INFECTIOUS BURSAL DISEASE: A CASE REPORT

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SUMMARY: This paper reports an outbreak of Infectious Bursal Disease (IBD) occuring in broiler chickens derivated from immunized breeders, housed in four neighbouring poultry farms and having a vaccinated or unvaccinated status to IBD virus. High mortality, bad performance and occurence of miscellaneous infections were the main field findings. IBD was experimentally reproduced in SPF and commercial chickens by Bursa of Fabricius homogenate inoculation. IBDV - antibodies and virus particles (33 to 38 nm) were detected in the experimentally inoculated birds.

UNITERMS: Gumboro disease ; Poultry diseases

INTRODUCTION

The occurrence of miscellaneous infections in chickens can be associated with an immunesuppresion state caused by Infectious Bursal Disease (IBD) as well as by many other diseases (MANDELLI [17], 1986). IBD, also named Gumboro Disease and firstly described in USA (COSGROVE [9], 1962), is in many countries, an important disease of chickens that affects bursa of Fabricius. It is induced by a Birnavirus (LUKERT & HITCHNER [14], 1984 e ABDU et alii [1], 1986) which replicates into the bursal lymphocytes, producing inflammation, enlargement and damage of the bursa of Fabricius (LUKERT & HITCHNER [14], 1984 e RIDDELL [21], 1987). IBD virus infection in potentially susceptible chickens can elicit high economic losses related with heavy mortality and/or bad performance. In Brazil, IBD was first described by NAKANO et alii [19] (1972). SAUKAS [22] (1978) was the first to isolate and characterize the IBDV. The incidence of IBD in the country seems to be very high if the prevalence of serologically positive chickens (SAUKAS [22], 1978), the several communications of positive laboratorial diagnoses and the systematic use of vaccines were considered. In 1983, seven cases of IBD were reported by the BRASIL. Ministério da Agricultura [4]. Also, there is some evidence that IBD is becoming more difficult to control in the last years since intermediate strains of IBDV have been introduced into the country aiming the vaccination of broilers produced by the immune breeders.

Based on these data, this work was done to verify the spread of the IBDV among the broiler flocks constituted of chicks hatched from immune breeders having unvaccinated or vaccinated status to IBDV.

MATERIAL AND METHOD

- *Field survey*: Four broiler chicken flocks housed in different neighbouring poultry farms were chosen to study the occurrence of IBDV. Historically, since 1986, many broiler flocks produced in that area showed high incidence of miscellaneous disease processes (about 4 to 8 weeks of age) and/or bad performance, as such: Flock A - chickens were vaccinated against IBDV and presented diarrhoea at 35 days of age; 46 days of age many birds presented tracheal rales; total mortality was 4.5% (Tab. 1) and about 1% of the birds were culled; Flock B - birds were also vaccinated against IBDV (Tab. 1); some birds presented tracheal rales at 23 days of age, mortality was 2.5%. Flocks C and D - birds were not vaccinated against IBDV (Tab. 1). Birds from both flocks presented high mortality after 3 weeks of age and signs of prostration and

diarrhoea were found at 39 days of age; 7.2% and 9.0% of mortality were respectively found. All broiler chickens were hatched from hyperimmunized breeders.

- *Sample collection*: Twenty chickens were taken at a random from each house and were bled to obtain serum samples. Ten sentinel birds, vaccinated against Newcastle Disease (ND) and Infectious Bronchitis (IB) viruses were used in the flock A. The sentinel birds were necropsied fifteen days after exposure. Ten birds were also taken at random from flocks B, C and D and necropsied. Bursa of Fabricius were collected from all the birds.

Sample preparation and viruses.

- *Bursa of Fabricius (BF)*: The BFs were ground in a pest and mortar and homogenized in Ten Broeck (Pyrex). BFW/V (Weight/volume) suspension was prepared using Tryptose Phosphate Broth (TPB) and stored at - 70°C until use.

- *IBDV - strains*: The following strains were used throughout the experiments: G9 - Kindly supplied by Dr. J.B. McFerran (Veterinary Research Laboratories of Stormont, Northern Ireland), Lukert and Ag2 both supplied by Salsbury Laboratories (Campinas - SP) and the commercial vaccine Bursine II strain (Salsbury).

