The Pathogenesis of the Lesions Produced by Eimeria zuernii in Calves

P. H. G. Stockdale*

ABSTRACT

The pathogenesis of the lesions caused by Eimeria zuernii in calves is described. The gross lesions and the development and resolution of the microscopic lesions of the large intestine are described in detail. The development of the first asexual generation causes few changes in the lower ileum. The second asexual generation and gametogony of E. zuernii appear to be the pathogenic stages of its development. It is during these stages of the life cycle that epithelium is lost, capillaries are exposed and that hemorrhage into the lumen of the large intestine occurs. Resolution of these lesions takes place in approximately ten days in calves which survive.

RÉSUMÉ

L'auteur décrit la pathogénèse des lésions attribuables à Eimeria zuernii, chez le veau. Il décrit aussi en détail les lésions macroscopiques, ainsi que le développement et la résolution des lésions microscopiques du côlon. Le développement de la première génération asexuée de la coccidie provoque peu de changements dans la partie distale de l'iléon. La deuxième génération asexuée et la gamétogénèse de cette coccidie semblent correspondre aux stades pathogènes de son développement. C'est au cours de ces stades que l'épithélium se desquame, que les capillaires sont mis à nu et que survient l'hémorragie, dans la lumière du côlon. Les lésions se cicatrisent dans environ dix jours, chez les survivants.

*Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Animal Diseases Research Institute (W), P.O. Box 640, Lethbridge, Alberta T1J 3Z4.

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INTRODUCTION

The lesions of the intestinal tract of cattle caused by coccidia have been described by a number of authors (2, 5, 6, 7). Most of these authors described lesions which they assumed to be mainly due to *Eimeria zuernii* although some were described as being due to mixtures of species of *Eimeria* or a combination of *E. zuernii* and *E. bovis*.

None of these studies examined the pathogenesis of the enteric lesions caused by *E. zuernii*. The following study was made to investigate the pathogenesis of this disease.

MATERIALS AND METHODS

Calves used in this study were male Holsteins from the Agriculture Canada Research Station, Lethbridge. The calves varied in age at the time of infection from 24 hours to three months. The calves were reared as described earlier (8).

Calves were infected with a standard dose of 600,000 sporulated oocysts of approximately 100% pure *E. zuernii* or its equivalent in numbers of sporocysts or sporozoites (1, 3, 8). This inoculum was given via a stomach tube. The calves were injected with 20 mg dexamethasone[1] daily for three days on days 13, 14 and 15 post-infection if the particular calf was to be killed on day 16 or later (9). Calves killed earlier in the series received no corticosteroid. Calves were killed on the following days after infection: day 10, day 12, days 14, 15, 16, 17, 18, two calves on day

[1]Azium, Schering Corp., Pointe Claire, Quebec.

19, two calves on day 20, days 21, 22, 23, 24, day 26, two calves on day 28, day 30 and day 32. Most of the calves killed between days 18-28 were killed while moribund.

The calves were killed by electrocution. The abdominal cavities were opened immediately and the ileocecal junction located. The small intestine was fixed in Serra's fixative in one metre segments beginning at the ileocecal junction up to the abomasum. The cecum and colon were fixed in Serra's fixative and areas of the cecal and colonic mucosa were fixed in 4% glutaraldehyde. Tissues placed in Serra's fixative were transferred to 90% alcohol four hours later and tissues from glutaraldehyde were transferred to Millonig's buffer also four hours after fixation. Tissues fixed in Serra's were embedded in paraffin wax, cut at 5 μ and stained with haematoxylin and eosin. Tissues fixed in glutaraldehyde were embedded in epon, cut at 1 μ and stained with Toluidine blue.

