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OUTBREAKS OF INFECTIOUS BURSAL DISEASE IN BROILERS IN MALAYSIA

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SUMMARY: Two outbreaks of acute infectious bursal disease in broilers are reported for the first time in Malaysia. The clinical signs are sudden death, whitish diarrhoea, inappetance and uneven growth. Age of affected birds varied from 19-28 days and mortality ranged from 20% to 60%. The most significant post mortem lesion was the enlarged and often haemorrhagic bursa of Fabricius. Histologically, there were haemorrhages and necrosis of lymphoid cells in the bursae. Diagnosis was based on clinical signs, histopathological lesions and virus isolation in specific pathogens free (SPF) eggs. The isolate was found to be pathogenic.

Keywords: infectious bursal disease, broilers, Malaysia.

INTRODUCTION

Infectious bursal disease (IBD) has been reported from most poultry producing areas of the world. Although the disease has been previously known to occur in this country, it was generally regarded as a mild disease without causing serious economic losses. Mortalities with clinical signs and pathological changes associated with acute IBD has been confirmed for the first time during the middle of 1991. This report describes the clinical signs, pathological lesions, virus isolation and a pathogenicity study from these two outbreaks.

MATERIALS AND METHODS

Case Histories

a. **Case 1** The farmer having 10,000 broilers of Arbor Acre breed aged 28 days had a total mortality of 20% over a four-day period. The birds had been vaccinated with Newcastle disease "F" at day 4. No IBD vaccination was done on the farm. The prominent clinical signs were drowsiness, anaemia, uneven growth and inappetance.

Six live birds that were submitted to the laboratory for examination appeared dull. They were killed by humane slaughter by severing the neck. The proventriculus and gizzard of all six birds were devoid of feed. The kidneys were slightly enlarged and the bursa of Fabricius was grossly enlarged with prominent bursal folds (Figure 1).

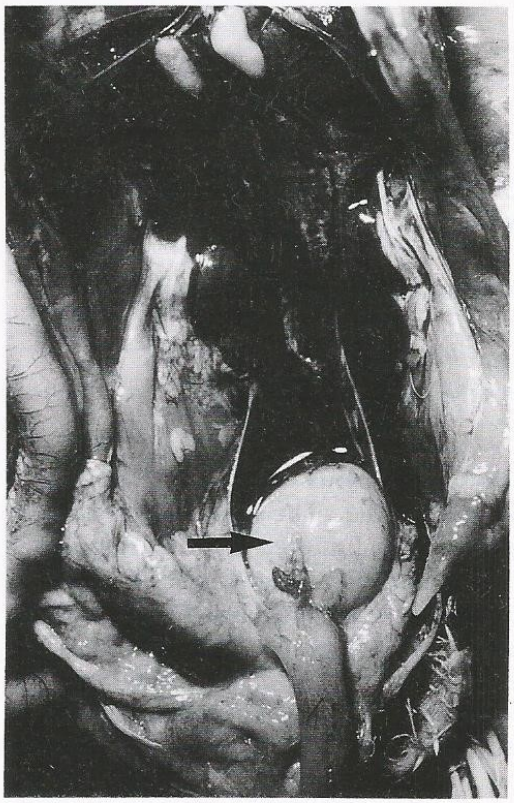

Figure 1: Enlarged bursa of Fabricius (arrow)

b. **Case 2** The farm had 13,000 Arbor Acre broiler birds aged 19 days. Total mortality was 60% during the last four days. Infectious bronchitis/Newcastle disease vaccination was carried out at day old. No IBD vaccination was done on the farm. There was evidence of diarrhoea and uneven growth in the flock.

Post mortem lesions of five birds submitted included petechial haemorrhages of the thigh muscle and small intestinal mucosa, swollen kidneys with distended renal tubules and enlarged and haemorrhagic bursa of Fabricius.

Laboratory Examination

a. **Agar-gel precipitation test (AGPT):** The field bursae submitted for diagnosis were homogenised in tryptose phosphate broth with 1% of an antibiotic solution and used in an AGPT. The AGP plates were made with 2% noble agar in phosphate buffer pH 7.2 with 0.2% sodium azide. Antibody to IBD was a convalescent serum pooled from IBDV-infected minimal-disease-free(MDF) chickens. A normal bursal homogenate and serum from specific pathogens free (SPF) chickens were also tested simultaneously as controls.

b. **SPF embryonated-chicken-egg (ECE) inoculation:** The bursal suspension was centrifuged at 2,000 rpm for 10 mins. and the supernatant harvested. 0.2 ml each of 1/10 dilution of the supernatant was each inoculated onto the chorio-allantoic-membrane (CAM) of five SPF ECE and incubated for 7 days at 37°C. Eggs that died within 24 hrs. after inoculation were discarded.