- *IBDV - antiserum*: Hyperimmune serum was obtained from 5-week-old Specific Pathogen Free (SPF) chickens experimentally inoculated by oral route with Bursine II strain (1 dose/bird) and G9 strain of IBDV. Virus suspensions were administered two times at 2 week-interval. The chickens were kept in an isolated room outside of the University Campus. Blood samples were obtained 15 days after virus reinoculation. Antiserum against G13 and G23 strains of IBDV were Kindly supplied by Dr. J.B. McFerran.

- *Experimental chickens*: SPF chickens with about 6 weeks of age were supplied by Biovet Laboratories (Cotia, SP) or obtained by hatching SPF embryonated chicken eggs supplied by Granja Rezende (Uberlândia - MG). Unvaccinated commercial day-old chicks (Hyssex Brown) (supplied by Cooperativa Agrícola of Cotia CAC/SP) were reared until 6 weeks of age and also used to reproduce the disease.

- *Agar gel precipitation (AGP) test*: AGP test was carried out according to SAUKAS [22] (1978) using Agar Noble (Difco) prepared at 0.8% in phosphate buffered saline pH 7.2.

- *IBDV detection in BF*: BF W/V suspension, twice frozen and thawed, was centrifuged at 3,000 rpm for 30 minutes. The supernatant was tested against IBDV antiseruns and SPF chicken serum by AGP test.

- *Serology*: AGP test were used to detect serum antibodies against G9 and Ag2 strains of IBDV prepared according to SAUKAS [22] (1978). Microtest serum neutralization tests were carried out using Lukert strain of IBDV adapted to chicken-embryo fibroblast cell culture (Kindly supplied by Salsbury Laboratories, Campinas, SP) according to HITCHNER et alii [12] (1975). IBDV antiserum (G13 and G23) and SPF chicken serum were used as controls.

- *Chicken-embryo Fibroblast culture (CEF)*: SPF chicken Embryos (Granja Rezende, Uberlândia, MG) with 9 days of age were used to obtain fibroblast cultures by the procedure described by HITCHNER et alii [12] (1975).

- *Histology*: *BF*, intestine and proventriculus with gross lesions, fixed in formol buffered saline (10%), were included in paraffin. Thin sections were stained by hematoxilin-eosin and mounted on glass slides.

- *Electron microscopy*: Small pieces of BF, fixed in 2% glutaraldehyde and post-fixed in 1% osmium tetroxide, were dehydrated in a graded series of acetone before inclusion in Araldite 502. Ultra-thin sections, stained with uranyl acetate and lead citrate, were examined at Phillips EM 201 electron microscope.

Experimental procedure

Trial 1 - Experimental inoculation of BF suspension in SPF chicks: BF W/V suspension diluted in TPB (10^{-1}) was inoculated by oral route (0.5 ml/bird) in five SPF chickens. The chickens were necropsied four days after inoculation. BF was examined for the presence of gross, microscopic and ultrastructural lesions. A small piece of BF was used to search IBDV precipitating antigen and stored as the virus seed.

Trial 2 - Serological study: Six SPF chickens were orally inoculated with 10^{-1} dilution of virus seed obtained above (flock C). Four uninoculated chickens were housed together (contact infection). Serum samples were obtained before and 7, 14 and 28 days after inoculation. Twenty eight days after inoculation all birds were necropsied. BFs were carefully collected and weighed.

Trial 3 - Experimental disease in commercial chickens: Serologically negative four-week-old commercial chickens were randomly divided in five experimental groups of ten birds. BF virus seeds obtained in the

first trial were inoculated by oral route; one group was used as control; serum samples obtained at 7 and 12 days post-inoculation were submitted to the AGP test; BF were collected at 5 and 12 days after inoculation and searched for the presence of IBDV precipitating antigen.

- *Statistical analysis*: The Coefficient of Variation was calculated according to GOMES [11] (1984). BF's weight was submitted to analysis of variance using the Duncan's test ($p < 0.05$).