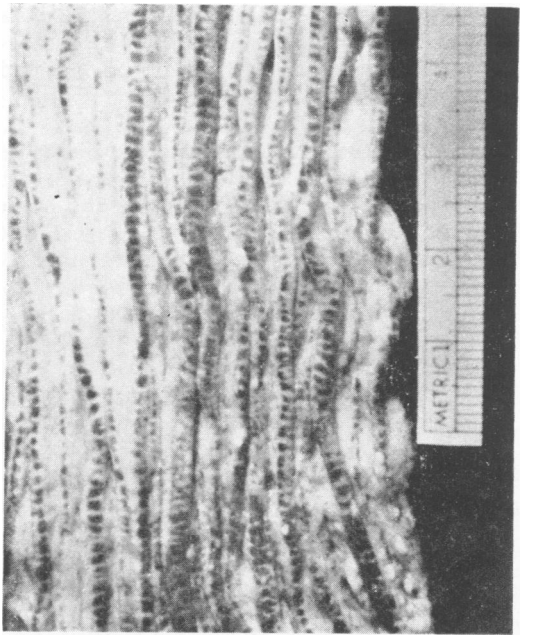

Fig. 1. Colon with "tiger-striping" on longitudinal mucosal folds.

RESULTS

Gross Pathology

In the calves killed up to 17 days after infection no gross lesions were seen in either the small or large intestines. The contents of the large intestine of the calf killed on day 16 were fluid.

The lesions seen in calves killed from day 18 to day 26 were similar although more or less severe in the individual animals. The contents of the colon and cecum were very fluid and varied from almost black to the bright red colour of fresh blood. The area of the large intestine that was usually most affected was the proximal 30 cm of the spiral colon and the whole of the cecum. In most of the calves killed at this time the epithelium was completely absent from this area, the lamina propria being covered by a diphtheritic membrane. In two of the calves killed (one at day 19 and one at day 20) large fibrinous casts with transverse constrictions were found up to 5 cm in diameter and up to 50 cm long. Petechiae were seen on the remaining mucosa appearing randomly or arranged in a "tiger-stripe" pattern (Fig. 1). The colonic and cecal walls were greatly thickened by yellow gelatinous edema usually found in greatest quantity in the submucosa.

The fibrinous casts found in the two calves had apparently acted as large bowel obstructions as in both calves the small intestine was hyperemic, dilated and flaccid.

Calves killed after 26 days postinfection had large intestines containing fluid, normal coloured contents with no obvious lesions of the colon or cecum.

Histopathology

Small intestine: First generation schizonts were found in the last 3 m of the small intestine of the calves from day 10 to day 21 after infection. The schizonts appeared to be in connective tissue cells of the lamina propria and were usually close to the muscularis mucosa (Fig. 2). The schizonts were usually surrounded by lymphocytes which sometimes were up to three cells deep. In sections from calves killed on days 15, 16 and 17, schizonts were found which also contained a few neutrophils. The outlines of former schizonts filled with leucocytes were seen in the lamina propria of the

calf killed on day 16. Generally the schizonts appeared to cause remarkably little reaction in the small intestines of the infected calves (Fig. 2).

Large intestine: No differences were noted between the lesions occurring in the cecum and colon. The histological lesions were divided arbitrarily into three phases. The first phase was those lesions seen early in infection in the calf killed on day 16. The second phase was the lesions seen in calves killed from day 18 to day 21 inclusive. At this stage the lesions were most extensive and active and a number of calves died or became moribund during this period. The final phase is that of organization of the lesions and the reconstitution of the epithelium of the large intestine.

The lesions of the large intestine of the calf killed 16 days after infection were mild. The epithelium, although it contained many trophozoites and second generation schizonts, was intact and unchanged from the surface of the ridges to the depths of the glands (Fig. 3). The lamina propria was edematous and infiltrated by many plasma cells (Fig. 3), neutrophils, lymphocytes and macrophages. The submucosa and external muscular layers were also edematous and there were many lymphocytes in the submucosa.

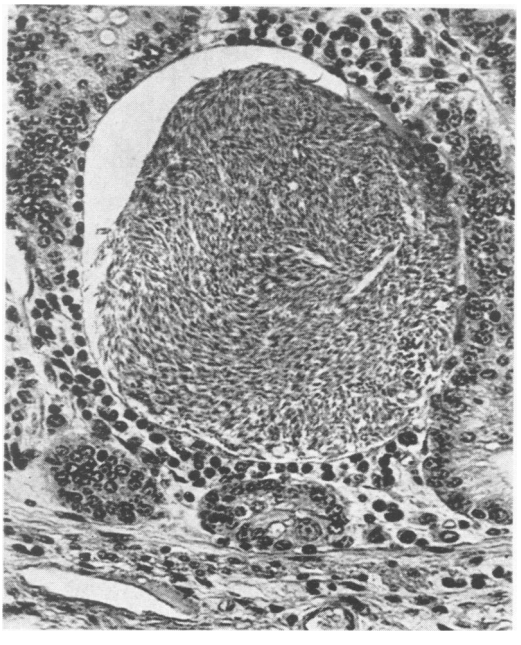

Fig. 2. First generation schizont of E. zuernii in ileum of calf killed 16 days after infection. H & E. X200.

