PATHOLOGY OF NATURALLY OCCURRING INFECTIOUS BURSAL DISEASE IN CHICKEN

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ABSTRACT

Fifteen natural outbreaks of Infectious Bursal Disease (IBD) in layers of 4 to 6 week age were studied. The mean mortality in these outbreaks was 21 per cent. Six week old chicks were most commonly affected with the mean mortality of 27 per cent. Prevalence of IBD virus serotype 1 in Namakkal area was ascertained by Agar Gel Precipitation Test and the outbreaks of IBD were confirmed by observing several fold increase of IBD antibody titre (64 – 230) in convalescent birds. Ailing birds were seronegative. Peak mortality was observed on 5th day and the birds recovered 7 to 10 days after the first clinical sign was noticed. On necropsy, bursal enlargement with haemorrhage, muscular haemorrhages, sphenomegaly, pale liver, congested and atrophic thymus, catarrhal enteritis, pale kidneys and congested Harderian gland were observed. Lymphocytic depletion and glandular transformation with infiltration of heterophils and mononuclear cells in bursa, lymphoid depletion in thymus and spleen, and depletion of plasma cells in of Harderian gland were observed. The study revealed the pathotypic shift of IBDV, serotype 1 due to the extreme vaccination pressure that might have resulted in more virulence causing mortality up to 50 per cent in Namakkal area.

Key words: IBD, Natural outbreak, Serotyping, Pathology, Pathotypic shift

Infectious Bursal Disease (IBD) caused by Birna virus, is known to occur in many poultry producing areas in the world. It was first reported by Cosgrove (1962) at a place called Gumbo in USA. In India, it was first reported by Mohanty et al. (1971) in Uttar Pradesh. Outbreaks of IBD were reported in Namakkal, a major egg producing area of Tamil Nadu, since 1988 (Purushothaman et al., 1988; Reddy, 1994).

In Namakkal, mortality due to IBD did not exceed two per cent till 1992, but in the year 1993, a multifactorial disease complex involving IBD resulted in heavy mortality, ranging from 20 to 90 per cent which caused heavy economic loss to the poultry farmers of this area (Poultry Disease Episode, 1993; Reddy, 1994). Hence, this present study was undertaken to identify the serosurveillance (serotype) and to assess the pathogenicity of the field IBD virus.

MATERIALS AND METHODS

Fifteen IBD affected farms in and around Namakkal were taken for the study. Five each, ailing and convalescent birds 21 days after the observation of first sign of disease were selected from each farm for the study. Blood samples were collected from

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ailing birds and the sera separated. Filter paper blood samples were collected from the convalescent birds (Brugh and Beaud, 1980) and the sera were eluted by using 0.1% (v/v) polyoxyethylene-23-lauryl-ether (Brij-35) in normal saline. Sera samples from ailing birds and the elutes from convalescent birds were used in quantitative agar gel immunodiffusion test (Cullen and Wyeth, 1985) to estimate the antibody titres. The IBD virus serotype – 1 (CVL 87, type-1, Faragher strain) and hyperimmune serum obtained from Central Veterinary Laboratory, Weybridge, UK were used to estimate the antibody titre and to confirm the presence of IBDV in the IBD affected birds, respectively.

Birds were sacrificed by cervical dislocation and complete necropsy was conducted to collect the bursae aseptically. A 50 per cent bursal suspension in saline was made. The resultant homogenate was subjected to three freeze–thaw cycles and centrifuged at 1500 rpm for 10 minutes. The supernatant was collected and antibiotics viz. penicillin (10,000 IU/ml), streptomycin (10,000 μg/ml) and gentamicin (320 μg/ml) were added and incubated at 37°C for 30 minutes. The samples were subjected to AGPT (Wood et al., 1979). Bursa from healthy chicks served as negative control. The supernatant positive by AGPT was treated with 10 per cent chloroform (1:chloroform) : 4 (supernatant) ratio for 15 minutes (Dash et al., 1991). The chloroform treated suspension was centrifuged at 3000 rpm for 10 minutes and the supernatant was used as inoculum for virus isolation in chicks. Fifty per cent pooled suspension (50 μl) was inoculated in fifty cockerels through cloacal and ocular routes. The birds were sacrificed 72 hours post-inoculation and the bursae were processed to isolate the virus. Presence of IBDV antigen was tested by AGPT.

On necropsy, gross lesions were recorded. Representative pieces of bursa, spleen, thymus, Harderian gland, caecal tonsils and liver were fixed in 10 per cent formal saline and embedded in paraffin. Sections of 5-6 micron thickness were cut and stained by haematoxylin and eosin (H&E) method for histopathological examinations.

