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EXPERIMENTAL COCCIDIOSIS IN MULE DEER FAWNS

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Abstract: Five mule deer fawns *(Odocoileus hemionus)* ranging in age from 3 to 6 weeks were given sporulated *Eimeria mccordocki* oocysts orally. Four of the five fawns developed coccidiosis. Initial clinical signs appeared by 8 to 9 days post-inoculation and included elevated body temperature and bloody diarrhea. Dehydration and limited emaciation occurred as the disease progressed. The disease was allowed to run its course in one fawn and oocysts were passed in the feces on the 16th day following inoculation. Oocyst passage continued until the 27th day, at which time the animal appeared to be completely recovered. The remaining animals demonstrated all signs of coccidiosis and were euthanized prior to passing oocysts. Intracellular stages of *E. mccordocki* in these animals were confined to the last 75 to 100 cm of the ileum. The cytoplasm of each infected epithelial cell contained one to five meronts. The intestinal epithelium was inflammatory and contained many necrotic foci.

INTRODUCTION

The first account of *Eimeria* in an *Odocoileus* sp. was the report by Honess[4,5] for a coccidium, *E. mccordocki,* from the black-tailed deer, *O. hemionus hemionus,* in Wyoming. A preliminary description of the oocyst was given.

E. mccordocki subsequently was found in white-tailed deer, *O. virginianus virginianus,* in a number of localities. Anderson and Samuel[2] isolated the parasite from white-tailed deer in Wisconsin, Pennsylvania, and Texas, and gave the first detailed description. The major trend of these and other studies was taxonomic, and virtually nothing was mentioned regarding the biology of the parasites or the possible role they could play as disease agents.[6,7,8] The present work was done to increase knowledge of the biology of the parasite as well as record the effects of controlled infections on mule deer fawns.

MATERIALS AND METHODS

A survey for natural infections of *E. mccordocki* was carried out on two populations of captive mule deer, *Odocoileus hemionus,* near Fort Collins, Colorado. Forty animals were examined at least once. Examination included visual assessment of physical body conditions, general demeanor and fecal diagnosis.

Fecal samples were identified for each animal and collected in paper bags. Oocysts were concentrated for microscopic examination using a modification of a technique suggested by Benbrook and Sloss.[3] Oocysts were collected on cover glasses mounted at the top of centrifuge tubes completely filled with the sugar solution. Fecal specimens with oocysts were transferred to 3% aqueous potassium dichromate solution, strained and placed in sporulation containers and incubated at 30-32 C for various periods of time until sporulation was complete.

Five mule deer fawns were obtained for the study from Colorado and Wyoming. Fawns were in poor body condition, which necessitated careful husbandry over a period of 3 to 6 weeks before experimentation. A milk mixture diet, consisting of two parts whole bovine milk to one part evaporated milk, was

used over the entire rearing period. Alfalfa hay and concentrates were added to the diet during infection.

Each fawn was examined the first day for previous coccidial infection and then every week over the acclimatization period. Fawns were marked with an identifying ear tag and the health of each individual was followed closely.

Sporulated oocysts were cleared from dichromate and debri by centrifugation at 1,500 rpm 5-8 times, pouring off the supernatant each time. Oocyst suspensions were added to the daily milk ration of the fawn selected for experimental infection. One animal was infected with two species of *Eimeria (E. mccordocki* and *E. madisonensis)* collected from a field infection of an adult mule deer. A dose calculated to contain 15,500 sporulated oocysts of *E. mccordocki* and 75 oocysts of *E. madisonensis* was fed to fawn 97 (Table 1). This was done to separate the two species through expected incubation period differences and obtain a pure isolate of *E. mccordocki* for the remainder of the study. Fawn 97 was housed in an iron cage with a sliding floor to facilitate fecal collection. The fawn was observed closely for signs of coccidial infection. The body temperature was recorded and fecal collection and examination done twice a day at 12 h intervals. This procedure was adopted also in handling the other experimental animals. The *E. mccordocki* oocysts obtained from the pilot experimental infection were used for infecting the four other fawns (Table 1).

Results of the primary infection of fawn 97 were used to establish a necropsy time for the other fawns according to predetermined dates. Fawns 95, 94 and 93 were killed at 7, 9 and 13 days post infection, respectively (Table 1), to relate the clinical manifestation of coccidiosis with the difference stages of the life cycle.

Animals were euthanized with dry ice (CO_2) inhalation in a mask. The entire intestinal tract of each animal was dissected free from the other visceral organs. Boundaries and major anatomic features of the intestines were located for proper sample collection.

Macroscopic intestinal lesions were noted and the intestinal lumen was examined for abnormal contents such as free blood or mucus. Tissue specimens were taken at 30 cm intervals, starting at 4 cm from the pyloric sphincter. Intestinal rings were rinsed gently in tapwater and fixed in 10% neutral saline-formalin in labelled jars. Histological samples were also taken from the liver, kidneys and the lungs. Specimens were dehydrated in ethyl alcohol, embedded in paraffin, cut at 5μm and stained with hematoxylin and eosin.