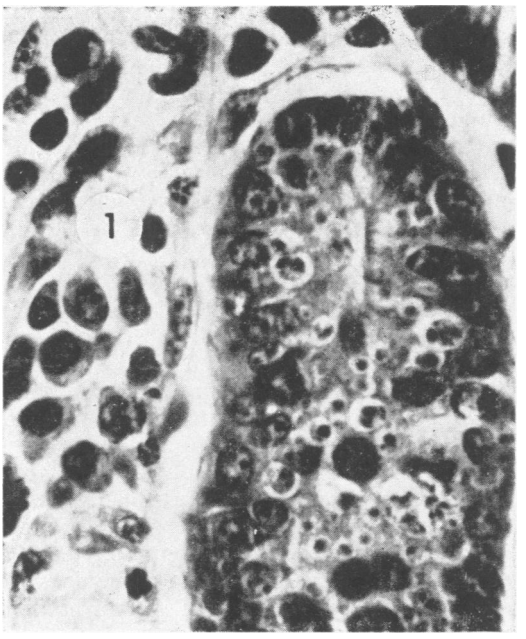

Fig. 3. Epithelium and lamina propria of large intestine of calf killed 16 days after infection. Trophozoites and merozoites in the epithelium; mononuclear and plasma cells in the lamina propria (1). H & E. X1000.

The lesions in the calves killed from day 18 to day 21 after infection were very active and extensive. The mucosal epithelium was present in the affected areas of the large intestine of the calf killed on day 18 but was absent from those areas of the calf killed 21 days after infection. On day 18 the epithelium was covered by a layer of fibrin which in some areas was twice as thick as the epithelium itself. Enmeshed in the fibrin were erythrocytes, mononuclear cells, granulocytes and oocysts (Fig. 4). The columnar epithelial cells covering the lamina propria in affected areas were rounded up and in others had altered to squamous epithelium (Fig. 4). In some places epithelial cells were completely absent thus exposing lamina propria. However, the glands could still be discerned. Some glands were distended with oocysts and inflammatory debris. In the calves killed on days 19 and 20 epithelium was absent from much of the lamina propria. A diphtheritic membrane covered the epithelium or, where absent, the lamina propria. The fibrin of the membrane enmeshed large numbers of erythrocytes and polymorphs.

The epithelial cells of the glands were more difficult to discern and sometimes the outlines of the glands could only be iden-

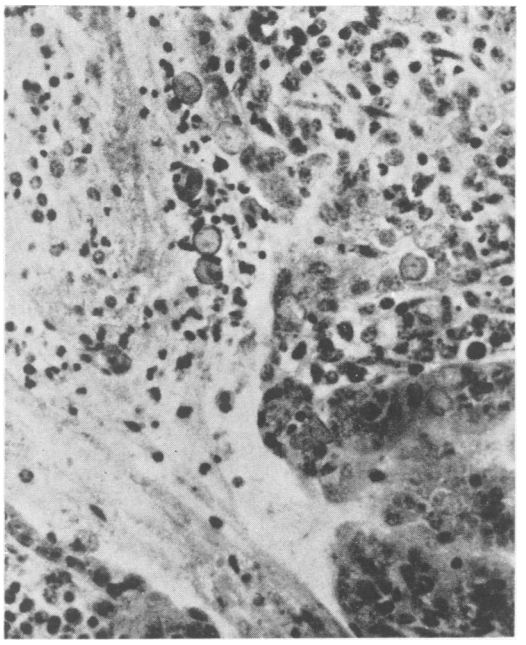

Fig. 4. Section of large intestine of calf killed 18 days after infection with E. zuernii. Oocysts at junction of epithelium and overlying fibrinous cast. H & E. X200.

tified by lines of oocysts or zygotes. The epithelium of the affected large intestine of the calves killed on day 20 was no longer identifiable as a complete epithelium. In some areas islands of five to 20 epithelial cells were visible, in others, a layer of squamous epithelial cells covered the surface. By day 21 only a few isolated foci of epithelial cells were visible within the lamina propria.

