Impression Smear of Liver Tissue from a Rabbit

What Is Your Diagnosis?

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Case Presentation

A 7- to 11-week-old female rabbit from a commercial rabbit farm with a history of diarrhea and sudden death was submitted alive to the Animal Disease Diagnostic Laboratory at Purdue University for euthanasia and necropsy. On presentation, the rabbit was thin with reduced fat stores and muscle wasting. The rabbit weighed 2 lb 4 oz. The hair coat was rough, and fecal material was adherent to hair on the perineum.

Primary gross pathologic changes were observed in the small intestines and liver. The small intestines were distended and filled with gray-green semisolid ingesta. Undigested food pellets were present in the colon. The liver had multiple 1- to 3-mm–diameter, slightly raised, discrete to coalescing, yellow-white nodules scattered throughout the parenchyma. Many of these nodules contained yellow-white caseous material. The remaining parenchyma was severely congested and edematous. Air-dried scrapings and impression smears of the nodules were prepared and stained with an automated Wright's stain (Hematek, Bayer Diagnostics, Elkhart, Ind; Figure 1).

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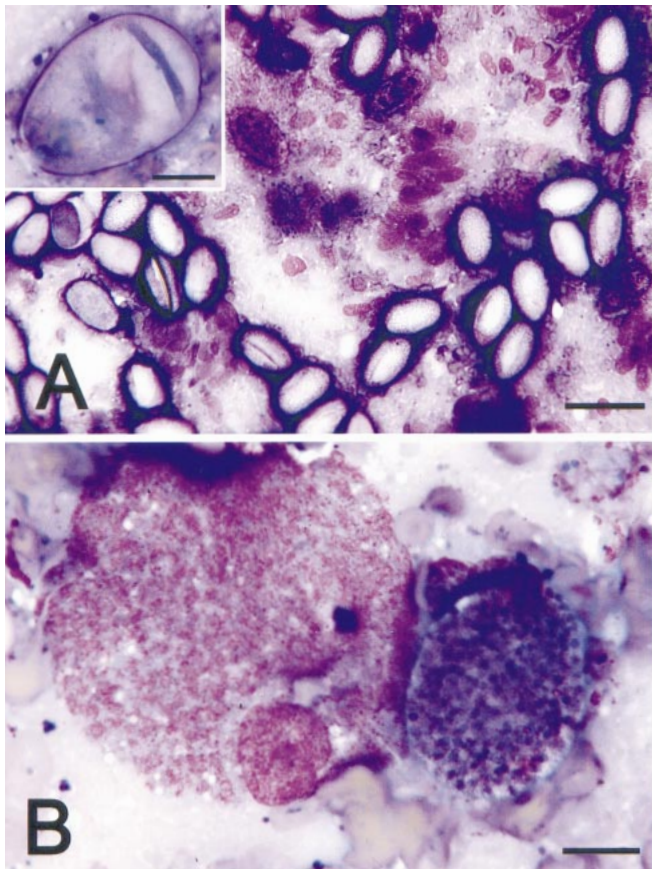

Figure 1. Impression smear of liver nodules from a rabbit. Wright's stain. (**A**) Bar = 40 µm; **Inset**: bar = 10 µm. (**B**) Bar = 10 µm.

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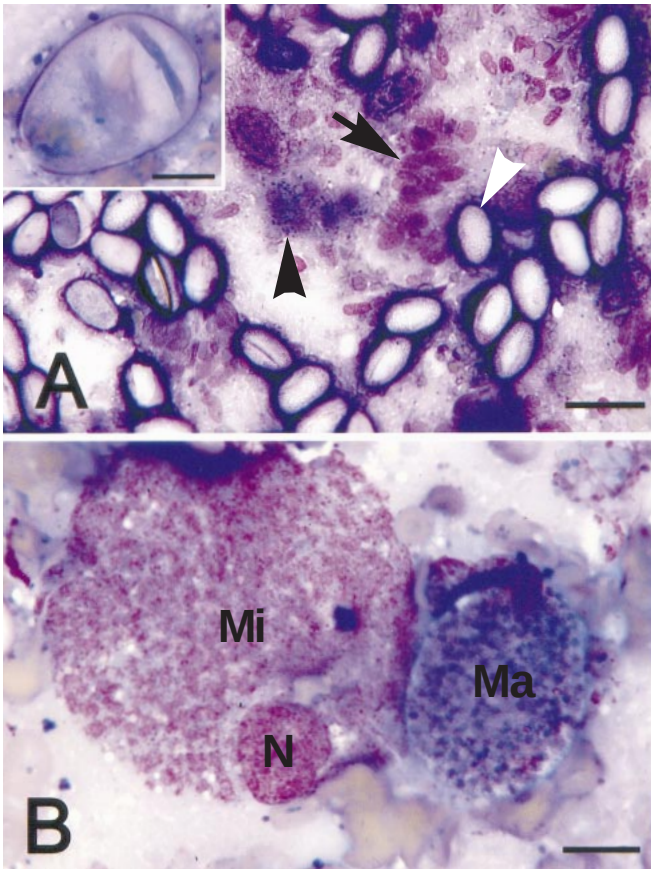

