Severe Intestinal Coccidiosis in a Newborn Lion (Panthera leo)

J. P. DUBEY¹ and J. E. JARDINE²

1Animal Parasitic Diseases Laboratory, United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Beltsville, MD, USA; 2VET PATH Laboratory Services, Belmont, Western Australia

Summary. Severe coccidiosis was found in sections of small intestine of a less than 2 day old lion (Panthera leo) born in captivity. Schizonts, merozoites, gamonts, and unsporulated oocysts were located in epithelial cells of ileum. Ultrastructural examination indicated that schizonts divided by schizogony. Merozoites were 5.1–7.0 × 1.4–1.6 \( \mu m \) in size and contained numerous micronemes arranged in rows. The rhoptries had a long neck with long bulbous ends that were turned around in a loop. Organisms did not stain with antibodies to Toxoplasma gondii and Neospora caninum. This is the first report of intestinal coccidiosis in a neonatal lion.

Key words: Coccidia, epithelial cells, ileum, parasites, ultrastructure

INTRODUCTION

Isospora felis, I. rivolta, Toxoplasma gondii, Hammondia hammondi, Cryptosporidium spp. and Sarcocystis spp. are the known intestinal coccidia of domestic cats and probably of other felids (Bowman 1999). Little is known of coccidiosis in lions. In the present paper, intestinal coccidiosis in a newborn lion (Panthera leo) cub is reported.

MATERIALS AND METHODS

Formalin-fixed tissues of a captive born lion cub were submitted to the Department of Veterinary Pathology, University of Pretoria, South Africa in 1995. The cub was less than 48 hours old and born to a mature wild-caught lioness; no other information was available. Specimens included samples of the intestinal tract and other organs fixed in buffered neutral 10% buffered formalin. Paraffin-embedded tissues were cut at 5 \( \mu m \) thickness, stained with haematoxylin and eosin (H and E), and examined microscopically. A paraffin-block, and an H and E-stained slide, were forwarded to the Animal Parasitic Diseases Laboratory, U. S. Department of Agriculture, Beltsville, Maryland for further evaluation.

Paraffin-embedded sections were stained with polyclonal rabbit antibodies to T. gondii, and N. caninum using methods and reagents used previously (Lindsay and Dubey 1989). A portion of paraffin-embedded block was deparaffinized, embedded in methacrylate, and 1–3 \( \mu m \) sections were stained with H and E. A sample of paraffin-embedded section of ileum was deparaffinized and processed for transmission electron microscopy.
RESULTS

Protozoa were evident in epithelial cells of the mid-jejunum to the ileum with no organisms seen in the stomach, duodenum, proximal jejunum and colon. Organisms were not seen in extra-intestinal organs. Protozoa were in enterocytes of the villi with little evidence of parasitism in the crypt epithelial cells or the goblet cells. No parasites were seen in the lamina propria or other gastro-intestinal tissues. Occasional dilated crypts contained accumulated neutrophils and debris. The submucosa contained moderate numbers of lymphocytes and scattered neutrophils and eosinophils with some evidence of congestion and edema.

Enteroctyes were multiply infected with a coccidian parasite (Figs 1, 2). Most parasites were in various developmental stages of schizogony, from merozoites to mature schizonts (Figs 1–4). Parasites were situated above the host cell nucleus (Figs 1, 2). The intestinal lumen also contained parasites and cellular debris (Fig. 1). Fluid-filled spaces were located immediately beneath the epithelium. All stages of schizogony and gametogony were seen. Schizonts were up to 15 µm wide and contained up to 20 merozoites. In some schizonts, merozoites were arranged around a central residual body (Figs 1, 2). Microgamonts contained up to 10 microgametes (Fig. 1). Unsporulated oocysts were approximately 10 µm in diameter and were seen in the epithelium as well as the intestinal lumen (Fig. 1). Protozoa did not stain with anti- T. gondii and anti-N. caninum antibodies.

Ultrastructurally, parasites developed intracellularly within a parasitophorous vacuole. Merozoites measured 5.1–7.0 × 1.4–1.6 µm (n = 6) from posterior pore to conoidal tip and had all the organelles and inclusion bodies characteristically found in the coccidia. Merozoites had a few ultrastructural features that were somewhat unusual. For example, the rhoptries, numbering 2–7 in most sections, were homogeneously electron-dense with relatively long necks. Also, rhoptries were oriented so that their tapered ends and necks initially projected posteriorly, but then curved anteriorly 180° toward the conoidal tip. Another somewhat unusual feature included micronemes that were relatively long (up to 333 nm in length) that were frequently arranged in rows (Fig. 4). Cross-sections and longitudinal sections of merozoites revealed up to 96 micronemes. In addition to other organelles associated with the apical complex, merozoites contained several lipid bodies, several mitochondria, a few amyllopectin granules, micropores, several dense granules, and a nucleus with a prominent nucleolus.

