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Source: Journal of Wildlife Diseases, 28(3) : 386-390

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-28.3.386

Journal of Wildlife Diseases, 28(3), 1992, pp. 386–390

RODENTS ARE NOT A SOURCE OF ENDOGENOUSLY-PRODUCED, FECALLY-TRANSMITTED *CARYOSPORA BIGENETICA* OOCYSTS

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ABSTRACT: Fifteen Swiss-Webster mice (*Mus musculus*) and eight cotton rats (*Sigmodon hispidus*) were inoculated orally with *Caryospora bigenetica* oocysts. Feces from these animals were collected from 0 to 180 days postinoculation (DPI) and examined for endogenously-produced oocysts using Nomarski microscopy. Oocysts were recovered from mouse feces at 0, 1, 2, 3, 5, 7, 8, 10, and 14 DPI, and from cotton rat feces at 1, 2, and 9 DPI. The recovered oocysts were determined to be from the original inocula due to the presence of thick walls, polar granules, and Stieda and substieda bodies. All animals exhibited clinical signs at 8 DPI. Developmental stages of *C. bigenetica* were identified in various tissues of seven cotton rats found dead at 9, 10, 11, 12, and 13 DPI. Caryocysts were found in muzzle, tongue, footpad, scrotum, and rectum of mice and cotton rats at 30 DPI. Fecal samples collected from mice on 0, 8, 10, 12, 14, 16, and 18 DPI, and from cotton rats on 0, 9, 11, 13, 15, and 17 DPI were injected subcutaneously into 13 mice. Of the 13 mice, a *Caryospora* infection was observed only in the mouse inoculated with 0 DPI mouse feces. We propose that endogenously-produced *C. bigenetica* oocysts are not fecally-transmitted by Swiss-Webster mice or cotton rats.

Key words: *Caryospora bigenetica*, coccidia, cotton rat, *Crotalus horridus*, fecal transmission, *Mus musculus*, *Sigmodon hispidus*, Swiss-Webster mice.

INTRODUCTION

Coccidian parasites of the genus *Caryospora* once were considered to have monoxenous life cycles. However, workers in the early 1980's demonstrated that three species, *C. bubonis*, *C. bigenetica*, and *C. simplex*, possess facultative heteroxenous life cycles that distinguish them from other coccidial species within the Eimeriidae and Sarcocystidae (Stockdale and Cawthorn, 1981; Cawthorn and Stockdale, 1982; Wacha and Christiansen, 1982; Upton et al., 1984).

The life cycle of *C. bigenetica* involves asexual and sexual development of the parasite in duodenal and jejunal epithelial cells of various crotalids (primary host). Unsporulated oocysts are excreted in the feces of snakes and sporulate in the external environment. Following ingestion of sporulated oocysts by the secondary host (rodents, dogs, and swine), *C. bigenetica* undergoes asexual and sexual reproduction and produces oocysts in extraintestinal tissue sites. Oocysts sporulate in the tissues and release motile sporozoites which penetrate other host cells and form caryocysts. Because *C. bigenetica* oocysts sporulate in a variety of secondary host tissues, including the lamina propria and submucosa of the rectum (Lindsay et al., 1988), our objective was to determine if these endogenously-produced oocysts are excreted in the feces of experimentally inoculated Swiss-Webster mice and cotton rats, and if secondary hosts could be a source of infective fecal oocysts.

MATERIALS AND METHODS

Twelve (6 males and 6 females) 3-wk-old Swiss-Webster mice (*Mus musculus*) and eight (2 males and 6 females) 9-wk-old cotton rats (*Sigmodon hispidus*) were housed in ten autoclaved metal cages containing sterile wood shavings for bedding when feces were not being collected, and in ten autoclaved metal hanging cages during the collection of feces. Each cage contained two animals of the same sex. Three additional 3-wk-old female Swiss-Webster mice (mouse-60, mouse-120, and mouse-180) were housed individually in autoclaved metal cages containing bedding, and in hanging cages for the collection of feces. Uninoculated male and female mice and cotton rats served as controls

for the study. Commercial rodent pellets and water were supplied *ad libitum*.