Figure 2. Impression smear of liver nodules from a rabbit with hepatic coccidiosis (same images as Figure 1). (**A**) Numerous oocysts (white arrowhead) are admixed with hepatocytes (black arrowhead) and clusters of bile duct epithelial cells (arrow). Wright's stain. Bar=40 µm. **Inset**: Higher magnification of an oocyst that has become wrinkled from drying and fixation. Bar=10 µm. (**B**) A microgametocyte (Mi) and macrogametocyte (Ma). N indicates the nucleus of an epithelial cell. Wright's stain. Bar=10 µm.

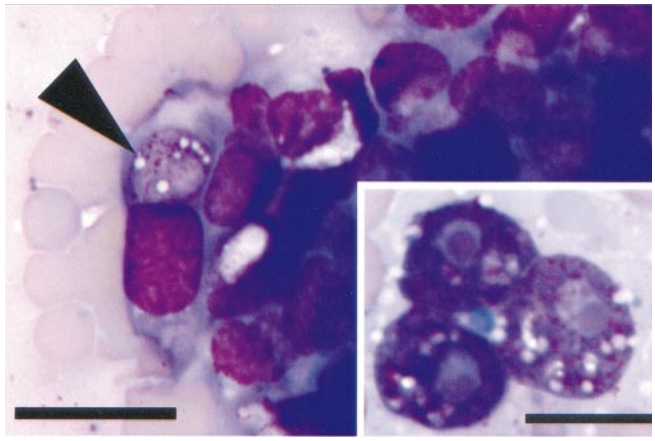

Figure 3. Impression smear of liver nodules from a rabbit with hepatic coccidiosis. Intracellular undifferentiated stages of *Eimeria stiedae* are located within hyperplastic bile duct epithelial cells (arrowhead). Bar = 20 µm. **Inset**: Higher magnification of extracellular undifferentiated stages of *E stiedae*. Bar = 10 µm.

Cytologic Interpretation

The impression smears of rabbit liver were of high cellularity and excellent diagnostic quality. The samples included high numbers of coccidial organisms intermixed with hepatobiliary parenchymal cells and low numbers of inflammatory cells (Figure 2A). The coccidial organisms were in various stages of development that included early gametogonous stages, microgametocytes, macrogametocytes, and fully formed oocysts.

Oocysts predominated and were ovoid to ellipsoidal and 30-39 by 18-22 µm in size (Figure 2A). They were characterized by a flat micropylar end and a smooth, usually bright pink wall sometimes wrinkled by fixation and drying. Macrogametocytes were present in lower numbers. These structures were oval to round and 25-34 by 20 µm and were filled with large homogeneous, rounded, bright pink or orange to bluish cytoplasmic granules (referred to as wall-forming bodies) (Figure 2B). Single large, centrally located nuclei occasionally were seen.

Microgametocytes were present in very low numbers within epithelial cells (Figure 2B). These structures were large, round, and up to 50 µm in diameter, and were filled with high numbers of magenta to purple, round to comma-shaped microgametes, each up to 5 µm in their greatest dimension. Morphologically undifferentiated stages were present in moderate numbers. These structures were blue, rounded, and 8-18 µm in diameter with large prominent, eccentric nuclei. Some of these structures contained fine purple intracytoplasmic granules and several tiny, distinct vacuoles. These stages were present within bile duct epithelial cells and extracellularly as a result of rupture of the epithelial cells (Figure 3).

Intimately associated with the organisms were numerous densely cellular, cohesive clusters of moderately hyperplastic bile duct epithelial cells (Figures 2A and 3). These cells were round to cuboidal to columnar in shape with moderate amounts of deeply blue cytoplasm. Overall, anisocytosis, anisokaryosis, and variation in nucleus:cytoplasm ratios were mild to moderate. Fewer cohesive clusters of hepatocytes were seen; some hepatocytes had mild vacuolar change consistent with glycogen accumulation and/or hydropic degeneration. Some cells contained intracytoplasmic green-blue granules consistent with bile pigments (Figure 2A).

