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Experimental *Cryptosporidium parvum* Infections in Opossums (*Didelphis virginiana*)

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ABSTRACT: Five nursing opossums (*Didelphis virginiana*) were each inoculated with 5×10^6 *Cryptosporidium parvum* oocysts of calf origin. Following inoculation, endogenous stages of *C. parvum* were observed in the ileum, cecum, and colon of these opossums. Two of three noninoculated pouch mates acquired infections during the study based on examinations of feces and tissue sections of all eight opossums. Mild diarrhea was observed in four of seven opossums harboring *C. parvum*, although none died as a result of the infection. Under the conditions of this study, *C. parvum* appeared to be only mildly pathogenic for opossums.

Key words: Cryptosporidium parvum, Cryptosporidium sp., coccidia, opossum, *Didelphis virginiana*, experimental infection, neonates.

Several authors have reviewed the literature on the coccidian genus *Cryptosporidium* (Levine, 1984; Navin and Juranek, 1984; Current, 1985; O'Donoghue, 1985; Tzipori, 1985; Fayer and Ungar, 1986). There is little information on cryptosporidial infections in marsupials; only O'Donoghue (1985) mentioned infection of a marsupial (the marsupial mouse, *Antechinus* sp.) with *Cryptosporidium* sp. Other data were not presented. Therefore, it is not known whether the species infecting the marsupial mouse was *C. parvum* or *C. muris*, the two valid species of *Cryptosporidium* found in mammals (Upton and Current, 1985; Anderson, 1987). The present study was undertaken to determine if nursing opossums (*Didelphis virginiana*) were susceptible to infection following oral inoculation with *C. parvum* oocysts.

A female opossum was captured in Lee County, Alabama (32°36'N, 85°36'W), and transported to the Laboratory Animal Care Facility (College of Veterinary Medicine, Auburn University, Alabama 36849, USA). The opossum was housed in a stainless steel dog cage and given commercial dry cat food (Purina Cat Chow, Ralston Purina Company, St. Louis, Missouri 63164, USA) and water, ad libitum. After 22 days in captivity, the opossum was observed to have nursing pouch young. Thirty-six days after the female opossum was captured, the pouch young were inoculated with *C. parvum* oocysts. Fecal examinations using Sheather's sugar solution (Ernst and Benz, 1981) on samples collected from the mother opossum were negative for *C. parvum* oocysts 1 wk and at 1 day before the pouch young were inoculated with *C. parvum* oocysts. Based on the report of Jurgelski and Porter (1974), we estimated that the opossums were approximately 75 days old when inoculated.

Oocysts of *C. parvum* used in this study were isolated originally from the feces of a naturally infected dairy calf (Lindsay et al., 1987), and maintained in our laboratory by periodic passage in 2- to 3-day-old calves. Oocysts were separated from fecal debris by flotation in Sheather's sugar solution and stored in Hanks' balanced salt solution (HBSS) containing 100 IU/ml penicillin and 100 μg/ml streptomycin (GIBCO, Grand Island, New York 14072, USA) at 4 C. Oocysts had been stored for <2 mo prior to these experimental inoculations.

Five nursing opossums were each inoculated orally with 0.5 ml of HBSS containing 5×10^6 oocysts using an 18 ga animal feeding needle. They were then weighed and returned to their dam. Their mean weight at the time of inoculation was 46.7 ± 0.8 g. Although we initially observed only these five nursing opossums, three other pouch mates were observed when opossums were being removed from

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the dam for examination at necropsy. Because the opossums were not identified at the time of inoculation, we were not able to differentiate between inoculated and noninoculated opossums. Therefore, only general conclusions can be drawn from the results of our examinations. One nursing opossum each was examined at necropsy 6, 7, and 8 days postinoculation (PI), two were examined 9 days PI, and three were examined 10 days PI. The following tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin for light microscopic examinations: lung, liver, kidney, spleen, pancreas, stomach, duodenum, jujunum, ileum, cecum, and colon. Feces from the colon of each opossum were divided and a portion was stained with Kinyoun's carbol fuschin (Heine, 1982) and the remaining portion examined by flotation using Sheather's sugar solution (Ernst and Benz, 1981).

None of the opossums died as a result of infection with *C. parvum*. The only observed clinical sign was semi-formed, dark brown fecal material in the colons and rectums of four of seven opossums harboring cryptosporidia. All opossums continued to nurse, as evidenced by milk in their stomachs at necropsy, and weight gains during the study. Their mean weight at necropsy was 86.5 ± 4.3 g.

Only one of the eight opossums remained *C. parvum* free during the course of this study. Five of seven infected opossums had developmental stages of *C. parvum* in the ileum, cecum, and colon, whereas two had stages only in the cecum and colon. Mild villous atrophy was observed in the ilea of opossums examined 7 and 8 days PI. Developmental stages of *C. parvum* and microscopic lesions were not observed in the other tissues of these or the other opossums examined. Oocysts of *C. parvum* were observed in the feces of six of the eight opossums examined. However, one of the opossums negative for fecal oocysts had developmental stages of *C. parvum* in the cecum and colon.

Results of this study indicate that pouch young opossums are susceptible to oocyst-induced infections with *C. parvum*. However, because of the lack of severe clinical disease and minimal microscopic lesions following inoculation of 5×10^6 oocysts, *C. parvum* appears to be only mildly pathogenic for opossums. Two of the three opossums initially overlooked and not inoculated developed *C. parvum* infections during the course of the study. It is possible that they were infected by ingesting oocysts of *C. parvum* that passed, unexcysted, through the digestive system of the inoculated pouch mates.

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