RESULTS

- Field findings

Performance data: Considering the performance data of the clinically healthy birds reared in the same area with the same management procedure, birds of the flocks A, B, C and D showed high mortality rate, poor feed conversion and low daily gain. Also it was found that the age of slaughter was delayed among the diseased birds if considered the age of the clinically healthy birds versus body weight or the performance scores. The expenses with medication was high in the flock A, B and C (Tab. 2).

Necropsy: Flock A - birds at 49 days showed haemorrhagic trachea, enlarged spleen, focal haemorrhages in the small intestine, caecal ulcers, whitish focus on the serosae surface of the swollen proventriculus and a large variation in the size of the BFs. *Eimeria* sp. oocysts were detected in some gut samples. Some broilers from flock B showed complicated airssaculitis, haemorrhagic trachea, gut and small BF. Flock C - IBD - unvaccinated chickens presented lower body weight and paleness at the time of the necropsy; haemorrhages into the pectoral muscles and gut, swollen kidneys and BF athrophy were also found. Flock D - unvaccinated chickens presented thyphilitis, small BF and gut haemorrhages.

Serology: All serum samples obtained from A, C and D broiler flocks had precipitating antibodies. Flock B - serum samples did not have precipitating antibodies.

IBDV: IBDV - precipitating antigens were not detected in the pooled BFs from each flock, tested against different antiseruns.

- Experimental findings

- Trial 1 and 2 - BF inoculation in SPF chicks.

Lesions: The SPF chickens inoculated with A, B, C and D - BF homogenates were slightly pale and presented grey - coloured - enlarged spleen at necropsy. Small BF with lymphoid tissue rarefaction was a common finding among the chicks inoculated with B and D homogenates. Enlarged BF, covered with yellowish gelatinous exudate were found in the A and C - inoculated chicks. Bursal lymphoid tissue depletion, edema and haemorrhages in the serosae layer, serous exudate in the BF lumen. Necrosis and cystic cavities in the medulla of BF lymphoid follicles (Fig. 1) were also found.

IBDV: All BF - samples collected from experimentally inoculated chicks produced strong lines of precipitation when tested against G13, G23, G9 and Bursine II hyperimmune seruns.

Electron microscopy: Aggregates of uenveloped virus particles (Fig. 2) were observed in the cytoplasms of slightly changed and/or foamy mononucleated cells of all BFs. Foamy globules and myelin figures were commonly observed in the degenerated cells (Fig. 3). The individual diameter of virus particle ranged from 33 to 38 nm.

Serological response: Neutralizing and precipitating antibodies were detected in the serum samples collected at different times after inoculation of BF homogenate derivated from flock C. Contact birds showed slight lower antibody titers than the experimentally inoculated birds (Tab. 3).

BF weight: Low BF weight (0.373 ± 0.178 g) related with a large range of size (VC = 47%) was observed between SPF chicks inoculated with BF homogenate. Contact birds showed significantly higher BF weight (0.533 ± 0.086 g) (VC = 1.5%) than the inoculated chicks ($p < 0.05$).

- Trial 3: BF inoculation in commercial chicks:

Lesions: BF and spleen lesions, like the described above for SPF chicks were found in the commercial chicks five days after inoculation. BF athrophy was observed 12 days after inoculation. At this time, swollen proventriculus and haemorrhagic intestine were also found. The gross lesions detected in the proventriculus were: whitish areas on the serous surface, abnormally swollen glandular follicles and ulcers in the mucosal surface. Mononuclear cell infiltration into the mucosa and submucosa, epithelium necrosis and lymphoid tissue proliferation in the appropriat tunic, were microscopically observed. Loss of glandular epithelium, fibrosis and infiltration of

mononuclear cells were found in many glandular follicles. Focal haemorrhages were observed in the mucosa of the duodenun and jejunum. Clusters of bacteria and an intense inflammatory reaction were not microscopically observed in those tissues.

IBDV: Precipitating antigens related with IBDV were detected in the BF W/V suspension obtained on day 5 post-inoculation.

Serology: Precipitating antibodies against IBDV were detected in the serum samples collected at 5 and 12 days after inoculation.

DISCUSSION

The clinical and necroscopic findings and the field performance data from birds of the flocks A, B and D are not usually described for IBD but they resemble the cases of miscellaneous infections associated with IBD-induced immunosuppresion (MANDELLI [17], 1986). On the other hand, the findings concerning flock C could be related to classical IBD described by LUKERT & HITCHNER [14] (1984).

