

Coccidiosis in the Gallbladder of a Goat

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ABSTRACT: Coccidiosis, probably due to *Eimeria* sp., was found in the gallbladder of a goat. Meronts, gamonts, and oocysts were found in the epithelium of villous and submucosal glands. Meronts were $11.7 \times 7.7 \mu\text{m}$ and contained 3-20 merozoites; meronts were 8-12 μm long and 1.5-2.3 μm wide. Macrogamonts and unsporulated oocysts were $9.6 \times 8.7 \mu\text{m}$ and $11.6 \times 9.5 \mu\text{m}$, respectively. There was generalized cholecystitis characterized by necrosis and infiltration by mononuclear cells. Liver was normal.

Several species of *Eimeria* parasitize the intestines of goats, sheep, and cattle, but none is known to invade the gallbladder (Levine, 1973; Lima, 1980). This report describes severe coccidiosis in the gallbladder of a dairy goat.

Materials and Methods

The goat was a 118-day-old purebred Alpine female from a commercial goat dairy in Darby, Montana and had been fed 50,000 sporocysts of *Sarcocystis capracanis* 33 days prior to necropsy. The goat showed clinical signs of acute sarcocystosis between 23 and 30 days postinoculation (Dubey et al., 1981).

The goat was killed by electrocution, exsanguinated, and necropsied immediately. Portions of gallbladder and other tissues were fixed in 10% neutral-buffered formalin (NBF). A small piece of the gallbladder was also fixed in Helly's fixative. Paraffin-embedded sections were examined after staining with hematoxylin and eosin (HE). After finding coccidian stages in the gallbladder, several pieces of gallbladder fixed in NBF for 18 months were embedded in glycol methacrylate for light microscopy and also processed for transmission electron microscopy. Methacrylate-embedded sections were cut at 3 μm , and examined after staining with hematoxylin and eosin, Giemsa, iron-hematoxylin (IH) or periodic acid Schiff (PAS) hematoxylin. For transmission electron microscopy, sections were examined under a JEOL 100 cx microscope.

Results

Histologic examination of the gallbladder revealed an unidentified coccidium different from *Sarcocystis*. Because the unidentified coccidium, which may be *Eimeria* sp., has not been reported previously from the gallbladder of goats, the parasite is described below.

Parasitic stages

Meronts and gamonts were found in the epithelium of villous and submucosal glands of the gallbladder (Figs. 1-7). More parasites were found in glands than in the villous. Meronts were more

