Case Report —

Cryptosporidiosis of the Bursa of Fabricius of Chickens

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SUMMARY

Light-microscope and electron-microscope studies of a coccidial organism found in the bursa of Fabricius from 3 chickens clearly established the parasite as belonging to the family Cryptosporiidae. Hyperplasia and heterophil infiltration were associated with the presence of organisms attached to the microvillus border of epithelial cells lining the plicae of the bursa of Fabricius. Although there were no clinical signs or gross lesions common to the 3 cases described, all had similar histologic lesions in the epithelium lining the bursa of Fabricius.

INTRODUCTION

Coccidia of the family Cryptosporiidae have life-cycle stages intimately associated with the microvillus border of epithelial cells (5). The first recognized members of this family, Cryptosporidium muris and Cryptosporidium parvum from mice, were described by Tyzzer, who also reported the occurrence of organisms morphologically identical to C. parvum in the ceca of chickens (10). Coccidia found on epithelial cells in the terminal third of the small intestine of 10-to-14-day-old turkey poultis conformed to Tyzzer’s description of cryptosporidia of mice and were designated Crypto-
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Cryptosporidium meleagridis (9). Slavin (9) associated diarrhea in those poult with the presence of that parasite.

Cryptosporidia have been described in guinea pigs (3), calves (7,8), reptiles (5), and monkeys (4). Meuten et al. (7) reviewed the general biological properties and lesions associated with infection by cryptosporidia.

Cryptosporidia have not previously been reported in the bursa of Fabricius (BF). Lesions in the BF of 3 chickens were associated with organisms having the morphologic features of Cryptosporiidae. This report describes features found in studies with the light microscope and electron microscope.

MATERIALS AND METHODS

Case 1. Portions of BF and pancreas obtained from 6-week-old broilers suspected of having infectious bursal disease and fixed in 10% formalin were received on Feb. 4, 1974. The broilers were part of a flock of 21,000 chickens housed in a building previously occupied by turkey poult. According to the referring veterinarian, lesions of the BF were fluid accumulation and slight hemorrhage.

Case 2. Portions of BF, kidney, proventriculus, liver, spleen, small intestine, heart, sciatic nerve, and brain obtained from 17-week-old chickens with clinical signs of Marek's disease (MD) and fixed in 10% neutral buffered formalin were received on July 16, 1974. Approximately 6 weeks later (August 30, 1974) 4 chickens were selected at random from this flock and portions of all major organs (including duodenum, terminal small intestine, ceca, rectum, cloaca, liver, and gallbladder) were fixed in 10% neutral buffered formalin.

Case 3. Hematoxylin-and-eosin-(H&E) stained sections prepared from paraffin-embedded formalin-fixed portions of thymus and BF from 7 chickens were received on Nov. 13, 1974, from a regional poultry diagnostic laboratory. After the tissue sections were examined, formalin-fixed tissues were requested and received. Thymus and BF from a 7-week-old chicken were the only tissues available. Gross examination had revealed an enlarged thymus, and MD was tentatively diagnosed.

Tissue processing. Routine methods were used in preparing H&E-stained sections cut from paraffin-embedded tissues with steel knives. Formalin-fixed tissues from cases 2 and 3 were also embedded in glycol methacrylate and sectioned at 2 μ on glass knives by previously described (1) methods. These thin sections
were stained with acid-fuchsin and toluidine blue (AF-TB) or with H&E.

Portions of formalin-fixed BF from case 2 were washed in sodium cacodylate buffer, pH 7.2, further fixed for 1 hour in 1% osmium (OsO₄) in the same buffer, dehydrated in a graded series of methanol, and embedded in a mixture of D.E.R. 332 and 732 (6). Ultrathin sections stained with uranyl acetate and lead citrate were examined with an RCA EMU-4A electron microscope.

The sizes of various life-cycle stages were determined with photomicrographs of organisms in both plastic- and paraffin-embedded tissues made at identical magnification as a micrometer scale.

RESULTS

Examination of tissue sections of BF from case 1 revealed extensive hyperplasia of bursal epithelial cells with clumps of organisms and cellular debris in the bursal cavity (Fig. 1). Numerous small oval structures were attached to the surface of the epithelial cells (Fig. 2). Life-cycle stages were difficult to identify in sections from paraffin-embedded BF. Moderate numbers of heterophils were in the epithelial cell layer. No significant changes were noted in the bursal lymphoid follicles or in the interfollicular connective tissue. The pancreas was normal. Other organs were unavailable for examination and no further studies were done on this case.

Histologic examination of paraffin-embedded tissues from case 2 revealed tumors composed of pleomorphic lymphoid cells in ovaries, kidneys, and liver. A lymphoid cell infiltration was in the sciatic nerves, and a moderate number of lymphoid cells were in the interfollicular connective tissue of the BF. There was a non-suppurative encephalitis. MD was diagnosed. Subsequent exam-

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ination of plastic-embedded BF revealed numerous variable-sized ovoid structures attached to the microvillus border of bursal epithelial cells (Fig. 3). Life-cycle stages were present and identified as trophozoites, macrogametocytes, schizonts, and merozoites (Figs. 4, 6, 7). Many epithelial cells had cytoplasmic vacuoles containing amorphous material (Fig. 4). Heterophil infiltration was mild. Epithelial hyperplasia, although not as severe as in case 1, was in multiple focal areas. Cryptosporidia were not found in the proventriculus, duodenum, liver, or attached to any other epithelial cells examined.

Life-cycle stages revealed by electron-microscope examination of BF from case 2 included trophozoites, schizonts containing merozoites, and macrogametocytes (Fig. 8). Life-cycle stages were enclosed by extensions of epithelial cell membranes, with loss of microvilli at attachment sites (Fig. 9).

