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A *Hammondia*-LIKE COCCIDIAN WITH A MINK-MUSKRAT LIFE CYCLE[1]

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Abstract: A tissue cyst-forming coccidian morphologically resembling the known species *Hammondia* has a mink-muskrat life cycle. Cysts are found in skeletal muscle of muskrats *(Ondatra zibetheca).* Mink *(Mustela vison)* fed infected muskrat carcasses shed oocysts for 4 to 6 days after a prepatent period of 6 to 8 days. The oocysts, 99% of which are unsporulated in mink feces, measure 11.5 to 12 μm × 10 to 11 μm. Sporulated oocysts have 2 sporocysts, each with 4 sporozoites. The present work was insufficient to establish whether this *Hammondia*-like parasite is identical to the known *Hammondia* spp. or is a new parasite, although the evidence gathered supports the hypothesis that this parasite is a new member of the genus *Hammondia*.

INTRODUCTION

Recent investigations into the life cycles of tissue cyst-forming coccidians have shown that striated muscle cysts found in prey species are intermediate forms of organisms whose life cycles culminate in the formation of oocysts or sporocysts in the feces of a carnivore.[3] At present, three genera, *Toxoplasma*, *Hammondia* and *Sarcocystis*, are recognized as having the potential to infect striated muscle cells of the intermediate host.[3,11]

Sarcocystis spp. muscle cysts are recognized in mice and voles, *Sarcocystis putorii* (Railliet and Lucet, 1891) of the common European vole *(Microtus arvalis)* being of particular interest in this context because its definitive host is the European weasel *(Mustela nivalis).*[1,2,12,14]

Hammondia hammondi was discovered when *Toxoplasma*-like oocysts in cat feces were distinguished from *Toxoplasma gondii* by cross-infection studies.[5] In these studies, *Hammondia hammondi* oocysts from cats infected laboratory strains of mice, but a natural intermediate host was not found until Australian workers showed *Hammondia hammondi* infection in free-living rats *(Rattus rattus* and *Rattus norvegicus).*[10] In addition, *Hammondia pardalis* has been proposed as a new species in the ocelot *(Felis pardalis).*[6] *Hammondia heydorni* has been described, having both a cow-dog and dog-dog life cycle.[7,8]

This paper reports investigations into the life cycle of an *Hammondia*-like parasite of muskrat and mink.

MATERIALS AND METHODS

Animals

Mink. Mature female mink were obtained from commercial mink ranches. To ascertain coccidia-free status, fecal flotations, following the protocol of Dubey[4] were performed every other day for 30 days. No mink shed oocysts prior to experimental infection.

Muskrats. Skinned carcasses of muskrats trapped in Connecticut and New Jersey during the 1978-79 and 1979-

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80 seasons were obtained from trappers. Trappers checked their lines at least once daily, skinned carcasses within 24 h, and the carcasses were used for transmission studies within 24 h.

Cats. Kittens were obtained from a local animal shelter. Feces were examined by the fecal flotation method and schedule mentioned above. Only kittens that did not shed coccidian oocysts of a size compatible with *Toxoplasma*, *Hammondia* or *Sarcocystis* spp. designation were used in experiments, although cats shedding *Isospora felis* or *Isospora rivolta* were used.

Dogs. Puppies from a local shelter were obtained and feces examined by the same protocol as that for kittens and mink. No puppies had coccidian oocysts in their feces during the 30-day pre-infection period, although helminth eggs were found.

Rats and Mice. Fisher rats and BALB C mice were obtained from commercial sources.

Diets

Animals were maintained on commercial feed, with the exception of mink, which were fed 30% horse meat and 70% commercial chow with vitamin supplements, due to the unavailability of nutritionally complete commercial mink feed.

Housing

Mink, cats and dogs were housed individually while each group of mice and rats were housed in a separate shoe-box type cage.

Presence of Coccidian Cysts and Zoites in Muskrat Muscle

Rapid diagnosis of coccidian zoites in muscle was obtained by a modification of the pepsin digest technique of Jacobs and co-workers.[4] Diagnosis was confirmed by examination of routinely prepared histologic sections of skeletal muscle (Figs. 1 and 2).

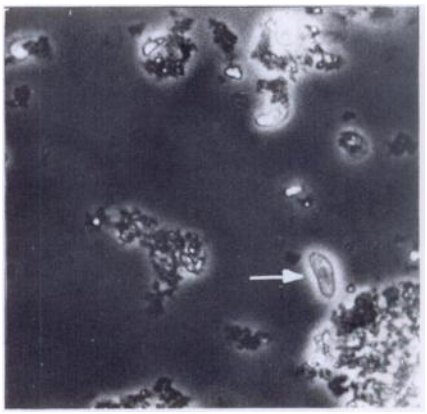

FIGURE 1. Pepsin-digested muskrat muscle viewed with dark-field optics. A coccidian zoite (arrow) can be seen near cellular debris. Unstained × 700.