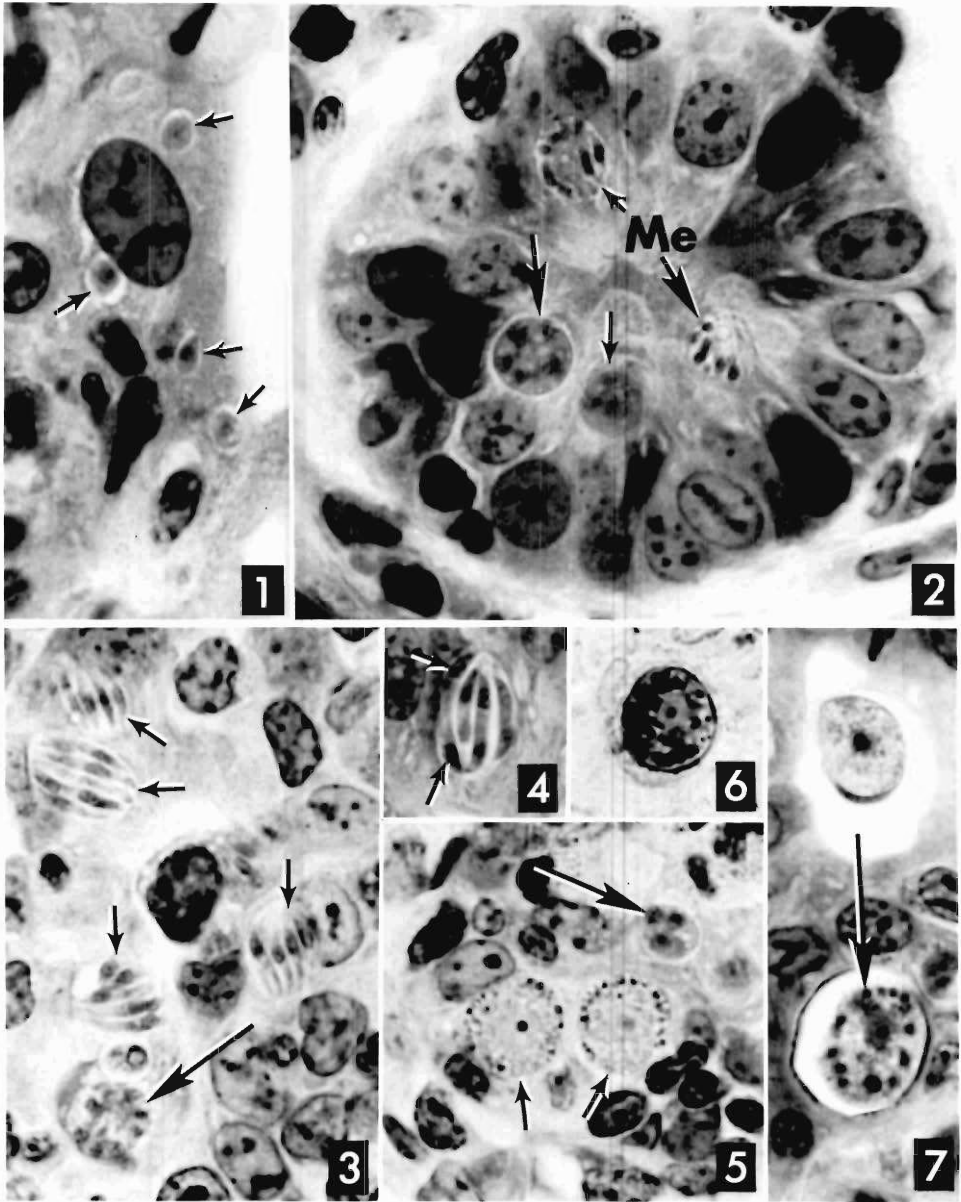
numerous than gamonts and the intensity of infection varied; some sections were heavily infected, whereas others had few parasites.

Meronts were in various stages of development (Figs. 1-4). Mature meronts were $9.3 \times 6.7 \mu\text{m}$ ($5-15 \times 4-11 \mu\text{m}$; $N = 58$) and contained 1-17 merozoites. The merozoites were 8-11 μm long and 1-2 μm wide; they occupied the entire length of the meront (Fig. 4). Merozoites were curved at the broader posterior end and pointed at the thin anterior end; the nucleus was usually located toward the posterior end. The nucleus stained prominently with hematoxylin and occupied the entire width of the merozoite (Fig. 4). A few fine granules were seen just anterior to the merozoite nucleus in sections stained with iron hematoxylin. Merozoites contained several PAS-positive granules; the intensity of reaction varied among merozoites. Merozoites within a given meront were arranged randomly often head to tail (Figs. 3, 4); thus, it was difficult to count them accurately or determine their length. Free merozoites or meronts were seen in the lumen of the glands and the gallbladder.

Most gamonts were in glands. Macrogamonts had a large nucleus with a prominent nucleolus, even in the youngest ($3 \times 2 \mu\text{m}$) macrogamont (Fig. 1). Mature macrogamonts were $9.6 \times 8.3 \mu\text{m}$ ($7-14 \times 6-12 \mu\text{m}$; $N = 21$) and contained PAS-positive wall-forming bodies (Fig. 5). Microgamonts were not identified with certainty. However, few 2- to 8-nucleated bodies (Fig. 2) interpreted as microgamonts were $7 \times 5.7 \mu\text{m}$ ($5-9 \times 4-7 \mu\text{m}$; $N = 5$); their nuclei were much smaller than those of macrogamonts. Unsporulated oocysts were $11.6 \times 9.5 \mu\text{m}$ ($9-15 \times 7-12 \mu\text{m}$; $N = 10$) and were seen in glands (Figs. 6, 7).