Trophozoites were in the tubular portion of the ceca from 1 of 4 chickens obtained from the source flock of case 2. These life-cycle stages were few in number, were the only stages observed, and were not found in the small intestine, rectum, cloaca, or atrophic BF.

Histologic examination of thymus and BF from the 8-week-old chicken in case 3 revealed destruction of thymic architecture by actively proliferating pleomorphic lymphoid cells which invaded the thymic capsule and extended into perithymic fat. Extensive epithelial hyperplasia was accompanied by heterophil infiltration in the BF. The spherical structures on the microvillus border of the epithelial cells were observed more readily in sections of plastic-embedded BF (Fig. 5). Life-cycle stages observed included tropho-

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Fig. 4. Two trophozoites (T) of different sizes and 2 stages believed to be macrogametocytes (M) are on the microvillus border. This is a higher magnification of Fig. 3. Note the intracytoplasmic vacuole containing amorphous debris (arrow). Plastic-embedded section, AF-TB stain. ×1417.

Fig. 5. Numerous cryptosporidia are on the microvillus border of epithelial cells from BF in case 3. Cells with cytoplasmic granules (arrow) are heterophils. Hyperplasia is present. Plastic-embedded section, AF-TB stain. ×740.

Fig. 6. Macrogametocytes and trophozoites are on the microvillus border of these bursal epithelial cells. The life-cycle stage in the upper right was not positively identified but may be a microgametocyte. Plastic-embedded section, AF-TB stain. ×1850.

Fig. 7. A macrogametocyte is associated with loss of microvilli. A thickened dark attachment zone is also present. Plastic-embedded section, AF-TB stain. ×2650.
Cryptosporidiosis of the bursa of Fabricius

Cryptosporidiosis of the BF from 3 chickens was associated with focal to diffuse epithelial cell hyperplasia and various degrees of heterophil infiltration. Numerous organisms were on the surface of the affected epithelium, and several life-cycle stages were identified. Thin sections prepared from plastic-embedded tissues were superior to sections prepared from paraffin-embedded tissues.

The organism was identified on the basis of comparisons of the observations in these 3 cases with those reported for cryptosporidia in chickens (10), guinea pigs (3), monkeys (4), and calves (7,8). Ultrastructural studies (2) of C. parvum in mice revealed the organism enveloped by epithelial cell plasmalemma. The organisms in the BF from the chicken in case 2 had the same type of association with epithelial cells. Ultrastructural features of various life-cycle stages in case 2 were similar to those reported (11) for C. wrairi in guinea pigs and with those observed in monkeys (4) and in a calf (7). These comparisons clearly establish the parasite found in the chicken BF as belonging to the family Cryptosporiidae. No further identification was attempted.

The presence of Cryptosporiidae in the BF of chickens is regarded as very unusual. There were no clinical signs or gross lesions common to all 3 cases. Two of the 3 chickens had MD, but any sig-

Fig. 8. Several stages of development are seen in this electron micrograph. Trophozoites (T) are at nearly the same stage. Note the membranous folds in the attachment zone (arrows). Schizonts (S) containing merozoites and a macrogametocyte (M) represent other life-cycle stages. The macrogamete contains polysaccharide granules (clear) and dense granules referred to by Vetterling et al. (9) as wall-forming bodies. ×6479.

Fig. 9. Two trophozoites are on the surface of the columnar cells lining bursal plicae. The organism at the left is at a younger or earlier stage of development. ×15,260.
Table 1. Comparison of the relative sizes (μM) of the life-cycle stages of *Cryptosporidium*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Calf, BF, plastic-embedded</th>
<th>Chicken, BF, plastic-embedded</th>
<th>Chicken, BF, paraffin-embedded</th>
<th>Turkey, Slavin (8) (dried smears)</th>
<th>Meuten et al. (6) (formalin-fixed tissues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tophozoite</td>
<td>2 to 6 x 2 to 6</td>
<td>4 x 4</td>
<td>4.0 to 5.0</td>
<td>1.9 x 1.7</td>
<td></td>
</tr>
<tr>
<td>Schizont</td>
<td>4 to 8 x 4 to 6</td>
<td>ND</td>
<td>4.0 x 5.0</td>
<td>3.0 x 2.6</td>
<td></td>
</tr>
<tr>
<td>Merozoite</td>
<td>2 to 4 long</td>
<td>ND</td>
<td>5.0 x 1.1</td>
<td>2.0 x 1.2</td>
<td></td>
</tr>
<tr>
<td>Macrogametocyte</td>
<td>6 x 6 to 8</td>
<td>4 x 4</td>
<td>4.5 to 5.0 x</td>
<td>3.1 x 3.0</td>
<td></td>
</tr>
<tr>
<td>Microgametocyte</td>
<td>4 to 6</td>
<td>ND</td>
<td>3.5 to 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>4 to 6 x 12 to 22</td>
<td>8 x 14 to 18</td>
<td>4.0</td>
<td>3.9 x 2.9</td>
<td></td>
</tr>
</tbody>
</table>

*ND = not done, because of difficulties in identifying stages in paraffin-embedded sections.*
nificance of this association is not known. It is of interest that a
diagnosis of infectious bursal disease was based on the gross find-
ings in case 1 and that chickens in this flock were in a house previ-
ously occupied by turkey poults. It is unfortunate that no sections of
ceca were available from any of the 3 cases. Finding Crypto-
sporiidae in the ceca from 1 of 4 chickens in the source flock from
case 2 raises the possibility that involvement of BF results from
chance infection by organisms passing from the ceca. The lesions
found in the BF of the 3 chickens are similar to those found in the
gallbladder and biliary tree of a juvenile Rhesus monkey with
cryptosporidiosis (4). The BF was the only organ involved in
chickens.

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