Very low numbers of inflammatory cells were noted and consisted of small reactive lymphocytes, plasma cells, and heterophils. A low to moderate number of erythrocytes also was seen. The cytologic findings were con-

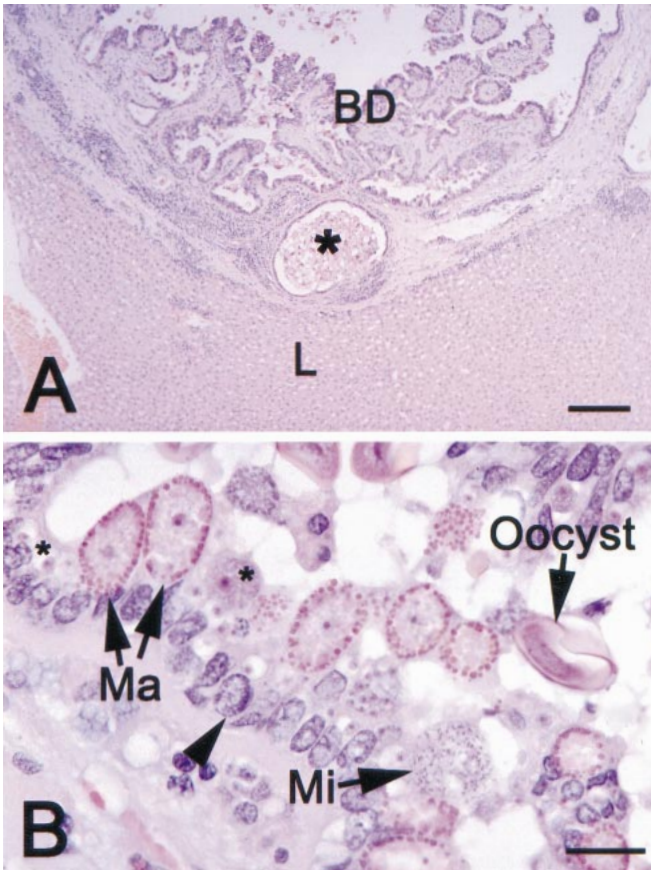

Figure 4. Histologic section of liver tissue from a rabbit with hepatic coccidiosis. (**A**) The liver parenchyma (L) is compressed by an enlarged bile duct (BD). Note the papilliferous fronds of hyperplastic bile ductular epithelium and the accumulations of extracellular oocysts (*). Hematoxylin and eosin. Bar = 200 µm. (**B**) Higher magnification of Figure 4A. Various developmental stages of the organism are present within the bile ductular cells (the arrowhead indicates the nucleus of an epithelial cell) and include macrogametocytes (Ma), microgametocytes (Mi), and undifferentiated stages (*). An oocyst is present extracellularly. Hematoxylin and eosin. Bar = 20 µm.

sistent with *Eimeria stiedae* infection (hepatic coccidiosis) and associated bile ductular hyperplasia, with minimal hepatocellular vacuolar degeneration and intracellular cholestasis.

Histologic Interpretation

Histopathologic findings in the liver consisted primarily of extensive biliary hyperplasia with numerous intralesional coccidia. Bile ducts were markedly dilated and lined by hyperplastic columnar epithelial cells thrown into multiple papillary fronds (Figure 4A). Numerous protozoal stages, including undifferentiated gamonts, microgametocytes, macrogametocytes, and developing oocysts, were within bile duct epithelial cells (Figure 4B). Duct lumina were filled with numerous thin-walled, ovoid oocysts. The hyperplastic bile ducts were surrounded by large amounts of fibrous connective tissue with mild lymphohistiocytic inflammatory infiltrates.

Discussion

Coccidiosis is primarily a disease of young rabbits.[1] Infected adults are carriers. The disease is seen most often in breeding and rearing establishments where sanitation is poor. Outbreaks of the disease are common under natural conditions. The most important species of rabbit coccidia is *E stiedae*, which affects the bile duct epithelial cells. All other species of coccidia are found in the intestine. Of these, the most important species are probably *E intestinalis*, *E irresidua*, and *E magna*.[2-4]

E stiedae commonly affects domestic rabbits throughout the world. Disease incidence varies depending on geographic location and type of operation, with smaller farms more likely to be affected.[5] Incidence has decreased in laboratory rabbits from modern, well-managed rabbitries.[2,6,7]

Most infections are believed to be mild, and although mild infections are often clinically inapparent, severe infections may be extremely pathogenic and may result in death.[8] Young rabbits are most susceptible; however, infected adults become carriers of the disease and are a source of infection.[8] Quantitative morbidity and mortality data are limited. Studies conducted in the 1930s revealed that approximately 9% of 2000 rabbits examined were affected by hepatic coccidiosis and that approximately 5% of deaths attributed to coccidiosis were specifically attributable to *E stiedae*.[9] In France, 22.5% of dead broiler rabbits were infected with *E stiedae*.[5] In Brazil, 48% of rabbits that died in breeding farms and 64% of rabbits killed in slaughterhouses were infected with *E stiedae*.[5] Young rabbits experimentally infected with 10,000 or 100,000 oocysts had 40% and 80% mortality, respectively.[10] In addition to the premature loss of rabbits in severe cases, *E stiedae* may predispose surviving rabbits to other diseases and may cause significant decreases in weight gain, fat-soluble vitamin metabolism, dry matter and lipid digestibility, and digestible energy.[11,12] Affected livers are condemned.[10] These problems all result in financial losses for commercial rabbitries.