Ultrastructurally, meronts were located within a parasitophorous vacuole (PV) in the cytoplasm of epithelial cells (Figs. 8-12). Of the 28 meronts studied, 5 were immature and 23 contained merozoites. The youngest meront observed was $5.7 \times$

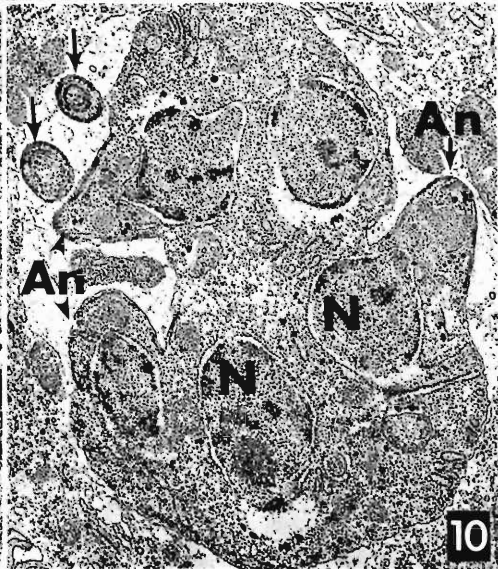
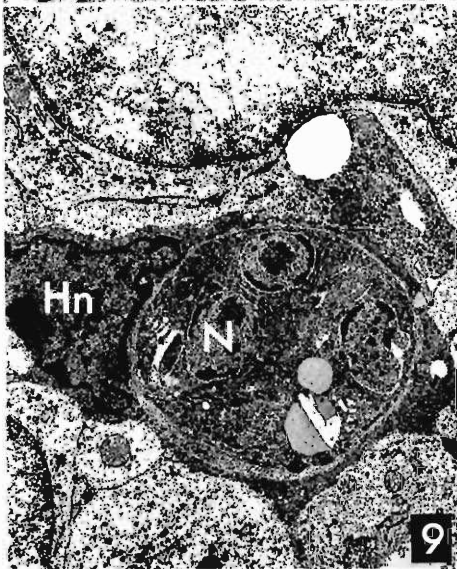
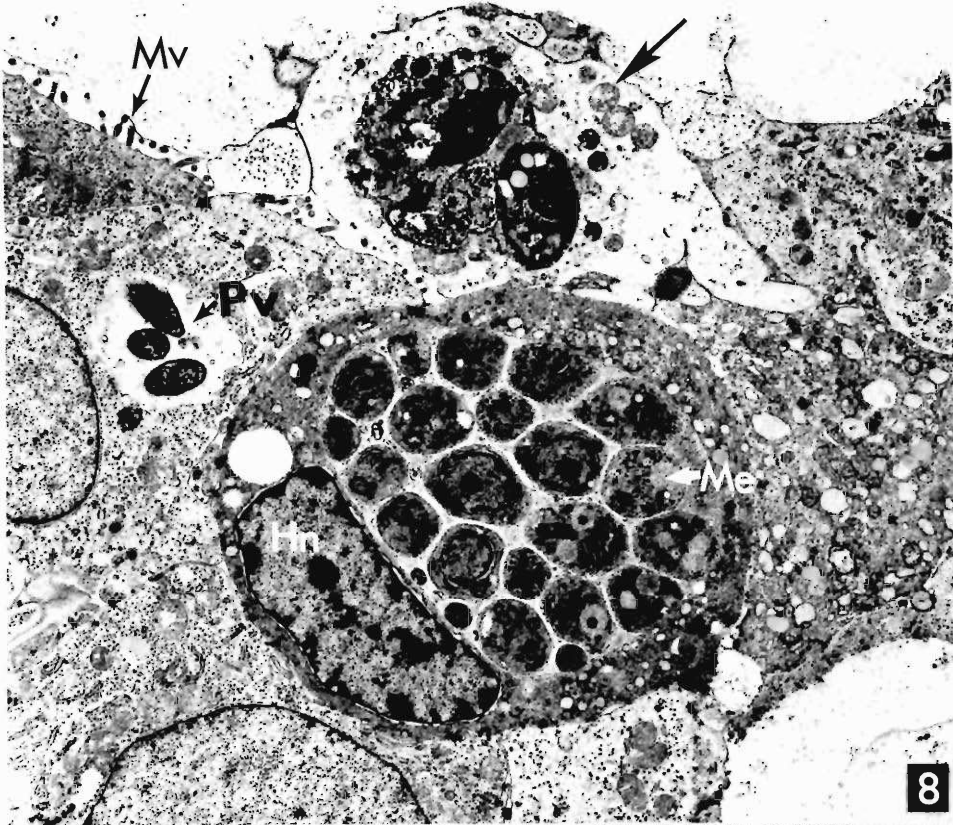
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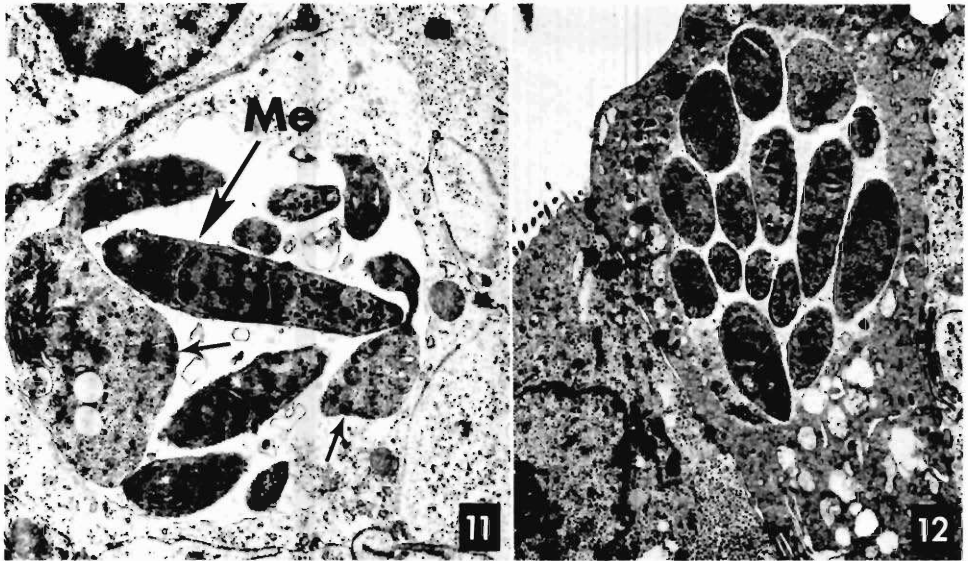
Figures 1-7. Stages of the coccidium in gallbladder of goat ($\times 1,000$). Figures 1-5 and 7, 3 μm , methacrylate; Figure 6, 1 μm , epoxy resin. 1. Uninucleate zoites (arrows) in parasitophorous vacuoles in epithelial cells of the villous. HE. 2. Two immature meronts (Me), a binucleate (small arrow) and a 5-nucleate (large arrow) meront or microgamont. IH. 3. Four mature meronts (small arrows) and an immature meront (large arrow) in epithelial cells of a submucosal gland. HE. 4. Meront with 6 merozoites. Arrows point to the nucleus of the merozoite. HE. 5. Two macrogamonts (small arrow) each with a central nucleus, and a 3-nucleate meront (large arrow). HE. 6. Partly formed oocyst with a central nucleus and wall-forming bodies. Toluidine blue. 7. Two unsporulated oocysts with wrinkled walls. Well-forming bodies are present in 1 oocyst (arrow) and absent in the other. IH.