Schizogony occurred by endopolygeny in which several nuclear divisions occurred prior to merozoite formation (Fig. 3). Merozoites formed initially internally with primordia appearing in association with each nuclear pole during the last nuclear division. Eventually, merozoites budded at the surface of the schizont. Frequently, parasites remained within the parasitophorous vacuole of the original host cell and underwent asexual or sexual development. Mature schizonts contained approximately 24 merozoites and a small residual body.

Microgamonts occurred within a prominent parasitophorous vacuole. Several nuclear divisions occurred with nuclei becoming situated near the schizont surface where microgametes budded from the gamont surface by process described previously for several coccidians. Microgametes were biflagellated and contained a nucleus, microtubules, two basal bodies, and a mitochondrion.

Mature macrogamonts were surrounded by a single plasmalemma that appeared tightly adhered to the parasitophorous vacuole and contained all the organelles and inclusion bodies characteristically present in the coccidian parasites including two types of wall forming bodies, lipid bodies, a centrally located nucleus, and mitochondria. Features that were somewhat unusual included relatively numerous, large type I wall forming bodies (1.1 × 0.7 µm; n = 12) that contained reticulated cores and type TI wall forming bodies that were small (56.2 to 225 nm; n = 12) and electron-dense. Most sections of a mature macrogamont contained a large (approximately, 1.35 µm in diameter) moderately electron-dense body with an electron-dense core.

Oocysts showed varying degrees of oocyst wall formation. Two oocysts measured 9.2 × 7.9 µm. The oocyst wall was multilayered including three prominent layers; an inner most moderately electron-dense layer; a middle, electron-dense layer, and an other moderately electron-dense and granulated outer layer. A single membrane separated the inner layer from the oocyst cytoplasm; a double membrane layer was tightly interposed between the inner and middle layers and appeared as an electron-lucent line; and a space separated the outer layer from the parasite plasmalemma, which tightly adhered to the parasitophorous vacuole.
Fig. 1. Stages of the coccidian parasite in sections of ileum of the lion cub. 1μm sections stained with H and E. A – note an oocyst (Oo) and a merozoite (Mr) in intestinal lumen. Also note heavy parasitization of the enterocytes by schizonts (Sc), macrogamonts (Ma), microgamonts (Mi), and an oocyst. B – a schizont with a residual body. C – schizonts with elongated merozoites. D – Mature microgamont with microgametes (Mg).
DISCUSSION

The parasite in the lion was neither *I. felis* nor *I. rivolta* because schizonts, gamonts, and oocysts were much smaller than those of *I. felis* or *I. rivolta* (Shah 1971, Dubey 1979). The parasite was not *T. gondii* or *N. caninum* because it did not stain with *T. gondii* or *N. caninum* antibodies. Furthermore, schizonts were structurally different than those of *T. gondii*, and tachyzoites were not found (Speer and Dubey 2005). The stages of the parasite were also different than those of *Hammondia hammondi* (Dubey and Sreekumar 2003). Additionally, *H. hammondi* is not transmitted congenitally.

Felids are not a definitive host for *N. caninum* (Dubey *et al.* 2002). We are not aware of a report of intestinal coccidiosis in a lion.

The age of the cub and the development of the parasites support the assumption that these were acquired as an intra-uterine infection.

Acknowledgements. We would like to thank John Jenkins, Armed Forces Institute of Pathology, Washington, D.C. for electron microscopy, Professor N.P.J. Kriek of the Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Republic of South Africa for referral of the case material, and Professor C. A. Speer, Cellular and Microbiology, The University of Tennessee, 373 Biotechnology Building, Knoxville, TN 37920, USA for advice.

Fig. 3. Transmission electron micrograph of an immature schizont in the ileum of the lion. Note a schizont with five nuclei, one of which (arrow) is dividing.

Fig. 2. Transmission electron micrograph of entrocytes in the ileum of the lion. Note hyperparasitazation of entrocytes. Also note the intestinal villus (V) border, uninucleate (U) schizonts or gamonts, several schizonts (Sc), one with a residual body (Rb), and merozoites (Mr).
Fig. 4. Transmission electron micrograph of two merozoites within a schizont in the ileum of the lion. Note conoid (C), large micro- nemes (M) some of them are present also in the non-conoidal end, rhoptrie (R), a nucleus (N), and a nucleolus (Nu).

REFERENCES


Received on 6th December, 2007; revised version on 21st January, 2008; accepted on 21st January, 2008