The lamina propria of the large intestine of the calf killed 18 days after infection contained many plasma cells, lymphocytes and neutrophils. The capillaries of the lamina propria, many directly exposed to the gut lumen, were distended (Fig. 5). Many contained large numbers of leucocytes and others were packed with distorted red cells. By day 20 after infection the lamina propria was much reduced in size and contained oocysts, plasma cells, lymphocytes, neutrophils and eosinophils (Fig. 6). On day 21 smooth muscle cells and capillaries were all that could be certainly identified among the inflammatory cells and fibroblasts of the lamina propria. There were many cells with large pale blue nuclei present which might have been regenerating epithelium or fibroblasts of the lamina propria.

In the calves killed from day 18 to 21 there was edema of the submucosa and in some, edema within the external muscle layers. Lymphocytes, plasma cells, neutrophils were seen within the edematous submucosa (Fig. 7).

Cross sections through the fibrin casts found in the large bowels of the calves killed on days 19 and 20 were examined and found to be varied in composition of fibrin in which were tangled large numbers of leucocytes (mainly neutrophils), erythrocytes and oocysts. The central areas of the cast were mainly composed of fibrin with few cellular elements but many bacterial colonies.

The changes seen in the large intestines of the calves killed from day 22 to day 32 were those of sequential resolution of the lesions seen earlier. The lamina propria of the large intestine of the calf killed on day 22 was covered by epithelium and the outline of glands lined by epithelium was clearly visible. These epithelial cells had

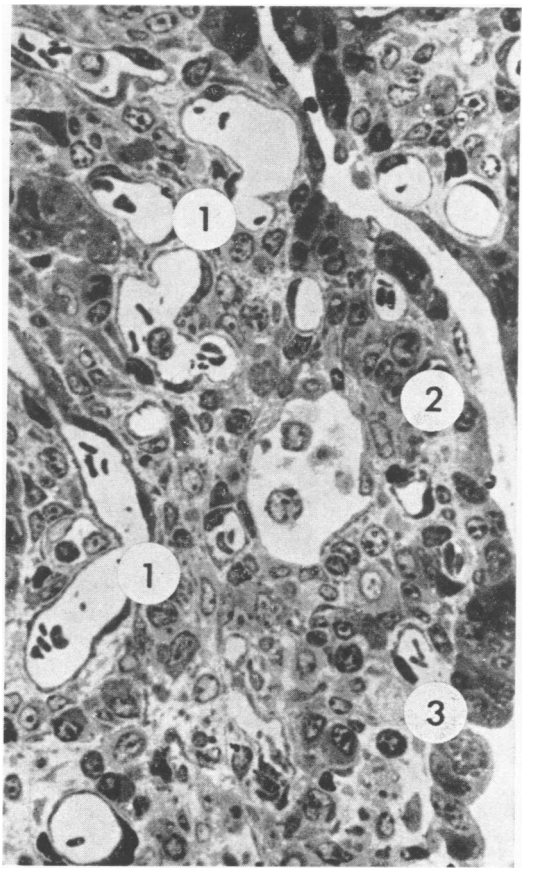

Fig. 5. Detail of large intestine to show dilated capillaries (1), rounded and flattened epithelium (2) and second generation schizonts (3). Toluidine Blue. X500.