RESULTS

Two species of *Eimeria (E. mccordocki* and *E. madisonensis)* were found in the

TABLE 1. Method used to infect mule deer fawns with *Eimeria mccordocki* and days post infection necropsy was performed.

Fawn No.	Age at Infection (wks)	Total Dose of Oocysts	Duration of Infection (days)
97	6	15,500	-*
95	4	18,000	7
94	3	16,800	9
93	4	18,000	13
100	5	24,000	15

*Fawn 97 was not examined by necropsy because infection proceeded for the entire patency period.

two populations of captive mule deer. Twenty one of 40 animals examined were infected with at least one of the two species. Eighteen deer had mixed *E. mccordocki* and *E. madisonensis* infections, three animals had *E. mccordocki* only. *E. madisonensis* was not encountered as a single infection. Oocyst counts ranged between 60 and 1,500 *E. mccordocki* oocysts per gram of feces. *E. madisonensis* oocysts ranged between 16 and 386 per gram of feces.

Clinical signs observed during the course of the infection in fawns 97, 94 and 93 were indicative of a severe gastroenteritis. There was elevation of temperature, diarrhea and blood-flecked feces (Table 2). Fawn 95 did not develop clinical signs attributable to coccidial infection. No oocysts were passed by fawns 94, 93 or 100 prior to euthanasia.

Carcasses of three animals (100, 93 and 94) were greatly dehydrated and emaciated. The intestinal tract had macroscopic lesions attributable to coccidiosis. Blood was present in the contents of the small intestines. The intestinal mucosa was thickened, severely congested and showed localized foci of an early necrotic nature. The lesions in the ileum were distributed irregularly for the first few centimeters then became more concentrated for the remaining length of the small intestine. The large intestine had hemorrhagic contents as the only abnormal finding. There was mild fatty change in the liver with scattered foci of congestion. No abnormalities were found in other internal organs. There were no macroscopic lesions in the internal organs or the intestinal tract in fawn 95.

Histologic evidence indicated that the parasitic stages of *E. mccordocki* were confined to the last 75 to 100 cm of the ileum in fawns 93, 94 and 100.[1] No microscopic evidence of parasitism was found in fawn 95.

Parasite development was limited to the epithelial cells of the secretory mucosa. Infection rate per epithelial cell, however, was found to increase towards the inner parts of the villi, but did not proceed further than a point just above the opening of the crypts of Lieberkühn. No developmental stages of the parasites were noticed in the crypts, muscularis mucosa, the lacteals or connective tissue cells.

Parasitic stages of various generations occupied parts of the cell above the nucleus and toward the brush border. Each infected epithelial cell contained one to five meronts (Fig. 1). The entire cell was usually occupied and the cytoplasmic area filled with developing asexual stages. The remainder of the host cell was often vacuolated, evidence of meronts having previously developed in that portion of the cell. Parasitized cells were not greatly enlarged. The host cell nucleus was often partially displaced and altered, becoming elongated and concave in some cases.

The life cycle of *E. mccordocki* was found to consist of two asexual generations followed by gametogony and subsequent oocyst development. First generation meronts contained

TABLE 2. Clinical signs of *Eimeria mccordocki* infection in mule deer fawns.

	Days After Infection			
Fawn No.	Temperature Rise	Diarrhea	Blood in Feces	Recovery
97	8-11	9-18	9-11	27
100	8-12	9-15	8-11	-
93	8-11	8-13	8-12	-
94	8-9	8-9	none	-
95	none	none	none	-

small residual bodies, and had a peripheral distribution of nuclei. Meronts had an average of 8 to 22 merozoites and measured 8.8 to 12.8 by 7.8 to 10.5 μm. Mature first generation merzoites were characteristically banana-shaped, with one end more blunt and the other end sharply pointed. Merozoites contained two refractile eosinophilic granules at both ends and had a conspicuous nucleus.

The life cycle of *E. mccordocki* was found to consist of two asexual generations followed by gametogony and subsequent oocyst development. First generation meronts contained small residual bodies, and had a peripheral distribution of nuclei. Meronts had an average of 8 to 22 merozoites and measured 8.8 to 12.8 by 7.8 to 10.5 μm. Mature first generation merzoites were characteristically banana-shaped, with one end more blunt and the other end sharply pointed. Merozoites contained two refractile eosinophilic granules at both ends and had a conspicuous nucleus.

Second generation meronts did not differ greatly in shape from first generation meronts but were distinguishable by their size as well as the larger size of the residium. They were generally larger, ranging between 9.8 to 14.4 μm by 9.5 to 13.5 μm. Each second generation meront contained 13 to 28 merozoites. Second generation merozoites were larger and longer than merozoites of the first generation. Their measurements ranged between 6.7 to 7.5 by 2.0 to 2.3 μm, their average being 7.4 to 2.1 μm.

