH et al., 1995). The present work deals with deadly outbreaks of IBD and aflatoxicosis in two adjoining organized poultry farms in Himachal Pradesh, which were further complicated by secondary bacterial infection.

## **Materials and methods**

In the present investigation, morbid materials comprising bursa of Fabricius, spleen, thymus, liver and kidney from necropsied birds were collected in 10% formal saline for histopathological studies. Tissue samples of liver, lung, spleen, kidney, intestine and heart blood were taken aseptically in sterilized vials for microbiological processing. These samples were inoculated on to 6% sheep blood agar (BA) both aerobically and under reduced O<sub>2</sub> tension at 37 °C for 3-5 days. Bacterial isolates were

characterized on the basis of their cultural, morphological and biochemical features (BUCHANAN and GIBBONS, 1974). The same samples were inoculated on to Sabouraud's dextrose agar (SDA) and Candida agar (CA) and incubated at 25 °C for two weeks. The cultures were examined daily for any mycobiotic growth during the incubation period. Visual examinations of the fungal colonies were made and their colonial morphology or characteristics, such as texture, pigments and rate of growth on media, were recorded. Morphology of the fungi was identified by examining a small aliquot of the growth in lactophenol blue under dry objectives after carefully separating the fungal mass with a fine straight dissecting needle on a grease-free slide. Agar gel precipitation test (AGPT) was conducted using finely triturated bursal tissue of infected chickens with IBD hyperimmune serum. Antigen was prepared from bursal homogenates collected from 22 infected chickens. The bursal homogenate was mixed 1:1 (weight:vol) with sterile saline, subsequently frozen and thawed three times. This homogenate was centrifuged at low speed (300xg); the supernatant was used as antigen. Random samples of feed were collected for microbiological processing and were also analysed for extent of mycotoxins.

### Results

Birds in age groups of 4 to 6 weeks developed symptoms of IBD on two poultry farms. Mortality was first noticed amongst 6-week-old broiler chickens, followed by 4-week-old birds, with 80 to 90% mortality over the following 3-4 days. Chickens that died were dehydrated, with blotches of haemorrhages on pectoral and thigh muscles (Fig. 1). Kidneys were enlarged, tubules and ureters were distended with urate deposits. The bursa of Fabricius was enlarged and hyperaemic, with petechial to echymotic haemorrhages and necrotic foci on its serosal surface (Fig. 2). Grossly, it appeared either creamy or greyish. On incision, a gelatinous, yellowish exudate was evident. The longitudinal laminae of bursa were hypertrophied, with extensive haemorrhages (Fig. 3). Spleen was slightly enlarged. In some of the chickens, the serosal surface of the proventriculus revealed mild congestion and haemorrhages. The liver was pale and enlarged, with



Fig. 1. Blotches of haemorrhages on pectoral and thigh muscles typical in IBD

a distended gall bladder. Histologically, normal follicular architecture was altered by degeneration and necrosis of lymphocytes in the cortex of bursa of Fabricius, characterized by interfollicular edema mixed with phagocytic cells. Lymphocytes were replaced by heterophils, cellular debris and hyperplastic reticuloendothelial cells. Haemorrhages were also present in



Fig. 2. Edematous and haemorrhagic bursa of Fabricius in acute IBD



Fig. 3. Incised bursa of Fabricius exhibiting haemorrhages, creamish exudate and thickened longitudinal laminae

the cortex and medullar regions. Fibroplasia was discerned in interfollicular connective tissue. Lesions in kidney were characterized by infiltration of large casts of homogenous material infiltrated with heterophils.

AGPT conducted on 22 samples of finely triturated bursal tissue with known positive hyperimmune serum of IBD revealed strong precipitation lines. Microbiological investigations led to the isolation of *E. coli* and *Streptococcus faecalis* from heart blood, and *Proteus* spp. from intestine. *Aspergillus flavus* was isolated from feed samples collected randomly from feed stocks. The aflatoxin level in different feed samples taken from the poultry farms ranged between 31-100 parts per billion (ppb).

### Discussion

IBD has a tremendous impact on the development of the poultry industry in India. The disease was first reported here by MOHANTY et al.

(1971). However, with mass immunization programs this disease regressed considerably but with the emergence of a variant virulent strain a sudden surge was observed in southern India in 1995 (SAH et al., 1995; VIJAYA et al., 1995). In 1995 and 1996, 779 and 423 IBD outbreaks with a mortality rate of 12-25% were reported from all over the country (Annual Report - Department of Animal Husbandry and Dairying 1996-97, Government of India).