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Figures 8-10. Transmission electron micrographs of the coccidium in epithelial cells of the gallbladder of the goat. Abbreviations: Hn = host cell nucleus, Pv = parasitophorous vacuole, Me = merozoites, N = nucleus



of the parasite, Mv = microvilli of the host cell, An = anlagen of the merozoite. 8. Three meronts in villus. The meront toward the far left contains the anterior ends of 3 merozoites. The meront toward the lumen (arrow) is being extruded into the lumen. The meront in the center contains 23 cross sections of merozoites. Note spaces among merozoites and absence of residual body ($\times 5,775$). 9. Three-nucleate meront ($\times 7,656$). 10. Developing merozoites in a meront. Note nuclei in close proximity of merozoite anlagen (arrows). Small arrows point to anterior ends of merozoites that have separated from the meront ($\times 9,628$).



Figures 11, 12. Transmission electron micrographs of meronts of the coccidium in the gallbladder of a goat. 11. Meront with merozoites separating from residual bodies (arrows). Note the position of the nucleus in a longitudinally cut merozoite (Me) ($\times 7,656$). 12. Mature meront with 15 merozoites ($\times 5,800$).

4.9 μm and contained 3 nuclei (Fig. 9). One immature meront contained 7 nuclei without evidence of merozoite formation. Prior to merozoite formation, the nuclei moved towards the periphery of the meront and each nucleus was incorporated into a merozoite anlage (Fig. 10). Merozoites separated asynchronously from the mother cell leaving 1 or 2 residual bodies (Fig. 11). The residual body was present in 10 of 23 meronts and was located usually toward one side of the meront; the residual bodies were $4.5 \times 2.4 \mu\text{m}$ ($2.5\text{--}5.7 \times 1.1\text{--}3 \mu\text{m}$; $N = 10$). Mature meronts were $11.7 \times 7.7 \mu\text{m}$ ($5.7\text{--}16 \times 3.9\text{--}8.3 \mu\text{m}$; $N = 23$) and contained 3–20 merozoites. Out of 23 meronts, 1 each had 3, 4, 5, 6, 15, 16, and 20 merozoites and 16 meronts had between 7 and 12 merozoites. Of more than 100 merozoites studied, only 3 were cut longitudinally and the position of the nucleus varied from terminal to central. The longest merozoite was 7.9 μm . Merozoites were 1.7 μm ($1\text{--}2.3 \mu\text{m}$; $N = 46$) wide. In all but 2 meronts, merozoites were arranged randomly within the meront with spaces among them. In 2 meronts, merozoites were tightly packed.

Gamonts were not identified with certainty under the electron microscope because of their rarity. Uninucleate organisms interpreted as macrogamonts were seen in epithelial cells; these contained more storage granules than those in meronts.

Lesions

The entire gallbladder was thickened due to edema and infiltration by mononuclear cells. The mononuclear cell infiltration was seen throughout the width of the gallbladder but was most pronounced in the lamina propria. There were minute hemorrhages in the lamina propria. The villi were stunted and occasionally fused. The villous epithelial cells were flat to low cuboidal. Focal necrosis was seen in the lamina propria, submucosal glands, and villous epithelium, and there was a focus of ulceration in 1 villus (Fig. 13). The epithelium in glands and the villi was hyperplastic as indicated by increased mitotic figures, and the crypts of the submucosal glands contained desquamated epithelial cells and parasites.

Neither lesions nor parasites were seen in bile ducts, liver, intestines, mesenteric lymph nodes, or other tissues.

Discussion

Lesions in the present study were considered to be due to the associated coccidium because the infection was localized in the gallbladder and parasites were seen in the lesions. Although species of *Eimeria* occasionally invade mesenteric lymph nodes of goats (Lotze et al., 1964; Lima, 1979), infection of the gallbladder in goats or other mammals has not been previously reported (Levine, 1973; Levine and Ivens, 1981).



Figure 13. Edema (arrow), necrosis (N), and mononuclear cell infiltration in the lamina propria ($\times 280$). IH, paraffin-embedded, 5 μm .

The coccidian parasite could not be identified further. Of the many species of *Eimeria* that are known to occur in goats, the oocysts of *Eimeria parva* (14–23 μm) are the smallest (Levine, 1973; Lima, 1980). The longest oocyst in the gallbladder in the present case was 15 μm long. The endogenous stages of *E. parva* in goats are unknown. Also, the meronts were the smallest of any eimerian species in goats (Levine, 1973). The parasite was different from *Sarcocystis*, although *S. capracanis* meronts are known to occur in the gallbladder of goats (Dubey et al., 1981); however, they occur in the vascular endothelium and are in direct contact with host cytoplasm whereas meronts in the present study were located in a parasitophorous vacuole. Eimerian stages were not identified in sections of intestines.

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Literature Cited

- Dubey, J. P., S. E. Weisbrode, C. A. Speer, and S. P. Sharma. 1981. Sarcocystosis in goats: clinical signs and pathologic and hematologic findings. *Journal of the American Veterinary Medical Association* 178:683–699.
- Levine, N. D. 1973. *Protozoan Parasites of Domestic Animals and of Man*, 2nd ed. Burgess, Minneapolis. IX + 406 pp.
- , and V. Ivens. 1981. *The Coccidian Parasites (Protozoa: Apicomplexa) of Carnivores*. Illinois Biological Monographs 51, University of Illinois Press, Urbana, Illinois. 248 pp.
- Lima, J. D. 1979. Development of *Eimeria* species in mesenteric lymph nodes of goats. *Journal of Parasitology* 65:976–978.
- . 1980. Prevalence of coccidia in domestic goats from Illinois, Indiana, Missouri and Wisconsin. *International Goat and Sheep Research* 1:234–241.
- Lotze, J. C., W. T. Shalkop, R. G. Leek, and R. Behin. 1964. Coccidial schizonts in mesenteric lymph nodes of sheep and goats. *Journal of Parasitology* 50:205–208.