INFECTIONOUS BURSAL DISEASE: A CASE REPORT

INTRODUCTION

The occurrence of miscellaneous infections in chickens can be associated with an immunosuppression state caused by Infectious Bursal Disease (IBD) as well as by many other diseases (MANDELLI, 1986). IBD, also named Gumboro Disease and firstly described in USA (COSGROVE, 1962), is in many countries, an important disease of chickens that affects bursa of Fabricius. It is induced by a Birnavirus (LUKERT & HITCHNER, 1984 e RIDDLE, 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 (1972). SAUKAS (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, 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. 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 BFVs were ground in a pest and mortar and homogenized in Ten Broeck (Pyrex). BFU/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 (Hysex Brown) supplied by Cooperativa Agrícola de 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 antisera 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 formalin buffered saline (10%), were included in paraffin. Thin sections were stained by hematoxylin-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. BFU/V suspension obtained above was tested against 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 un inoculated 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. BFU/V suspension was 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. BFU/V suspension was 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 (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 BF. *Eimeria sp.* oocysts were detected in some gut samples. Some broilers from flock B showed complicated airsaculitis, haemorrhagic trachea, gut and small BF.

Flock C - IBDV 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 BF from each flock, tested against different antiserums.

- 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 chickens produced strong lines of precipitation when tested against G13, G23, G9 and Bursine II hyperimmune serums.

Electron microscopy: Aggregates of unenveloped virus particles (Fig. 2) were observed in the cytoplasms of slightly changed and/or foamy mononucleated cells of all BF. 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 derived 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 chickens 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 chickens 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 appropriate 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 duodenum 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.

**Serosity:** 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 immunosuppression (MANDELLI, 1986). On the other hand, the findings concerning flock C could be related to classical IBD described by LUKERT & HITCHNER (1984).

Although ADENE et alii. (1985) described successful detection of IBDV antigens in the BF obtained from field diseased chicks, precipitating antigens were not detected in the original pooled BF's. 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, 1984; ABDU et alii, 1986; SIVANANDAN et alii, 1986).

The presence of aggregates of virus particles in the bursal cells closely related to foamy cells and myelin figures development, seems to be similar to that described for IBDV (CHEVILLE, 1967; LUNGER & MADDU, 1972; BURTONBOY et alii, 1974; SAUKAS, 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, 1984) but smallest particles has also been described (MANDELLI et alii, 1986) and ABDU et alii (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 - antiserums, 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 proventricular 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, 1984) could be due to a clotting problem elicited by IBDV - infection (SKEELES et alii, 1979; SKEELES et alii, 1980) in some types of chickens. Also the proventricular lesions are not typical for IBD. It has been described that IBDV produces haemorrhages in the proventricular mucosa at the junction of the proventricular and gizzard (LUKERT & HITCHNER, 1984; ABDU et alii, 1986), proventricular glands congestion and glandular cell necrosis (OKOYE, 1985). The presence of whitish areas on the surface of the proventriculus, macroscopically related to the presence of dense aggregates of small lymphocytes, has been associated with a systemic effect of Avian Encephalomyelitis virus (BUTTERFIELD et alii, 1969; LUGINBUHL et alii, 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, 1977). As the bursectomy results in an increase of the number of ileal lymphoid foci (BACK, 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 atrophy and/or from an intense stimulus on the immune system and/or from virus presence in the tissue. These hypotheses could account for the proventricular lesions found in the broiler chickens early stimulated with intermediate strains of vaccinal IBDV (ITO, 1988)*.

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

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr. J.B. McFarran for his helpfull criticism and for supplying the virus antisera and samples used in this study, to Salsbury for supplying virus samples; to Mr. Claudio Arroyo for preparing the sections; to Mrs. Livia Rodrigues da Silva for the technical assistance; to Biovet Laboratories; Granja Rezende and Cooperativa Agrícola de Cotia for supplying experimental animals.

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.


<table>
<thead>
<tr>
<th>Broiler Flock</th>
<th>Housed chickens</th>
<th>Sex</th>
<th>Age (days)</th>
<th>Mortality (%)</th>
<th>Schedule of vaccination&lt;sup&gt;(a)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23500</td>
<td>Mixed</td>
<td>46</td>
<td>4.5</td>
<td>NDV 9-28</td>
</tr>
<tr>
<td>B</td>
<td>11000</td>
<td>Female</td>
<td>30</td>
<td>2.5</td>
<td>NDV 9-28</td>
</tr>
<tr>
<td>C</td>
<td>7500</td>
<td>Female</td>
<td>39</td>
<td>7.2</td>
<td>NDV 10-28</td>
</tr>
<tr>
<td>D</td>
<td>7500</td>
<td>Male</td>
<td>39</td>
<td>9.0</td>
<td>NDV 9-28</td>
</tr>
</tbody>
</table>

<sup>(a)</sup> — The vaccines were administered by drinking water
<sup>(b)</sup> — Strain of low pathogenicity (Bursine I)
<sup>(c)</sup> — 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.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Broiler Flock</th>
<th>Age (days)</th>
<th>Mortality (%)</th>
<th>Body weight (kg)</th>
<th>Daily weight Gain (kg)</th>
<th>Feed conversion</th>
<th>Medication expense (%)</th>
<th>Performance score (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>54</td>
<td>4.43</td>
<td>1.25</td>
<td>39.35</td>
<td>2.35</td>
<td>1.54</td>
<td>160</td>
</tr>
<tr>
<td>Mixed</td>
<td>B</td>
<td>55</td>
<td>5.09</td>
<td>2.118</td>
<td>38.51</td>
<td>2.41</td>
<td>1.75</td>
<td>172</td>
</tr>
<tr>
<td>Female</td>
<td>C</td>
<td>53</td>
<td>3.54</td>
<td>2.257</td>
<td>42.13</td>
<td>2.27</td>
<td>0.49</td>
<td>177</td>
</tr>
<tr>
<td>Healthy</td>
<td>D</td>
<td>54</td>
<td>11.07</td>
<td>2.387</td>
<td>44.20</td>
<td>2.31</td>
<td>0.81</td>
<td>170</td>
</tr>
<tr>
<td>Male</td>
<td>Healthy</td>
<td>49</td>
<td>4.32</td>
<td>2.289</td>
<td>45.8</td>
<td>2.20</td>
<td>1.94</td>
<td>199</td>
</tr>
</tbody>
</table>

Notes:
- **(a)** Percentage of expense with medication calculated in relation to the total expense of the production.
- **(b)** Performance score = Daily weight gain x viability / Feed conversion x 100.
- **(c)** The results represent the mean obtained from ten flocks constituted of clinically healthy broiler chickens housed in poulty farms located in the same region.

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental Serologic test</th>
<th>DAYS AFTER INOCULATION</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Contact</td>
<td>AGP/G9</td>
<td>0.7 ± 1.5a (1.6)b</td>
</tr>
<tr>
<td></td>
<td>AGP/Ag^2</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>SN/Lukert</td>
<td>5.8 ± 0.7 (56)</td>
</tr>
<tr>
<td>Inoculated</td>
<td>AGP/G9</td>
<td>0.8 ± 0.9 (1.7)</td>
</tr>
<tr>
<td></td>
<td>AGP/Ag^2</td>
<td>2.0 ± 1.7 (4)</td>
</tr>
<tr>
<td></td>
<td>SN/Lukert</td>
<td>7.9 ± 0.5 (242)</td>
</tr>
</tbody>
</table>

(a) — Geometric mean titer (log2 of the reciprocal dilution) ± standard deviation
(b) — Antilog2 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 arrangement 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).
REFERENCES


Infectious bursal disease: a case report.

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