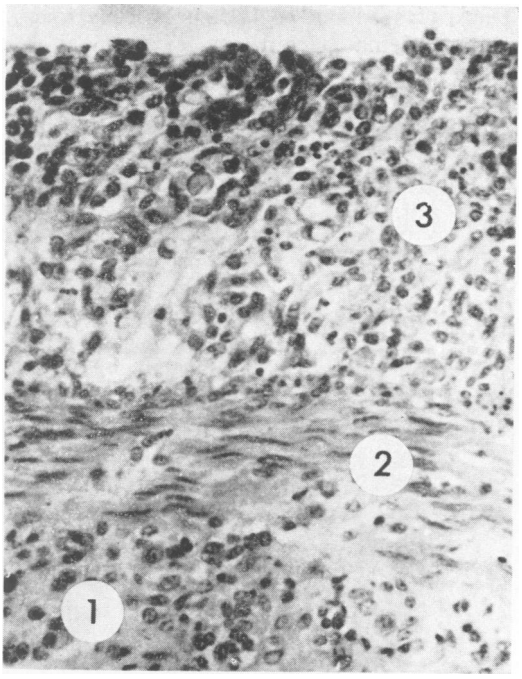

Fig. 6. Section through large intestine wall from submucosa to lumen. Note absence of any epithelium leaving lamina propria with connective tissue elements, leukocytes and oocysts. (1) submucosa, (2) muscularis musoca, (3) lamina propria. H & E. X200.

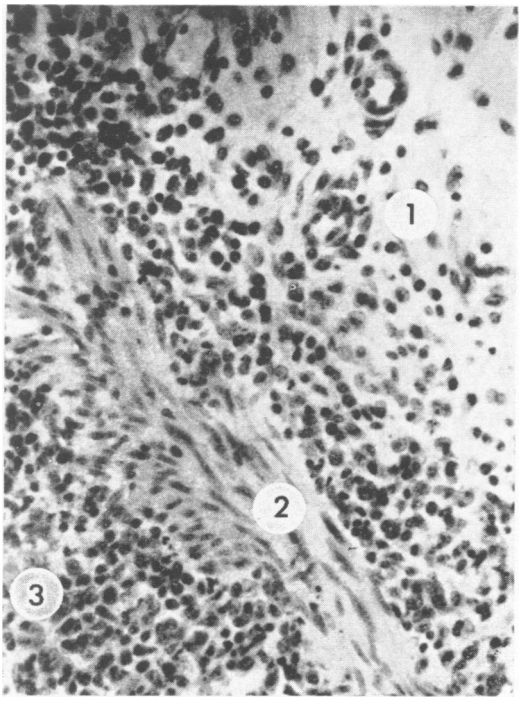

Fig. 7. Inflammation of the submucosa of the large intestine. (1) submucosa, (2) muscularis mucosa, (3) lamina propria. H & E. X200.

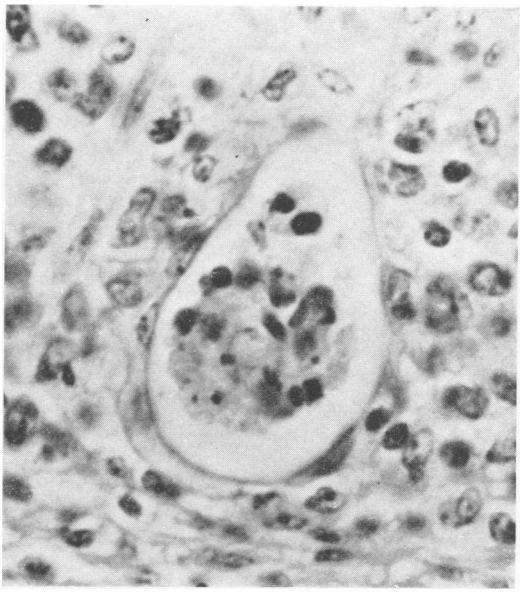

Fig. 8. Crypt abscess in lamina propria of calf infected with E. zuernii. H & E. X500.

large pale nuclei with little cytoplasm and cells varied from being cuboidal to columnar in shape. The epithelial goblet cells were producing mucus in the calf killed on day 28 and the epithelium appeared normal. Distended glands lined by squamous epithelium and filled with oocysts and inflammatory debris (Fig. 8) were seen in all the calves killed during this time but were less frequent in calves killed later in the series.

Granulocytes and mononuclear cells were still present in the lamina propria and the mucosa generally appeared to consist of more lamina propria than epithelium. By the 32nd day after infection the ratio of lamina propria to epithelium within the mucosa appeared more normal but monocytes, lymphocytes, plasma cells, eosinophils and neutrophils were still obvious within the lamina propria. In the calves killed on days 22 and 28 there were numbers of oocysts within the lamina propria. There was submucosal edema in calves killed on days 22 and 24.