c. **Pathogenicity study in SPF chickens:** A preliminary study on the pathogenicity of the isolate was undertaken. Four 4 weeks-old SPF chickens were inoculated orally with 0.2ml/bird of the field bursal homogenate and observed for clinical signs. Two birds were kept as the control. The birds were kept in SPF isolation units.

d. **Histopathology:** Portions of the spleen, kidney, liver, small intestine and bursa of Fabricius were fixed in 10% buffered formol saline, embedded in paraffin wax and sections were cut at 5 µm. They were stained with haematoxylin and eosin.

e. **Bacteriology**: The lung, liver, kidney, small intestine and bursa of Fabricius were submitted for routine examination.

f. **Parasitology:** Blood and intestines were subjected for routine examination for blood parasites and coccidial occysts.

RESULTS

No significant bacteria were isolated from any of the birds. Examinations for coccidiosis and leucocytozoonosis proved negative.

AGPT

The homogenised bursae was found to contain IBD as evidenced by a clear line of precipitation with antibody to IBD. The normal bursae and serum did not show any line of precipitation.

SPF ECE inoculation

All the eggs inoculated died between the 4th to 6th day after inoculation. The spleen of the embryos was enlarged. There was necrosis of the liver, atrophy and necrosis of the bursae. The CAMs were also thickened.

Pathogenecity study

Three birds died at 3 days after inoculation. The live bird was killed after 3 days inoculation. All birds were observed to be dull with ruffled feathers after the 2nd day of inoculation. Post mortem lesions include slight to moderate petechial haemorrhages on the medial thigh areas and abdominal muscles with oedema of the bursae. No clinical signs or lesions were observed in the control birds.

Histopathology

Significant changes were confined to the bursa of Fabricius. There was oedema of the bursal folds with degeneration and necrosis of lymphocytes in the medullary areas of the bursal follicles (Fig. 2). Heterophils often infiltrated and were mixed with the degenerative lymphocytes. In some sections, there was an increase in the interfollicular connective tissue with atrophy of the bursal follicles and marked depletion of lymphocytes in the follicles. Extensive haemorrhage in the bursal folds was a consistent finding (Fig. 3).

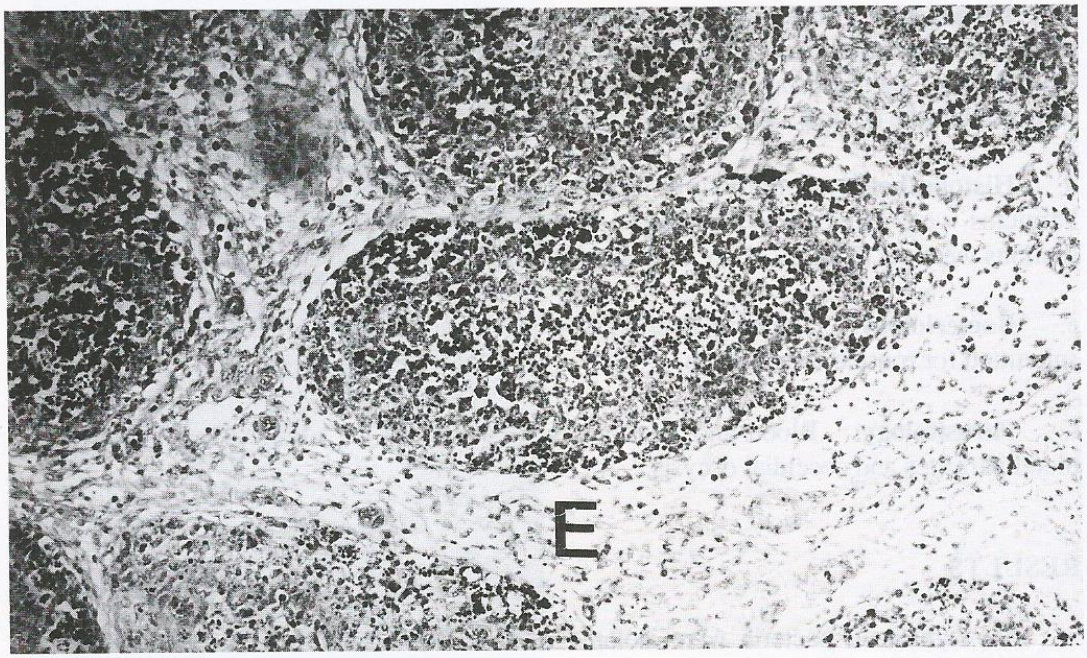

Figure 2: Necrosis of lymphoid cells in a follicle in the bursa of Fabricius. Note also the marked interfollicular oedema (E). (HEx250)