Feces containing *C. bigenetica* oocysts were collected from a captive timber rattlesnake (*Crotalus horridus*) and prepared for inoculation as described by Douglas et al. (1991). All animals were inoculated orally using a 20-gauge metal esophageal intubation tube. Twelve mice each were inoculated with 0.1 ml of Hanks' balanced salt solution (HBSS) (GIBCO Laboratories, Grand Island, New York, USA) containing 2.5×10^5 *C. bigenetica* oocysts. Four of the six female cotton rats each were inoculated with 0.2 ml of HBSS containing 1.75×10^5 oocysts and the remaining two female and two male cotton rats each received 0.2 ml of HBSS containing 2.0×10^4 oocysts. Mouse-60, mouse-120, and mouse-180 each were inoculated with 0.1 ml HBSS containing 2.6×10^5 oocysts.

Mice and cotton rats were examined daily for clinical signs of dermal coccidiosis. At 30 days postinoculation (DPI), all surviving animals, except for mouse-60, mouse-120, and mouse-180, were killed. Mouse-60, mouse-120, and mouse-180 were killed at 60, 120, and 180 DPI, respectively, following fecal collection. Fresh smears of muzzle, tongue, footpad, rectal, and scrotal (in males) tissues were examined with Nomarski interference contrast (NIC) microscopy for the presence of developmental stages of *C. bigenetica*.

Animals were placed in hanging cages for alternate 12-hr periods/day from 0 DPI until the conclusion of fecal collection at 18 DPI. Feces were collected in autoclaved metal pans containing a 2.5% (w/v) potassium dichromate solution. During the remaining time each day, the animals were returned to their original cages containing bedding. Mouse-60, mouse-120, and mouse-180 were placed in hanging cages and feces were collected over a 24-hr period at 60 DPI, 120 DPI, and 180 DPI, respectively. Feces collected daily were combined into two samples (male and female mouse feces, male and female cotton rat feces). Daily samples were blended individually for 30 sec, cleaned by flotation in Sheather's sugar solution (2.3 kg sucrose, 27.8 ml phenol, 1,447 ml distilled water), and examined for the presence of oocysts using NIC microscopy. The number of oocysts present in each sample was determined directly by counting individual oocysts or indirectly using a hemacytometer (Reichert Scientific Instruments, Buffalo, New York, USA) when oocysts were numerous. Measurements of sporulated oocysts and their sporocysts obtained from mouse and cotton rat feces collected at 1 DPI were made with an ocular micrometer.

Following microscopic examination, the daily samples were aerated for 7 days at 25 C, centrifuged for 8 min, and washed with distilled water. The centrifugation and water rinses were repeated three times followed by two rinses in sterile HBSS. The final volume of each sample was adjusted to 1.0 ml using sterile HBSS.

Thirteen 4-wk-old male mice were housed individually in autoclaved metal cages containing sterile wood shavings. Commercial rodent pellets and water were supplied *ad libitum*.

The inocula prepared from the male and female mouse feces collected at 0, 8, 10, 12, 14, 16, and 18 DPI were injected subcutaneously into the dorsocervical region of seven male mice, one mouse for each day. Inocula prepared from the male and female cotton rat feces collected at 0, 9, 11, 13, 15, and 17 DPI were injected into six male mice, one mouse for each day. At 30 DPI, these mice were killed and fresh smears of muzzle, tongue, footpad, scrotum, and dorsocervical tissue were examined with NIC microscopy for developmental stages of *C. bigenetica*.