Several second generation meronts appeared to have developed into macrogametocytes and several others into microgametocytes as early as the thirteenth day of infection. However, fully developed gamonts were observed only in fawn 100, which was examined 15 days after infection. Gamonts developed in large numbers in this animal, and multiple infection of the host cells was common. Macrogametes had a wide range of variation in size. Their measurements ranged from 2.5 to 3.5 μm in diameter. Microgamonts ranged from 9.5 to 15.5 μm. The sexual stages parasitized the same region of the small intestine as the asexual stages. They also occupied the same intracellular positions, away from the basement membrane of the host cells and towards its brush border.

Developing stages of the parasite in all three animals examined at necropsy were surrounded by zones of heavy leucocytic infiltration, with neutrophils and eosinophils most frequently observed. Loss of epithelium occurred in areas containing mostly asexual stages. Occasionally large hemorrhagic areas, or necrotic foci, were present in the ileal mucosa in fawns 93 and 100, in which the infection continued for 15 and 13 days, respectively. Some villi were devoid of glandular epithelium.

DISCUSSION

The survey made during the present study indicated that *E. mccordocki* and *E. madisonensis* are common in mule deer. *E. madisonensis* was less prevalent and was never found as a single infection. *E. madisonensis* was isolated originally from white-tailed deer.[2] Observations of *E. madisonensis* in the present study is the first demonstration of this coccidium from mule deer.

Developmental stages of *E. mccordocki* occupied practically every cell in a specific site of coccidial development in mule deer fawns (Fig. 1). The space which the parasites occupied represented a small part of the entire length of the small intestine.[1]

Effect of concurrent infection with *E. mccordocki* and *E. madisonensis* is not known, but the present study demonstrated that both can infect mule deer. It is not known whether they both occupy the same sites in the alimentary tract of deer or whether they show mutual site specificity. However, the

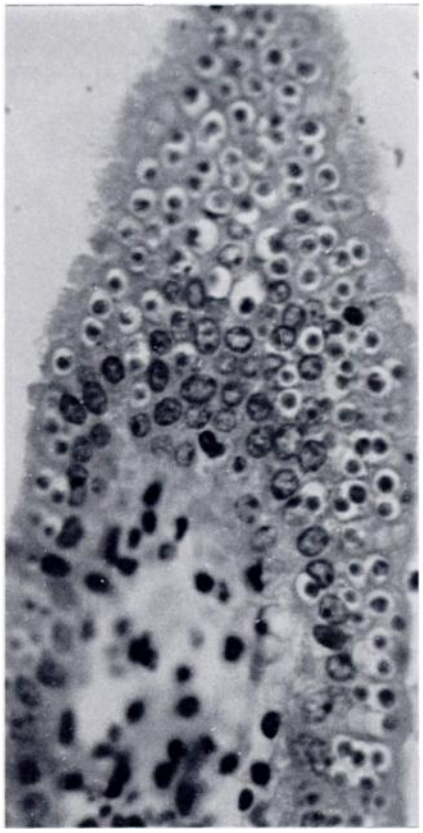

FIGURE 1. *Eimeria mccordocki* was present in most cells of villar epithelium in the last 75 to 100 cm of the ileum. Each infected cell contained one to five meronts.

developmental stages of *E. mccordocki* appear to be highly site specific. Chances that both species will develop in the same intestinal cells or region are very unlikely. One possible effect of concurrent infections is cross-protection. While this concept is highly possible among other coccidia, its existence in deer *Eimeria* requires investigation.

The prepatent period of *E. mccordocki* was found to be 15 days (384 hours) from the day occysts were administered orally to fawn 97. None of the other experimental animals passed oocysts in the feces before necropsy. The infection remained patent in fawn 97 for another 10 days (242 hours). Total patency period was 26 and a half days (636 hours).

E. madisonensis infection in fawn 97 had a prepatent period of 18 days. The infection remained patent for another nine and a half days.[1]

Asexual stages of *E. mccordocki* development in fawns 93, 94 and 100 coincided with typical clinical signs demonstrated in fawn 97. Clinical signs in fawn 94 were comparatively mild. First generation meronts, though quite numerous, occupied almost every cell in the parasitized region, but are probably not the principal pathogenic stages. Clinical manifestations of coccidiosis in the fawns increased with the development of second generation meronts which were comparatively fewer in number. Possibly the pathogenic state is precipitated by the invasion of new host cells with mature first generation merozoites, development of second generation meronts and invasion of new host cells by mature second generation merozoites.

Copious diarrhea started to appear in all fawns on the 9th day and was most severe in all fawns on the 13th and 15th days of infection. Blood flecks were voided in the feces during the 13th, 14th and 15th days of infection in fawns 93, 97 and 100. Tissue sections taken from animals at day 13 and day 15, post infection, demonstrated the highest number of second generation meronts. These meronts were larger than the first generation and had more merozoites per meront.[1] It is very likely that the second generation meronts were the most pathogenic.

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