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Hepatic Coccidiosis in a Native Korean Goat

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Abstract : A case of chronic cholangiohepatitis associated with *Eimeria* spp. is reported in a 6-month-old, male, native Korean goat. The goat died after having a 1-week history of diarrhea, anorexia and weight loss. At necropsy, numerous multifocal to coalescing, pale and mottled red foci were present throughout the liver. Histologically, numerous coccidian parasites in both sexual and asexual stages were found in the intrahepatic biliary epithelia and bile duct lumens. Based on the light microscopic and ultrastructural features, the parasites present in the liver were compatible with the genus *Eimeria* ; however, the species was not speciated.

Key words: coccidiosis, goat, hepatitis

Hepatic coccidiosis is known to be very rare in mammals other than rabbits. Only a few sporadic cases of hepatic coccidiosis have been documented in calves, dogs, ferrets and minks.[1-4] In goats, 3 reports regarding hepato-biliary infection of *Eimeria* spp. have been published previously. Among them, two cases were naturally occuring hepatic coccidiosis, and the other represents an experimental infection rather than a natural case.[5-7] Another case of coccidiosis in a goat involved the gall bladder but not intra- or extrahepatic bile ducts.[8] This paper describes a case of naturally occurring carprine hepatic coccidiosis. To our knowledge, this is believed to be the first such case reported in Korea.

A 6-month-old, male, native Korean goat had a 1-week history of anorexia, weight loss, and diarrhea and was given symptomatic antibiotic and fluid therapy for 5 days but remained depressed and unresponsive. He was found dead and referred to the National Veterinary Research and Quarantine Service for the postmortem evaluation.

At necropsy, the goat was very thin, emaciated and dehydrated with sunken eyes. The liver had well demarcated multifocal to coalescing, grayish white and mottled red foci. Approximately 30% of the hepatic surface were necrotic. The necrotic foci extended into the underlying hepatic parenchyma on the cut section. The small intestine was dilated and contained a moderate amount of semifluid ingesta. No significant gross abnormalities were observed in any other organs examined.

Representative parenchymal tissue samples were collected and fixed in 10% neutral phosphate- buffered formalin, processed routinely, and stained with hematoxylin and eosin for light microscopical examination. For electron microscopy, the formalin-fixed liver samples were minced into 1-mm cubes and post-fixed in 2% glutaraldehyde with 2% sucrose in 0.1 M sodium cacodylate buffer and subsequently in osmium tetroxide. Fixed specimens were epo-araldite embedded, and ultrathin sections were stained with lead citrate and uranyl acetate and examined under a transmission electron microscope (Zeiss model 109). Tissues from the liver and small intestine were collected aseptically, and routine aerobic and anaerobic bacterial cultures were performed.

Microscopically, major histological changes were confined to the portal and periportal areas of the liver. Fibrosis and severe infiltration of lymphocytes and macrophages with fewer eosinophils were noted throughout the affected portal areas and occasionally extended into the adjacent periportal area causing hepatocellular necrosis (Fig. 1). The bile duct

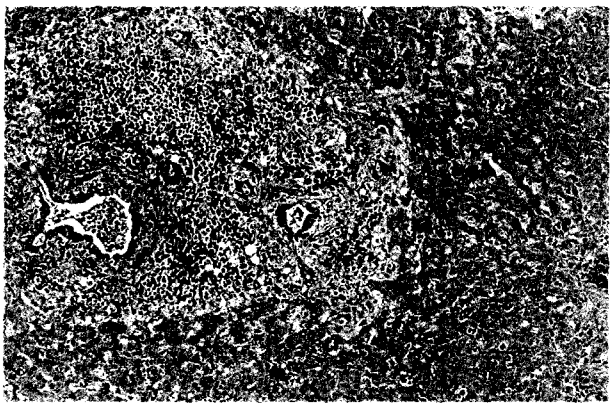

Fig. 1. Liver : goat. Note hyperplastic bile ducts that are surrounded by mixed mononuclear cells and a few eosinophils and fibrosis. The adjacent hepatic parenchyma is necrotic. HE. Bar= 80 μm.

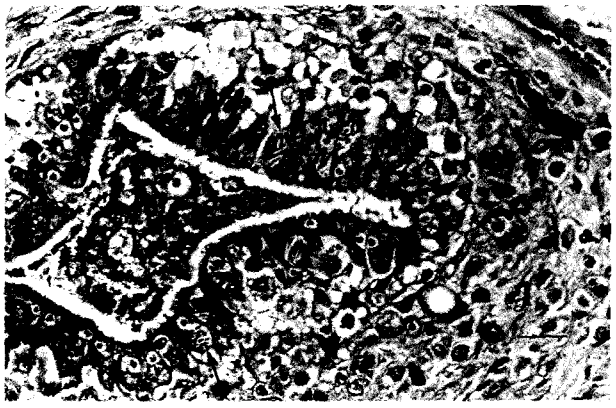

Fig. 2. Bile duct epithelium: goat. Note meront (arrow) with multiple merozoites in the cytoplasm of bile duct epithelium. Also note immature meronts (arrow head) and a macrogamete (open arrow). HE. Bar=20 um.

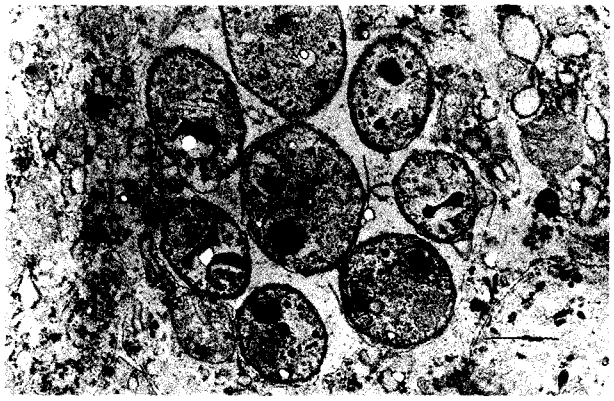

Fig. 3. Bile duct epithelium: goat. Nearly matured meront contains a few merozoites and is still attached to the residual body (R). Uranyl acetate and lead citrate. Bar=1 um.