On both farms the birds were vaccinated with intermediate strain (Georgia strain) at 18-20 days with a booster dose at 28-30-day intervals. Feeding of aflatoxin containing feed to poultry has a detrimental effect on humoral and cell mediated immune response, leading to fulminating disease outbreaks even after vaccination. MOHIUDIN (1993) documented a marked decline in antibody titres and phagocytic activity representing an impairment of humoral and cell mediated immune systems in experimentally-fed aflatoxin as low as 0.2 ppm. Similarly, BAKSHI et al. (1997) recorded a significant decrease in serum globulin component by feeding aflatoxin level in different feed samples from these poultry farms ranging between 31 and 100 ppb. Such a high aflatoxin content in feed could have impaired the immune system, thereby resulting in vaccination failure. Earlier, ANJUM (1994) reported a severe IBD outbreak with a mortality rate of up to 35.8% in grower pullets that were vaccinated with IBD vaccine, ascribable to immunosuppression owing to aflatoxicosis.

A wide range of conditions can promote formation of mycotoxins in feed. A moisture content of more than 15% in feed and moderately high temperature is conducive to the rapid growth of *Aspergillus flavus* and production of mycotoxins (PIER et al., 1979). The area under study is located between 32°23'N and 33°13'N and 75°46' and 79°04'E at a height of 1200 meters above msl; it has an rainfall ranging from 1450 mm to 1500 mm with relative humidity as high as 90% and a temperature of 25 °C in July and August. Such climatic conditions are favourable for the growth of fungus in feed stocks stored improperly, especially in damp sites.

In IBD, marked histopathomorphological alterations occur in the lymphoid structures, primarily in the cloacal bursa. The degeneration and necrosis of lymphocytes in the medullar area of bursal follicles identified

in present investigations is similar to the initial findings of CHEVILLE (1967). IBDV multiplies in the lymphoid cells of bursa of Fabricius resulting in severe immunosuppression due to destruction and depletion of lymphoid cells (KAUFER and WEISS, 1980). The virus replicates in B lymphocytes and primarily immature or precursor B lymphocytes (HIRAI and CALNEK, 1979).

Microbiological investigations yielded isolation of *E. coli*, *Streptococcus faecalis* and *Proteus* spp. These microbes, as opportunistic pathogens, could have further augmented mortality in these immunosuppressed chickens as a consequence of aflatoxicosis and IBD.

Strong precipitation lines were recorded in AGPT conducted on all 22 bursal homogenates of broiler chickens with group specific IBD antiserum. For detecting anti-IBD antibodies a number of serological procedures have been used, viz. AGPT, virus neutralization test (SKEELES et al., 1979) and enzyme linked immunosorbent assay (MARQUARDT et al., 1980). CULLEN and WYETH (1975) suggested AGPT even to quantify IBD antibodies in serially diluted serum samples.

The course of treatment recommended included segregation of affected birds, change of poultry sheds, change in feed, liver tonics, multivitamins and mineral supplements. Various approaches, such as the use of activated charcoal (HARVEY and ROTTINGHAUS, 1997), liver tonics (PARIDA et al., 1995), mineral supplements (UMESH et al., 1995) and raising dietary proteins, amino acids, energy levels (ABDELHAMID et al., 1994) have been adopted to restrict losses due to aflatoxicosis. The therapeutic regimen and management practice adopted in this case reduced the mortality to some extent.

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**MAHAJAN, A., R. C. KATOCH, R. CHAHOTA, S. VERMA, S. MANUJA: Istodobna pojava zarazne bolesti Fabricijeve burze, aflatoksikoze i sekundarnih infekcija u tovni pilića. Vet arhiv 72, 81-90, 2002.**

**SAŽETAK**

Opisana je istodobna pojava aflatoksikoze popraćene uginućem, zarazne bolesti Fabricijeve burze te sekundarnih infekcija na dvije farme tovni pilića. Pobel i pomor u pilića u dobi od 4 do 6 tjedana iznosio je 80-90%. Mikrobiološka pretraga bila je provedena na uzorcima jetre, slezene, bubrega, Fabricijeve burze, pluća, krvi iz srca, žuči i crijeva. Izdvojene su bakterije *Escherichia coli*, *Streptococcus faecalis*, *Proteus* spp. Iz uzorka hrane izdvojena je gljivica *Aspergillus flavus*. Uzorci tkiva jetre, slezene, burze i bubrega pretraženi su patohistološki. Gel difuzijskim precipitacijskim testom dokazane su jake linije precipitacije između homogenata tkiva Fabricijeve burze (22 životinje) i hiperimunog seruma za virus zarazne bolesti Fabricijeve burze. Patohistološki nalaz odgovarao je za aflatoksikozu, zaraznu bolest Fabricijeve burze i sekundarne infekcije. Razina aflatoksina u hrani kretala se između 100 i 500 ppb.

**Ključne riječi:** aflatoksikoza, tovni pilići, zarazna bolest Fabricijeve burze

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