Although ADENE et alii [2] (1985) described successfull detection of IBDV antigens in the BF obtained from field diseased chicks, precipitating antigens were not detected in the original pooled BFs. However, when the same BF - homogenates were inoculated in the SPF chickens, bursal lesions were elicited like the ones previously described for IBD (CHINEME & CHO [8], 1984; ABDU et alii [1], 1986; SIVANANDAN et alii [23], 1986).

The presence of aggregates of virus particles in the bursal cells closely related to foamy cells and myellin figures development, seems to be similar to that described for IBDV (CHEVILLE [7], 1967; LUNGER & MADDUX [15], 1972; BURTONBOY et alii [5], 1974; SAUKAS [22], 1978). Despite the virus particles present 33 to 38 nm of diameter it could be classified as an IBDV because this virus shows a large size range: the IBDV has been classically reported as a 55 to 60 nm - particle (LUKERT & HITCHNER [14], 1984) but smallest particles has also been described (MANDELLI et alii [18], 1972) and ABDU et alii [1] (1986) recognized two distinct population of antigenically related IBDV measuring 60 and 20 nm of diameter. As the BF homogenate, rich in virus particles of 33 to 38 nm produced a strong line of precipitation when tested against IBDV - antiseruns, and serum antibodies for IBDV were also detected in the experimentally inoculated chicks, it can be concluded that Gumboro disease virus was the infecting agent of the broiler chickens.

Only the commercial chickens inoculated with serially passaged BF homogenate presented gut haemorrhages and proventriculus lesions still undescribed for IBD. Focal haemorrhages in the small intestine are a common finding in broiler chickens and usually attributed to a miscellaneous causes. This kind of lesion in the gut and also the muscular haemorrhages described for IBD (LUKERT & HITCHNER [14], 1984) could be due to a clotting problem elicited by IBDV - infection (SKEELES et alii [24], 1979; SKEELES et alii [25], 1980) in some types of chickens. Also the proventriculus lesions are not typical for IBD. It has been described that IBDV produces haemorrhages in the proventriculus mucosa at the junction of the proventriculus and gizzard (LUKERT & HITCHNER [14], 1984; ABDU et alii [1], 1986), proventricular glands congestion and glandular cell necrosis (OKOYE 20, 1985). The presence of whitish areas on the surface of the proventriculus, microscopically related to the presence of dense aggregates of small lymphocytes, has been associated with a systemic effect of Avian Encephalomyelitis virus (BUTTERFIELD et alii [6], 1969; LUGINBUHL et alii [13], 1984). Also the increased number of lymphoid cells in the gut is thought to be connected to a peripheralization of the secondary lymphoid tissues and/or maturation of lymphoid response. By this way the lymphoid foci in the alimentary tract appear to play a general role via antibody production, and a local role relative to bacterial and other antigenic substances in the gut (FIRTH [10], 1977). As the bursectomy results in an increase of the number of ileal lymphoid foci (BÄCK [3], 1970), IBD could have an influence on the peripheral lymphocytic tissue of the alimentary tract. Then the mononuclear cells infiltration and lymphoid tissue proliferation into the proventriculus could be resulting from IBDV - induced BF athrophy and/or from an intense stimulus on the immune system and/or from virus presence in the tissue. These hypotheses could account for the proventriculus lesions found in the broiler chickens early stimulated with intermediate strains of vaccinal IBDV (ITO, 1988)*.

Finally considering that proventriculus lesions are usually observed in broiler chickens suffering from Malabsorption syndrome (MAAS & VEENE [16], 1985; RIDDELL [21], 1987) the effect of the BF - related virus on the gastroenteric tract of chickens needs further study.

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RESUMO: Este trabalho descreve um surto de Doença Infecciosa Bursal (DIB) ocorrendo em frangos de corte oriundos de reprodutoras imunizadas, alojadas em quatro granjas vizinhas e vacinadas ou não vacinadas com vírus da DIB. Mortalidade elevada, mau desempenho e ocorrência de infecções múltiplas foram os principais achados de campo. A DIB foi reproduzida experimentalmente em galinhas SPF e comerciais através de inoculação do homogenato de Bursa de Fabricius. Anticorpos contra vírus da DIB e partículas virais (33 a 38 nm) foram detectadas nas aves experimentalmente inoculadas.