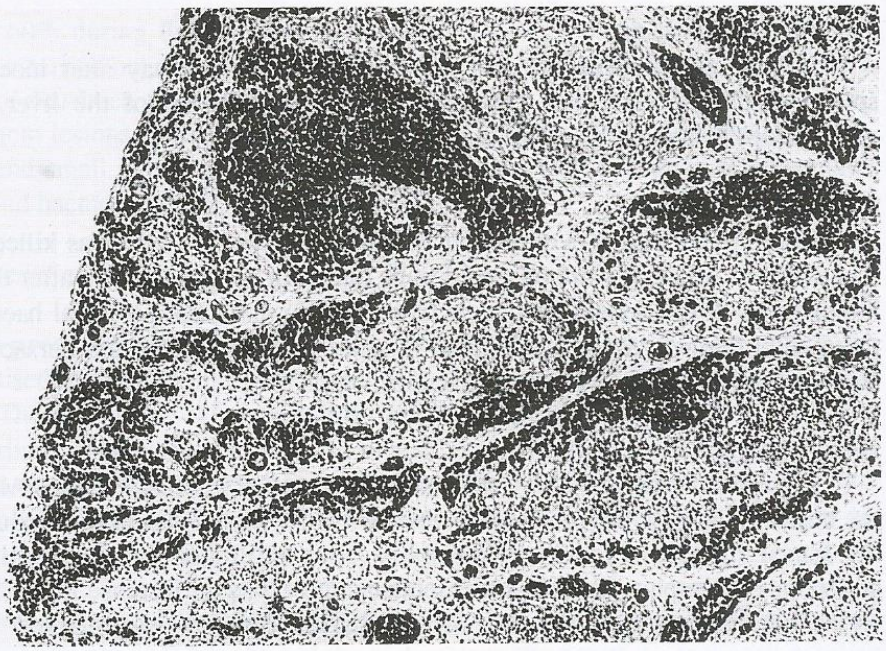

Figure 3: Diffused haemorrhage in the follicles of bursa of Fabricius. (HEx100)

DISCUSSION

Over the last few years, sudden outbreaks of IBD causing greater than usual mortality have been reported in England (Chettle *et al.*, 1989). Holland (Box, 1989), Belgium (Van den Berg and Meulemans, 1991) and more recently in Japan (Tsukamoto *et al.*, 1992). Highly virulent strains of type I IBD virus were isolated from the outbreaks in Europe and Japan. Tsukamoto *et al.*, (1992) was of the opinion that the IBD virus isolate that caused high mortality in the western parts of Japan was probably introduced into Japan.

In Malaysia, there are serological evidence and pathological studies to indicate that IBD is present (Joseph, 1986; Loganathan and Harizam, 1990). The disease has been associated with immunosuppression and most of the infections had been subclinical. Lim *et al.* (1992) carried out a national serological survey of IBD and concluded that IBD was widespread. In addition, two local viruses isolated from their studies were found to be mildly immunosuppressive.

Our study is the first confirmed report of virulent IBD causing heavy mortalities. Since this report, numerous outbreaks had occurred in both broilers and layers throughout the country. The sudden appearance of this virulent strain and its probable source is being investigated. More studies on the epidemiology and serotyping of this strain are also being pursued. It seems reasonable to assume, as in the case in Japan, that the virulent IBD virus was most likely introduced into the country.

The gross and histopathological changes in our outbreaks are consistent with those described by Chettle *et al.* (1989) and Tsukamoto *et al.* (1992) in which bursal changes were accompanied by necrosis and inflammatory response. This is in contrast to that reported in the United States by Snyder (1990) who observed that the variant strains produced rapid bursal atrophy with minimal inflammatory responses.

Commercially available IBD vaccines have been used in breeder farms and currently are being used in broiler farms as well. It appears that the vaccines provide only partial protection as outbreaks are still reported despite IBD vaccination.

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RINGKASAN

WABAK PENYAKIT BURSA BERJANGKIT VIRULENS DI MALAYSIA

Dua wabak penyakit bursa berjangkit akut pada ayam pedaging dilaporkan pada kali pertama di Malaysia. Petanda-petanda klinikal adalah mati mengejut, cirit-birit berwarna keputihan, kurang selera makan dan pertumbuhan berat badan tidak seimbang. Umur ayam yang terlibat diantara 19-28 hari dan kadar kematian dia antara 20% hingga 60%. Lesi paling tererti pada post mortem adalah bursa Fabricius yang terbesar dan kerap kali berhemoraj. Histologinya menunjukkan nekrosis sel limfoid dan hemoraj pada bursa tersebut. Diagnosis dibuat berasaskan petanda-petanda klinikal, lesi histopatologi, dan pemencilan virus dalam telur bebas patogen khusus (SPF). Virus ini didapati patogen.