Severe infections with *E stiedae* are characterized by anorexia, a distended abdomen, and weight loss. Diarrhea and icterus sometimes occur. The liver is greatly enlarged, and the bile ducts are dilated and appear on the surface of the liver as white nodules of various sizes containing creamy fluid packed with oocysts. The primary microscopic lesion consists of dilation and thick-

ening of the bile ducts due to extensive hyperplasia of the ductular epithelium. Developmental forms of the parasite are seen in the bile duct epithelial cells, and oocysts appear in the lumen. The liver parenchyma is destroyed by pressure from expanding biliary ducts and is gradually replaced by fibrous connective tissue.[1,7,13]

Rabbits are infected by ingestion of sporulated oocysts. The oocyst is ovoid to ellipsoidal and approximately 28-40 by 16-25 µm. It has a flat micropylar end, a smooth bright-pink wall, and a micropyle, but no polar granules or residuum. The 4 sporocysts are ovoid, contain 2 sporozoites each, and have a residuum.[1,7,13,14] Sporozoites penetrate the mucosa of the small intestine and pass via the mesenteric lymph nodes and hepatic portal system to the liver. In the liver, they enter the epithelial cells of the bile duct and occasionally the liver parenchymal cells, where they become trophozoites and then schizonts. The schizonts produce merozoites, but the number of asexual generations preceding gametogony is unknown. Oocysts pass out through the bile and appear in the feces 18 days after infection; sporulation occurs in 3 days.[3,4,15,16]

E stiedae infection can be diagnosed by identifying oocysts in the feces, based on size, shape, and structure. Oocysts must be differentiated from those of the intestinal coccidia, which also may be present in rabbits in large numbers without causing serious clinical signs. A more straightforward definitive diagnosis is made through postmortem examination of the liver.

Monoxenous coccidia have direct life cycles, and each coccidian is host specific.[6,17] The functional unit of the coccidian is the zoite (sporozoite, merozoite), which is a motile, banana- or cigar-shaped cell, rounded at one end and pointed at the other end. These earlier forms were not found in our cytologic preparations, probably because of the late stage of infection in this rabbit. The zoites migrate within the host and invade cells. They represent the beginning and end points of the coccidian life cycle.[17] Host infection begins with ingestion of sporulated oocysts. In *Eimeria* species, each sporulated oocyst contains 4 sporocysts, each of which contains 2 sporozoites. These sporozoites invade host cells and lead to the formation of many merozoites through an internal fission process called schizogony. Development of these and subsequent stages occurs within a parasitophorous vacuole in the cell cytoplasm. The life history of coccidia such as *Eimeria* includes both asexual (schizogonous) and sexual (gametogonous) reproduction.[3,6,17]

During asexual reproduction (schizogony), the sporozoites emerge from the oocyst and enter epithelial cells. Once inside the epithelial cells, the sporozoites round up to form trophozoites,which grow larger and become first-generation schizonts. Schizonts undergo nuclear fission followed by cytokinesis, forming many merozoites. First-generation merozoites released from this schizont invade intact cells and generate second-generation schizonts, and so on. Important consequences of schizogony include an exponential increase in the number of parasites arising from a single sporozoite, corresponding destruction of the host cells, and automatic halting of the asexual process after a fixed number of repetitive cycles.[3,6,16,17]

During sexual reproduction (gametogony), the merozoites produced by the final schizogony enter intact cells and develop into either female or male gamonts. The macrogametocytes (female gamonts) enlarge, store nutrients, and cause hypertrophy of the host cell. When mature, they are called macrogametes and contain many hyaline granules (wall-forming bodies) at their periphery.[18] The microgametocytes (male gamonts) undergo repeated nuclear divisions and become multinucleated; each becomes part of a biflagellated microgamete. The microgametes, freed by rupture of the host cell, seek out and penetrate macrogametes. Fertilization triggers formation of the cyst wall around the resulting zygote, producing the oocyst. The unsporulated oocysts are liberated from the body, usually in the feces. Under optimum conditions, the liberated oocysts need several days or more to sporulate in the environment, whereupon they are infective to other hosts.[3,6] ♢

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