RESULTS

Unsporulated and sporulated oocysts were observed in mouse feces collected on 0, 1, 2, 3, 5, 7, 8, 10, and 14 DPI (Table 1). The largest number of oocysts (6.0×10^4) was excreted at 0 DPI. Oocyst numbers varied during days 0-14 PI, but overall numbers declined after 0 DPI, and were absent after 14 DPI. No oocysts were found in the feces of mouse-60, mouse-120, or mouse-180. Unsporulated and sporulated oocysts were present in cotton rat feces at 1, 2, and 9 DPI, with the largest number (1.25×10^3) passed at 1 DPI (Table 1). Oocyst numbers declined after 1 DPI and were absent after 9 DPI.

The mean (range) values for sporulated oocysts ($n = 15$) from mouse feces collected at 1 DPI were 12.9 (12.0–14.3) μm by 12.2 (10.5–14.3) μm; sporocysts measured 10.7 (9.0–12.8) μm by 9.2 (8.3–10.1) μm. The mean (range) values for sporulated oocysts ($n = 15$) observed in cotton rat feces collected at 1 DPI were 12.7 (11.3–14.3) μm by 12.2 (10.9–13.9) μm and their sporocysts measured 10.8 (9.4–12.4) μm by 9.0 (8.6–9.4) μm.

All fifteen mice and eight cotton rats originally inoculated with *C. bigenetica* had clinical signs of dermal coccidiosis in-

TABLE 1. Total number of *Caryospora bigenetica* oocysts counted in the feces of Swiss-Webster mice and cotton rats during 12-hr sampling periods on days 0 to 18 postinoculation.

Days postinoculation	Swiss-Webster mice	Cotton rats
0	60,000	0
1	20	1,250
2	294	11
3	602	0
4	0	0
5	4	0
6	0	0
7	13	0
8	17	0
9	0	6
10	5	0
11	0	0
12	0	0
13	0	0
14	1	0
15	0	0
16	0	0
17	0	0
18	0	0

cluding swollen muzzles and footpads, and lethargy at 8 DPI. Cotton rats also had bloody ocular discharges, partially or completely closed eyelids, and labored breathing. Clinical signs were more pronounced in male cotton rats than in females. Female cotton rats inoculated with 1.75×10^5 oocysts were found comatose and subsequently died at 9, 10, 11, and 13 DPI respectively. Meronts were identified in the muzzle of the cotton rat dying 9 DPI. Gamonts, unsporulated oocysts, and sporulated oocysts were observed in the muzzles, tongues, footpads, and recta of the cotton rats dying 10 DPI and 11 DPI. Unsporulated and sporulated oocysts, free sporozoites, and caryocysts were identified in the muzzle, footpad, tongue, and rectum of the cotton rat dying 13 DPI. The two male cotton rats inoculated with 2.0×10^4 oocysts were found dead on 9 and 12 DPI. Gamonts were identified in the muzzle and scrotum of the male cotton rat dying 9 DPI while gamonts, unsporulated and sporulated oocysts, and free sporozoites were present in the muzzle, tongue, footpad, and scrotum of the male cotton rat dying 12 DPI. One female cotton rat that received 2.0×10^4 oocysts died at 12 DPI; gamonts, unsporulated and sporulated oocysts, and free sporozoites were observed in muzzle, tongue, and footpad of this cotton rat. At 30 DPI, the 15 mice and the one remaining female cotton rat all had caryocysts in muzzles, tongues, footpads, and scrota (male mice). Caryocysts also were seen in the recta of three male mice.

Twelve of the 13 male mice inoculated with fecally-derived samples had no clinical signs of dermal coccidiosis following inoculation. No stages of *C. bigenetica* were observed in fresh smears of the muzzles, tongues, footpads, scrota, and dorsocervical tissues from these 12 mice with NIC microscopy. The mouse inoculated with feces collected at 0 DPI from male and female mice exhibited swollen muzzle and footpads, and lethargy at 9 DPI. Caryocysts were found in the muzzle, tongue, footpad, scrotum, and dorsocervical tissue of this mouse at 30 DPI.