UNITERMOS: Doença de Gumboro; Aves domésticas, doenças

TABLE 1 – Management and field data concerned to the housing of broiler chickens. São Paulo, 1987.

Broiler Flock	Housed chickens	Sex	Age (days)	Mortality (%)	Schedulle of vaccination[a] (days)		
					NDV	IBV	IBDV
A	23500	Mixed	46	4.5	9–28	13	15[b]
B	11000	Female	30	2.5	9–28	14	9[c]
C	7500	Female	39	7.2	10–28	ND	ND
D	7500	Male	39	9.0	9–28	13	ND

(a) – The vaccines were administered by driking water
(b) – Strain of low pathogenicity (Bursine I)
(c) – Strain of intermediate pathogenicity (Bursine II)
NDV – Newcastle Disease Virus/LaSota
IBV – Infectious Bronchitis Virus/H–120
ND – not done

TABLE 2 – Performance data recorded from affected and unaffected broiler chickens. São Paulo, 1987.

Sex	Broiler Flock	Age (days)	Mortality (%)	Body weight (kg)	Daily weight Gain (kg)	Feed conversion	Medication[a] Expense (%)	Performance[b] score
Mixed	A	54	4.43	2.125	39.35	2.35	1.54	160
	Healthy[c]	53	3.54	2.257	42.13	2.27	0.49	177
Female	B	55	5.09	2.118	38.51	2.41	1.75	152
	C	52	8.16	1.974	37.96	2.34	1.46	149
	Healthy	51	2.65	2.090	40.74	2.27	0.73	174
Male	D	54	11.07	2.387	44.20	2.31	0.81	170
	Healthy	49	4.32	2.289	45.8	2.20	0.84	199

(a) – Percentage of expense with medication calculated in relation to the total expense of the production

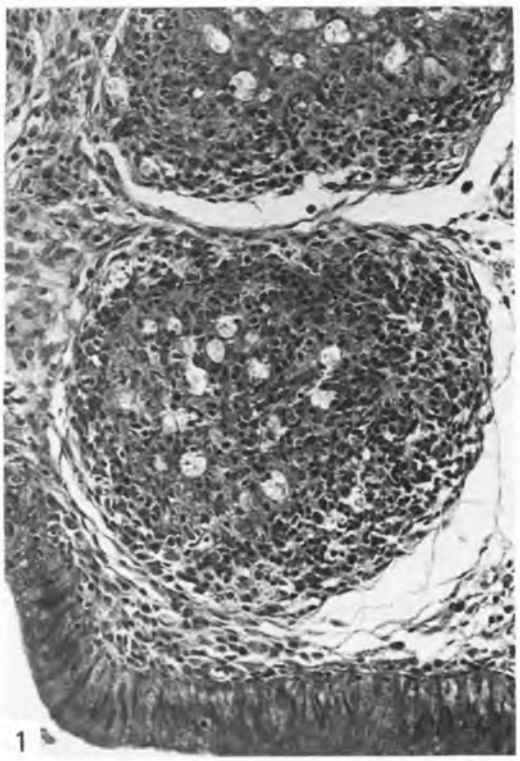
(b) – Performance score = $\frac{\text{Daily weight gain x viability}}{\text{Feed conversion}} \times 100$