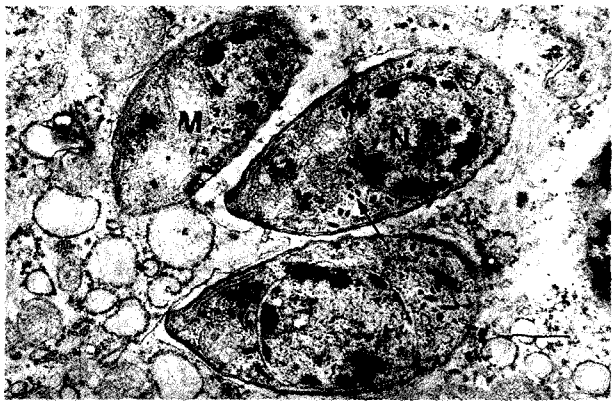

Fig. 4. Bile duct epithelium: goat. Merozoites with micronemes (arrow), mitochondria(M), and caudally located nuclei(N). Uranyl acetate and lead citrate. Bar=1 um.

epithelial cells were hypertrophic as well as hyperplastic and showed papillary projections into the lumina. Bile ducts were often dilated and filled with sloughed degenerated epithelial cells, cellular debris, neutrophils, and a few oocysts. Numerous coccidial parasites in different stages of the life cycle were present in the bile duct epithelium (Fig. 2). Coccidian meronts were in intact biliary epithelial cells, sloughed epithelial cells, and rarely found in the lumen of small bile ducts (Fig. 2). Meronts were elongated ovals and contained up to 16 crescent-shaped merozoites arranged random or head-to-tail orientation (Fig. 2). Macrogametes were oval-shaped with densely eosinophilic cytoplasmic granules (Fig. 2). Microgametes were round to oval with marginally basophilic granules around a light pink center. Few oocysts were seen within epithelial cells in the hepatic bile ducts and also free in luminal debris. They were oval to spherical and measured to be 2~16 by 8~13 um in size. No significant microscopic changes were present in the small intestine. Bacterial culture failed to isolate any significant bacterial pathogen from both liver and small intestine.

Ultrastructurally, meronts present in the bile duct epithelium were surrounded by parasitophorous vacuole and contained both mature and immature merozoites (Fig. 3). Meronts were approximately 8~15×5~10 um in size. Merozoites had features of an apicomplexan coccidia including a trilayered plasmalemma, a conoid, a polar ring, a few rhoptries, and numerous micronemes located anterior to the nucleus and occasionally attached to the residual body (Fig. 4). Longitudinally-cut merozoites were 3.5~5.4 um long and 1.2~2.0 um wide.

Except for rabbits, in other animals, infection of biliary epithelium by *Eimeria* spp. is rare and regarded as aberrant and often coincides with enteric coccidiosis. In rabbits, the lymphatic channel has been considered as a possible route of

hepatic invasion.[9] Coccidial schizonts have occasionally been found in the mesenteric lymph nodes of ruminants having enteric coccidiosis suggesting that schizonts may have spread to the mesenteric lymph node from the intestinal mucosa through lymphatic vessels.[10] In the second documented case of carprine hepatic coccidiosis, the goat had concurrent hepatic and enteric coccidiosis.[6] But due to a marked difference in size of the organisms present in the liver and small intestine respectively, it was not possible to conclude that the source of hepatic infection in that case was from the small intestine. Even the intestinal stages were not observed in our case, we could not conclude that the infection did not derive from the gut. *E. stiedae* in rabbits infects the liver with no intestinal stages but the infection results from ingested oocysts excysting in the intestine and the sporozoites penetrating the intestinal wall and entering the liver by the hepatic portal system. However, since fecal examination was not performed in this case, the exact route of hepatic invasion could not be determined. The macroscopic changes of the liver in this goat were similar to a previously reported case.[6] Extensive hepatic necrosis and marked dehydration were likely to be the cause of death in this goat.

Other Apicomplexa protozoans that have been proven to infect goats include *Cryptosporidium, Cystoisospora, Toxoplasma, Neospora, Besnoitia, Sarcocystis,* and *Hammondia.*[11, 12] All of them were able to rule out based on histologic and ultrastructural characteristics. *Cryptosporidium* differs from the organism in this case in several aspects. *Cryptosporidia* are located along the apical border of the host cell in a position described as intracellular but extracytoplasmic. Up to eight merozoites were usually present in a meront and a feeder organelle is also present. The meronts of *Sarcocystis* are located directly in the host cell cytoplasm not within a parasitophorous vacuole. The parasites in this case are not *Cystoisospora* because meronts have many organisms. Cyst-like stages were not observed as would be expected to occur in two-host coccidia such as *Toxoplasma*, *Neospora*, *Hammondia*, and *Besnoitia.*[11, 12] The asexual stages of them divide by endodyogeny, whereas asexual stages of this organism multiply by merogony.[11, 12] No *Isospora* species has been known to develop in the tissue of ruminant.[12]

The parasite was not able to speciate but ultrastructural features of both sexual and asexual stages of the organisms seen in bile duct epithelium is most compatible with a genus of *Eimeria*. Because of this newly documented case, coccidial infection should be included in the differential diagnosis of goats having cholangiohepatitis.

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