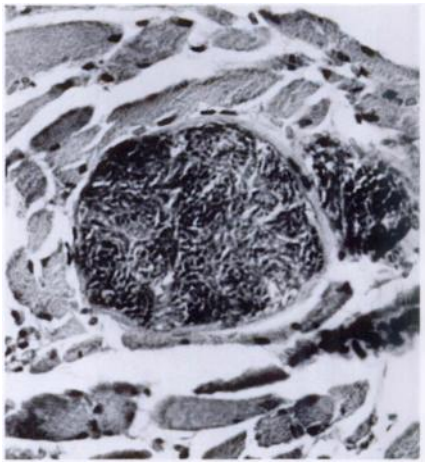

FIGURE 2. Muscle cyst in muskrat tongue. H&E × 250.

Experimental Design

Seven pairs of mink (Groups 1 through 7) were fed infected, eviscerated muskrat carcasses. Each of 7 infected muskrats was split along the spinal axis. The head and spinal cord were removed. Half of the carcass was placed in the appropriate

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mink cage so that both members of each group ate half a muskrat carcass over a 24 h period. Three pairs of mink (Groups 8 through 10) were similarly fed 3 uninfected muskrats. Feces of all mink in these groups were examined for coccidian oocysts daily for 30 days.

When coccidian oocysts were observed in the feces of some of the mink from Groups 1-7, daily fecal output was retained, oocysts separated by flotation, and sporulated following the method of Dubey.[3] Sporulated oocysts from both members of each oocyst-positive group were pooled but remained separate from the oocysts of other groups, and 200,000 oocysts were placed in the stomachs of each of four more pairs of coccidia-free mink (Groups 11 through 14) by intubation. In addition, 200,000 sporulated oocysts from each oocyst-positive group of mink were presented *per os* to a pair of rats and a group of 4 mice, while 4 rats and 4 mice were retained as uninoculated controls. Feces of mink in Groups 11-14 were examined daily for coccidian oocysts for 30 days following intubation of oocysts, while mice and rats were killed at 90 days postinoculation and muscle examined by the pepsin digest technique and histologic sectioning.

Mink from Groups 1-7 which shed oocysts and mink in Groups 11-14 were killed 30 days postinoculation and representative sections of the gastrointestinal tract, pancreas, ileocecal lymph node, liver, kidney, lung, heart, brain, diaphragm, tongue, esophagus and masseter muscles were examined for coccidian cysts. Pepsin digests were performed on skeletal muscle from these animals.

Four coccidia-free cats were fed two infected muskrat carcasses, divided in a manner similar to that described for the mink experiment, while 2 cats were fed one uninfected, divided muskrat carcass. Feces of all 6 cats were examined daily for 30 days.

Two coccidia-free dogs were fed one infected, divided muskrat carcass while two other dogs were fed a divided, uninfected carcass. Feces were examined daily for 30 days.

RESULTS

Four pairs of mink fed muskrats shed coccidian oocysts, while the other three pairs did not shed oocysts. The control mink (Groups 8-10) did not shed oocysts. Neither experimental nor control dogs and cats shed any oocysts similar to those seen in the four oocyst-positive groups of mink.

The oocysts appeared in feces after a prepatent period of 6 to 8 days, and the patent period lasted 4 to 6 days, with the largest numbers of oocysts appearing during the first two days of the patent period. Ten oocysts from each oocyst-positive mink were measured with a stage micrometer (*i.e.*, 80 oocysts). The range of dimensions was 11.5-12 μm · 10-11 μm, while the mean was 11.6 μm · 10.7 μm (Fig. 3). The oocyst wall had a double layer and a micropyle. Polar granules were absent. Sporonts consisted of spherical collections of granules. Rarely (<1%), sporulated oocysts were found in fresh feces, but sporulation occurred and

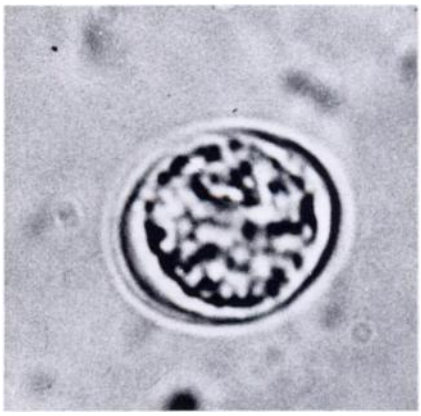

FIGURE 3. The unsporulated subspherical oocyst contained sporonts consisting of granules. Unstained × 2,500.