The lesions found in the calf killed on day 26 differed from those found in other calves killed later in the series. The lesions were still active. In some areas of the large intestine there was no epithelium. The lamina propria, muscularis mucosa and submucosa were confluent and indistinguishable. There were many eosinophils in the lamina propria. In these affected areas

there were numbers of neutrophils in the submucosal lymphatic vessels, the external muscle layers and the subserosal fat.

DISCUSSION

The gross lesions described here were described earlier by Smith and Graybill (7) and Railliet (6). The only feature that apparently had not been noted earlier was the production of large cecal and colonic casts. However, Smith and Graybill (7) reported that masses of fibrin were found on the epithelium. Hammond *et al* (5) described the production of a diphtheritic (bloody) membrane "...easily separable from the underlying mucosa..." in coccidiosis of calves due to *Eimeria bovis*. In fact, the description of the gross lesions these workers described as occurring in the large intestines of calves affected by *E. bovis* is almost identical to the description made here of the lesions caused by *E. zuernii*.

When calves are experimentally infected with *E. zuernii*, infection usually occurs but the animals do not become clinically ill. If corticosteroids are given as described here, then clinical disease also occurs (9). The corticosteroids are given during the development of the first generation schizont and the lesions of the large intestine occur during second generation schizogony and gametogony. Thus, it is considered that the lesions described here are essentially those caused by *E. zuernii* and independent of a direct effect of the corticosteroids. Similar lesions are seen in animals dying from coccidiosis in the field.

The histological sequence of the lesions found in the large intestine is interpreted as follows. Merozoites from the first generation schizonts in the ileum enter the colon and cecum from 14 days after infection onwards. They enter epithelial cells and rounding up rapidly become second generation schizonts. At this time lymphocytes and neutrophils enter the lamina propria in large numbers and the capillaries become dilated. As the schizonts mature the epithelial cells change in morphology, become rounded or flattened and in some areas become stripped off the underlying lamina propria.

At this stage (days 18, 19 and 20 after infection) second generation schizogony and gametogony are taking place. Generally schizogony in the large intestine appears to occur in the epithelium of the surface and upper areas of the glands. However, if the infection is very heavy schizonts are also found deep in the glands. Gametogony is usually found at the base of the glands. As the epithelial cells are lost into the intestinal lumen the dilated capillaries are exposed and large amounts of fibrin escape from intact vessels and all blood cellular and noncellular elements are lost from vessels that rupture. This occurs mainly from the superficial lamina propria on day 18 but by day 20, as the gland epithelia disappear, this presumably happens from all over the surface of the lamina propria. At the same time numbers of leucocytes and plasma cells are found within the mucosa.

Between days 18 and 21 the submucosa and external muscle layers are also involved in the colitis and typhlitis. There is edema and cellular infiltration of the submucosa and edema of the external muscle. From day 22 the lesions are resolving in surviving animals. Epithelium is replaced at first by cells that are cuboidal in type but which later appear to differentiate into columnar epithelium. However, the lamina propria appears more cellular than normal as late as 32 days after infection.

The dilated glands, often termed "crypt abscesses", appear early in infection (day 18) and are still present 30 days after infection. Presumably these occur because of blockage higher up the gland lumen towards the intestinal lumen. The glands fill with oocysts from the epithelium lining the gland and debris formed from leucocytes migrating through the epithelium into the gland lumen. Railliet (6) describes four stages of histopathological changes associated with bovine coccidia. These were arrived at from the examination of naturally occurring cases of the disease. His stages are similar to the phases of the infection described here: i.e. an initial hyperemia of the vasculature of the lamina propria. This is followed by destruction of the glands and the formation of "crypt abscesses". In his fourth stage the epithelium is entirely absent and the mucosa is reduced solely to lamina propria.

All of Railliet's stages are found during the most active phase of the disease from day 18 to day 21 after infection. Smith and

Graybill (7) and Davis and Bowman (2) also describe the loss of epithelium and haemorrhage into the large intestines of calves infected by *E. zuernii*. Hammond (4) described the migration of neutrophils into glands containing oocysts in calves infected with *E. bovis*. It is apparent that the histological lesions of *E. zuernii*, as well as the gross lesions, resemble those of *E. bovis* seen in the colons and ceca of calves (5).

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