**RESULTS AND DISCUSSION**

In the period of two years, though many farms with birds of 5 and 6 weeks of age were affected with IBD, fifteen farms were selected for the study. All fifteen IBD affected farms were having White Leghorn layer type chicken of 4 to 6 weeks age. The flock strength in these farms varied from 1100 to 3000. The mean mortality rate observed were 11, 18 and 27 per cent in 4, 5 and 6 weeks of age respectively. This is in agreement with earlier report on mortality rate (Numoya et al., 1992, Suryanarayana Murthy et al., 1994). Six week-old chicks were most commonly affected and this was also supported by the histopathological study of bursae. This observation is in agreement with earlier study conducted at Namakkal (Reddy, 1994). However, it was reported to occur in 2 to 5 weeks of age by other workers (Cosgrove, 1962; Faragher, 1972).

Bursa collected from all the 15 farms revealed the presence of IBDV specific antigen when tested with hyperimmune serum (CVL 87 type-I) in AGPT, the IBDV prevailing in Namakkal area might belong to the same serotype i.e., serotype-1 and was not a variant strain. The QAGID results revealed many fold increase of antibody titre 21 days after outbreak (estimated titre value ranged from 64 to 230). Lack of IBD antibodies in the ailing birds and several fold increase in the titre value of IBD antibodies in the convalescent birds also confirmed the prevalence of IBD.

The IBDV could be isolated in chicks from bursa on 3 days post-inoculation. It indicated that the bursa collected at the acute stage is ideal for demonstration of virus specific antigen. This findings are in agreement with Chang and Hamilton (1982).
Trembling was the first sign observed in the IBD affected flock, followed by whitish to greenish diarrhoea with soiled vent, ruffled feathers and anorexia. Severely affected birds did not respond to the environmental disturbances and unable to stand, whereas, birds with mild to moderate infection attempted to walk but with wobbling gait. Mortality started after 3rd day of first clinical sign, peaked on 5th day and subsided thereafter. The birds recovered after 7 to 10 days.

On necropsy, 73 per cent of chicks showed bursal enlargement and 27 per cent had haemorrhagic bursae. Sixty per cent birds had yellowish exudate in bursa, whereas 40 per cent had caseous exudate. Muscular haemorrhages in the thigh, leg and pectoral muscles were observed in all the cases. Proventriculus–gizzard junction showed haemorrhages in 25 per cent of the cases. Spleen was slightly swollen and mottled. In most of the cases, liver was pale and the thymus was severely congested or atrophic. Five per cent of the cases had congested Harderian glands. Other lesions observed were pallor muscles, catarrhal inflammation of intestines and congested or pale kidneys. More or less similar findings had been reported earlier (Cosgrove, 1962; Manjusha et al., 1995).

Bursa from the affected birds showed severe lymphocytic depletion in more than 50 per cent of the follicles, interfollicular oedema, central follicular necrosis (Fig. 1), moderate interfollicular fibrosis, interfollicular and intrafollicular infiltration of heterophils and mononuclear cells. In other cases, bursal follicles showed severe depletion of lymphocytes, loss of follicles, cystic and glandular transformation of follicles. Interfollicular areas showed severe fibrosis and heterophilic and mononuclear cellular infiltrations (Fig. 2). Regeneration of bursal lymphocytes could be observed in convalescent birds belonging to 4 and 5 week-old batches which was not evident in 6 week-old birds indicated more severity of the disease in 6 week-old age.

Thymus showed thinning of cortex, lymphoid depletion, haemorrhages and reticular cell hyperplasia in the medulla. Spleen revealed moderate to severe lymphoid depletion and reticular cell hyperplasia. Mild to moderate depletion of lymphocytes in the lymphoid nodules and in the diffuse lymphoid tissue of lamina propria and necrosis were noticed in the Harderian gland. Liver showed congestion and vacuolar degeneration. These findings are in agreement with earlier reports (Panisup et al., 1983; Manjusha et al., 1995).

From this study, it is inferred upon that the IBDV existing in Namakkal area produced acute pathological effects and belonged to serotype–1. Further, mortality rates of below 25 per cent were reported by earlier workers in Tamil Nadu due to IBD, serotype–1 (Johnson Rajeswar and Chandra Mohan, 1992; Reddy, 1994). But in this study, the same serotype caused up to 50 per cent mortality indicating the increased virulence (pathotypic shift) with out antigenic shift. Extreme vaccination pressure might have caused this pathotypic shift. Evidences are coming from Europe where pathotypic shift resulted in very high mortality rates (Van den Berg and Meulemans, 1991).

The predisposing and contributory factors viz., proliferation of poultry industry without proper health cover, overcrowding, vaccination stress due to unscheduled vaccination programmes, excess dose of vaccines reducing immune status, improper sanitary conditions, improper disposal of carcasses and transportation from infected to unaffected farms might be playing an important role in continued existence of the IBD in and around Namakkal area.

REFERENCES

Brugh, M. and Beard, C.W. (1980). Collection and processing of blood samples dried on paper for microassay of Newcastle disease virus...


Fig 1 Bursa - severe depletion of lymphocytes and central follicular necrosis in 5 week-old chick. HSE X 100

Fig 2 Bursa - glandular transformation of follicles, heterophilic and mononuclear cell infiltration into follicular areas is 6 week-old chick. HSE X 250