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was 95% complete in 96 h at room temperature.

Sporulated oocysts contained two sporocysts with four sporozoites each, and measured in the same size range as the unsporulated oocysts (Fig. 4). There was no oocyst residuum. The range of dimensions of 160 sporocysts was 8-9 μm · 6-7 μm, and the mean dimensions were 8.8 μm · 6.5 μm. There was a granular sporocyst residuum within the sporocyst.

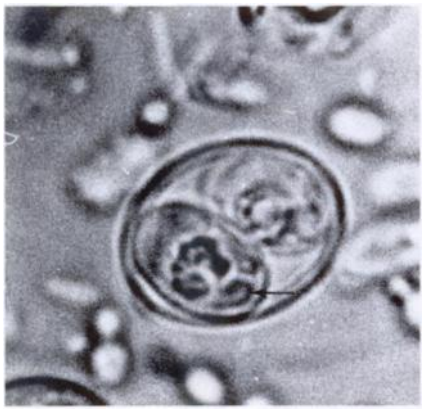

FIGURE 4. Sporulated oocysts had two sporocysts containing four sporozoites each. A sporocyst residuum was present. Part of a sporozoite can be seen (arrow). Unstained; phase-contrast optics × 2,500.

The 8 mink from Groups 1-7 that had shed oocysts had no coccidian forms in the gastrointestinal tract or other tissues examined histologically and skeletal muscle was negative for zoites by pepsin digestion. Identical results were obtained with mink from Groups 11-14.

Mink given sporulated oocysts by stomach tube (Groups 11-14) did not shed oocysts.

Rats and mice had no muscle cysts or zoites when examined by digestion and histologic examination 90 days after inoculation. No coccidian cysts were seen

TABLE 1. Summary of data from transmission experiments.

Animal species	Group number	No. in group	Fecal flotation results		
				Presence or absence of oocysts	Patent period (pid)*
Mink	1	2	Muskrat, zoite-positive	+	6-11
	2	2	As above	-	None
	3	2	As above	+	8-12
	4	2	As above	+	6-12
	5	2	As above	+	7-12
	6	2	As above	-	None
	7	2	As above	-	None
	8	2	Muskrat, zoite-negative	-	None
	9	2	As above	-	None
	10	2	As above	-	None

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TABLE 1. (continued)

	11	2	200,000 sporulated oocysts, from mink Group 1	-	None
	12	2	As above, but mink from Group 3	-	None
	13	2	As above, but mink from Group 4	-	None
	14	2	As above, but mink from Group 5	-	None
Cat	1	2	Muskrat, zoite-positive	-	None
	2	2	As above	-	None
	3	2	Muskrat, zoite-negative	-	None
Dog	1	2	Muskrat, zoite-positive	-	None
	2	2	Muskrat, zoite-negative	-	None

				Pepsin digestion results
				Presence or absence of zoites
Rat	1	2	200,000 sporulated oocysts, from mink Group 1	-
	2	2	As above, but mink from Group 3	-
	3	2	As above, but mink from Group 4	-
	4	2	As above, but mink from Group 5	-
	5	2	None	-
	6	2	None	-
Mice	1	4	200,000 sporulated oocysts, from mink Group 1	-
	2	4	As above, but mink from Group 3	-
	3	4	As above, but mink from Group 4	-
	4	4	As above, but mink from Group 5	-
	5	5	None	

*pid = postinoculation days.

in sections of brain and mesenteric lymph nodes of rats and mice.

Experimental design and results are graphically presented in Table 1.

DISCUSSION

These experiments demonstrate that a coccidian resembling *Hammondia* has a mink-muskrat life cycle. Since the oocysts of all known *Sarcocystis* spp. sporulate in the gut of the definitive host and are shed as sporocysts, this parasite is not a member of that genus. Failure of the cats to shed oocysts after feeding on infected muskrats, as well as absence of any type of coccidian cyst in the mice and rats, suggest that the parasite is not *Toxoplasma*.

Whether this *Hammondia*-like parasite is identical to *Hammondia hammondi* or *Hammondia heydorni* remains uncertain due to the inadequate number of dogs and cats examined, but the limited evidence supports the hypothesis that this is a new species of *Hammondia*. Failure to produce evidence of infection in mice and rats inoculated with sporulated oocysts suggests that this parasite is not *Hammondia hammondi*.