DISCUSSION

Transmission of *C. bigenetica* occurs from snake to rodent, canid, or swine by oocyst ingestion (Wacha and Christiansen, 1982; Lindsay et al., 1988; Sundermann et al., 1988; Douglas et al., 1992), from mouse to snake by predation (Wacha and Christiansen, 1982), and from rodent to rodent by predation and cannibalism (Sundermann et al., 1989). In the present study, we provide evidence that fecal transmission of endogenously-produced oocysts by Swiss-Webster mice and cotton rats is not a component of the facultative heteroxenous life cycle of *C. bigenetica*.

The appearance and time of occurrence of the clinical signs and developmental stages in the mice and cotton rats were similar to those reported for experimentally infected rodents (Wacha and Christiansen, 1982; Lindsay et al., 1988; Upton and Barnard, 1988) and canids (Sundermann et al., 1988). Mortality was higher

in cotton rats (83.3%) inoculated with 1.75 × 10^5 and 2.0 × 10^4 oocysts than in mice (0%) which received much higher oocyst doses (2.5 × 10^5, 2.6 × 10^5), indicating that *C. bigenetica* is more pathogenic for cotton rats than mice.

Mice and cotton rats excreted *C. bigenetica* oocysts in their feces from 0 to 14 DPI. These oocysts were considered to be from the original inocula even though developmental stages of the parasite were identified in rectal tissue of mice and cotton rats at 10, 11, 13, and 30 DPI. The oocysts possessed thick walls, polar granules, and Stieda and substieda bodies; these characteristics are known to be present only in snake-derived oocysts. In contrast, endogenously-produced oocysts in rodents have thin walls and lack polar granules, Stieda, and substieda bodies (Wacha and Christiansen, 1982; Upton and Barnard, 1988). The oocyst and sporocyst measurements also were in agreement with those for snake-derived oocysts (Wacha and Christiansen, 1982). If the rodents had excreted oocysts that were endogenously-produced in their tissues, we would have expected a rise in oocyst production with the maximum output of oocysts occurring at 10 to 14 DPI (Wacha and Christiansen, 1982; Sundermann et al., 1988; Upton and Barnard, 1988). The small number of oocysts passed 10 to 14 DPI probably came from the original inocula and were displaced into the intestinal lumen after entrapment in epithelial folds or crypts of the digestive tract. Excretion of oocysts as late as 14 DPI also could be explained by the coprophagic behavior of rodents, whereby oocysts present in the original inocula were recycled by the ingestion of excreted feces. The small number of oocysts (1.25 × 10^3; 0.16% of the original inocula for eight cotton rats) passed in cotton rat feces compared to the large number (6.0 × 10^4; 2.0% of the original inocula for twelve mice) found in mouse feces on the days of peak oocyst passage may indicate that sporozoites excyst from oocysts and are transported into cotton rat tissue more easily than into mouse tissue. This could be one reason why *C. bigenetica* is more pathogenic to cotton rats than mice.

Fecal samples collected at 8, 10, and 14 DPI from mice, and at 9 DPI from cotton rats, contained *C. bigenetica* oocysts but failed to produce clinical signs and tissue infections when injected into mice; this was due to the very small numbers of oocysts present in these samples. The fecal sample collected at 0 DPI from male and female mice contained viable oocysts which remained infective after passage through the digestive tract. These oocysts produced characteristic clinical signs of dermal coccidiosis and tissue infections when injected subcutaneously into a male mouse. Similar findings, where orally-administered oocysts remained viable after passage through the gut, have been demonstrated with *Toxoplasma* (Dubey and Frenkel, 1973) and *Sarcocystis* (Dubey, 1981; Box, 1983; Munday, 1984/85). In summary, we propose that endogenously-produced *C. bigenetica* oocysts are not excreted in the feces of Swiss-Webster mice or cotton rats, and thus, infections in animals in nature are probably not initiated by this route.

ACKNOWLEDGMENT

This is manuscript 15-913105 of the Alabama Agricultural Experiment Station.

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Received for publication 26 July 1991.