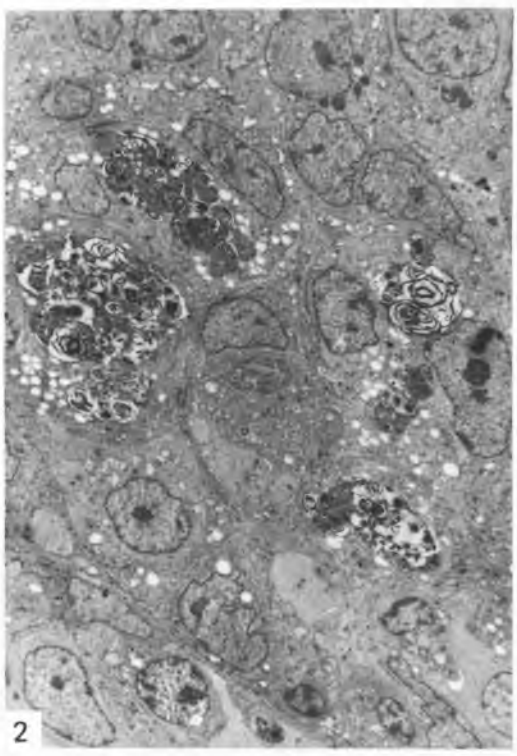
(c) – The results represent the mean obtained from ten flocks constituted of clinically healthy broiler chickens housed in poutry farms located in the same region.

TABLE 3 – Serologic response of SPF chickens orally inoculated with BF virus suspension obtained from flock C. São Paulo, 1987.

Group	Experimental Serologic test	DAYS AFTER INOCULATION 7	14	28
Contact	AGP/G9	0.7 ± 1.5[a] (1.6)[b]	0.7 ± 1.5 (1.6)	1.2 ± 0.9 (2.3)
	AGP/Ag^2	0.0	1.0 ± 1.1 (2)	1.5 ± 1.9 (2.8)
	SN/Lukert	5.8 ± 0.7 (56)	9.0 ± 0.9 (538)	8.3 ± 1.7 (320)
Inoculated	AGP/G9	0.8 ± 0.9 (1.7)	3.5 ± 0.5 (11.3)	2.8 ± 1.1 (7.1)
	AGP/Ag^2	2.0 ± 1.7 (4)	3.1 ± 0.7 (9.0)	3.5 ± 0.8 (11.3)
	SN/Lukert	7.9 ± 0.5 (242)	11.0 ± 1.2 (2033)	10.8 ± 1.8 (1810)

(a) – Geometric mean titer (log_2 of the reciprocal dilution) ± standard deviation
(b) – Antilog$_2$ of Geometric mean titer.
AGP – Agar gel precipitation test/G9 and Ag_2 strain of IBDV
SN – Serum neutralization test/Lukert strain of IBDV





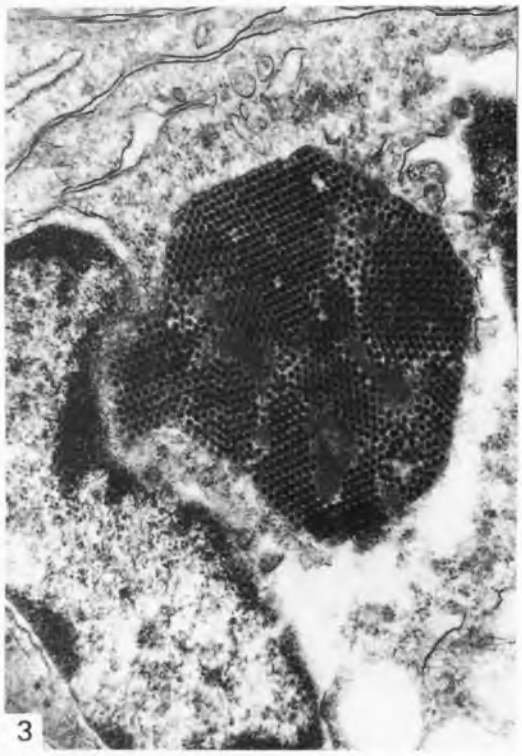


FIGURE 1 – Bursa of Fabricius of SPF chicken inoculated with BF suspension obtained from flock C on day 5 post-inoculation showing rarefaction of lymphoid cells and cystic degeneration in the medullar region. (H &E x 720).

FIGURE 2 – Ultrastructural changes in the Bursa of Fabricius of SPF chicken inoculated with BF suspension from flock C on day 5 post-inoculation. Mononucleated cells present cytoplasm vacuolization and myelin figures. (4.358 x magnification).

FIGURE 3 – Cristallin arrangment of electron dense particles within the cytoplasm of mononucleated cells of Bursa of Fabricius of SPF chicken inoculated with BF suspension from flock C on day 5 post-inoculation. (43.584 x magnification).

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