Emphasis was placed on the mink in the experimental work because mink are believed to be the only significant muskrat predators.[1] The failure to infect three of the seven pairs of mink fed infected muskrat carcasses suggests that the parasite may be transmitted with low efficiency, but immunity acquired previously by the mink remains a possibility. Since only 27 of 81 carcasses of muskrats examined by both digestion and histologic techniques were positive for muscle cysts, field data also support the hypothesis of low efficiency of transmission. Another possibility is that the muscle cysts seen in the skeletal muscle of muskrats is the cyst of a *Sarcocystis* sp. that does not complete its life cycle in mink, dog or cat, while the *Hammondia*-like cysts were never visualized. Close examination of the cysts seen in muskrat muscle shows the presence of septae between groups of zoites, and rounded zoites conforming to the published descriptions of metrocytes (Fig. 5). *Hammondia* cysts containing septae and metrocytes have never been described, while *Sarcocystis* cysts have both these morphologic features. A precedent for this situation exists in Heydorn's transmission experiments with *Hammondia heydorni*, in which he was able to produce infection in dogs after feeding bovine muscle in which only rare cysts could be demonstrated by histologic means.[2]

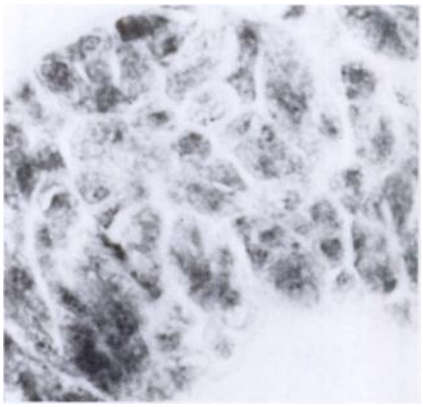

FIGURE 5. Cyst in skeletal muscle of muskrat. The cyst is septate and has metrocytes. H&E × 1,200.

LITERATURE CITED

1. CERNA, Z. 1977. Cycle de developpement sarcosporidien d'un Coccidie chez la souris obtenue apres infestation des animaux par des oocystes-sporocystes isoles de l'intestin de la Chouette-Effraie. Protistologica 13: 401-406.
2. CERNA, Z., I. KOLAROVA and P. SULC. 1978. Prispevek k poznani kokcidii tvovicick cysty. Folia Parasitol. Praha 25: 9-16. (English abstract: Contribu-

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tion to the problem of cyst producing coccidians. Biol. Abstr. 66, No. 28121, Sept. 1, 1978.)

3. DUBEY, J.P. 1977. *Toxoplasma, Hammondia, Besnoitia, Sarcocystis*, and other Tissue Cyst-forming Coccidia of Man and Animals. In: *Parasitic Protozoa*, Vol. III (J.P. Krier, Ed.), Academic Press, New York.
4. ERRINGTON, P.L. 1946. Predation and vertebrate populations. Quart. Rev. Biol. 21: 144-221.
5. FRENKEL, J.K. and J.P. DUBEY. 1975. *Hammondia hammondi:* A new coccidium of cats producing cysts in muscle of other mammals. Science 189: 222-224.
6. HENDRICKS, L.D., J.V. ERNST, C.H. COURTNEY and C.A. SPEER. 1979. *Hammondia pardalis* sp. n. (Sarcocystidae) from the ocelot, *Felis pardalis*, and experimental infection of other felines. J. Protozool. 26: 39-43.
7. HEYDORN, A.-O. 1973. Zum Lebenszyklus der kleinen Form von *Isospora bigemina* des Hundes. I. Rind und Hund als mögliche Zwischenwirte. Berlin. u. Münch. tierärztl. Wochschr. 86: 323-329.
8. ——, R. GESTRICK and V. IPCZYNSKI. 1975. Zum Lebenszyklus der kleinen Form von *Isopora bigemina* des Hundes. II. Entwicklungsstadien im Darm des Hundes. Berlin. u. Münch. tierärztl. Wochschr. 88: 449-453.
9. JACOBS, L., J.S. REMINGTON and M.L. MELTON. 1960. A survey of meat samples from swine, cattle, and sheep for the presence of encysted *Toxoplasma*. J. Parasitol. 46: 23-28.
10. MASON, R.W. 1978. The detection of *Hammondia hammondi* in Australia and the identification of a free-living intermediate host. Z. Parasitenkd. 57: 101-106.
11. MUNDAY, B.L., R.W. MASON, W.J. HARTLEY, P.J.A. PRESIDENTE and D. OBENDORF. 1978. *Sarcocystis* and related organisms in Australian wildlife. I. Survey findings in mammals. J. Wildl. Dis. 14: 417-433.
12. RUIZ, A. and J.K. FRENKEL. 1976. Recognition of cyclic transmission of *Sarcocystis muris* by cats. J. Inf. Dis. 133: 409-418.
13. TADROS, W. and J.J. LAARMAN. Note on the specific designation of *Sarcocystis putorii* (Railliet & Lucet, 1891) comb. nov. of the common European vole, *Microtus arvalis*. Parasitology 81: 466-468.

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