Parasites of Small Mammals in Grand Teton National Park: *Babesia* and *Hepatozoon*

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✦ Introduction

Babesia microti, a parasitic protozoan, is endemic in the *Microtus montanus* of Grand Teton National Park. It is transmitted by the tick *Ixodes eastoni* and reproduces in the erythrocytes of its vole host.

A second protozoan parasite, *Hepatozoon* sp., which is widespread in small mammals in Europe, is also found in reptiles throughout the world. The record of *Hepatozoon* in North American small mammals is not extensive.

Hepatozoon, unlike *Babesia*, may be a two-host or a three-host parasite. Also unlike *Babesia*, for which the intermediate host is always a tick, the intermediate host of *Hepatozoon* may be a tick, a mite, a flea, or a mosquito. The method of transmission by the vector also differs in the two parasites. *Babesia* is transmitted in the tick's saliva when it bites, whereas *Hepatozoon* infection requires the vertebrate host to ingest a vector.

In our 1996 studies, we sought more data on these two parasites. The objectives for 1996 focused on *Hepatozoon*: to sample specific populations of *M. montanus*, in which we have previously documented *Hepatozoon* infections; to determine whether differences exist in the infection rates at different study sites in the park; and to search for any additional vectors of *Hepatozoon* sp. infections in *M. montanus* by examining ectoparasites. These studies contributed to our long-term objectives of documenting the effects and cost of parasitism on *M. montanus* populations.

✦ Methods

All animals were trapped at sites within the boundaries of Grand Teton National Park using Sherman live-traps. The permit to collect small mammals in Grand Teton National Park was granted to A. J. Pinter. Upon removal from the traps, voles were killed by overanesthesia, then a 25 gauge needle was inserted into the left ventricle of the heart, and blood was collected in a heparinized tuberculin syringe. The blood was transferred to a micro-centrifuge tube. Several slides of peripheral blood smears were made from this blood. Slides were fixed in methanol and stained with Wrights-Giemsa stain. The peripheral blood smears were examined for the presence of *Babesia*, *Hepatozoon*, and other parasites.

The spleen, liver, and lungs were removed. Impression and squash smears of the organs were made and examined for the presence of parasites, especially for the schizonts of *Hepatozoon*, with a light microscope equipped with 15X oculars and a 100X oil objective.

Fleas were collected from the live-trapped animals, both before and after overanesthesia, whenever they were detected. They were either squashed and examined with a light microscope for the presence of oocysts, or they were placed in 70% ethanol and later sent for identification to Dr. Robert E. Lewis at Iowa State University.

✦ RESULTS AND DISCUSSION

Table 1 shows the results of trapping for 1988 through 1996. The extremes of infection rate for *Babesia microti* occurred in 1996 when there was a low of 16.7% in the spring and in 1995 when the high of 100% was recorded in the spring. Except for 1996, the spring rate of infection was always higher than that of the summer. This finding is consistent with *Ixodes eastoni*'s being a nest tick (personal communication, Richard G. Robbins) and *M. montanus*' living in the same nest until spring, with no dispersal occurring until the onset of the spring snowmelt and the breeding season.

Table 1. Incidence of *Babesia microti* and *Hepatozoon* sp. in *Microtus montanus*

Year	Season	Number caught	Number positive *Babesia*	Number positive *Hepatozoon*	Per cent positive *Babesia*	Per cent positive *Hepatozoon*
1996	Spring	18	3	3	16.7	16.7
	Summer	19	14	0	73.7	0
1995	Spring	19	19	0	100.0	0
	Summer	31	13	0	41.9	0
1994	Spring	9	5	0	55.6	0
	Summer	59	27	0	45.8	0
1991	Spring	23	17	4	73.9	17.4
	Summer	74	19	5	25.7	6.8
1990	Spring	16	9	0	56.3	0
	Summer	58	19	5	32.8	8.6
1989	Spring	35	16	12	45.7	34.3
	Summer	34	13	1	38.2	2.9
1988	Spring	20	13	0	65.0	0
	Summer	53	16	9	30.2	17.0

Samples of *Babesia*-infected blood were given to Yolanda Peck, a biology graduate student at the University of Nebraska at Omaha, for work-up and subsequent sequence analysis of the 16S-like rRNA gene of the parasite. Working with the guidance of Dr. Bruce Chase, Biology Department, University of Nebraska at Omaha, she sequenced the gene, and analysis of the results is in progress (personal communication, Dr. Bruce Chase; Peck and Chase, 1997).

Originally, *Babesia* was thought to be restricted to small mammals; however, in 1970 the first human cases were diagnosed in residents of Nantucket Island, Massachusetts (Western et al, 1970). In the United States, human babesiosis is caused primarily by *B. microti* but also by a recently recognized, as yet unspeciated, organism designated WA-1 (Thomford et al., 1994). As humans insert themselves into places where they have historically been present little, they sometimes contract new diseases, and the proximity of humans to ticks in Grand Teton National Park suggests that the status of babesiosis in small mammals of the park should be monitored.

Hepatozoon sp. is the other endoparasite of *M. montanus* that we continued to study in 1996. The infection rates for 1996 and previous collection years are displayed in Table 1. Rates of infection of *M. montanus* with *Hepatozoon* are characteristically lower than with *B. microti*; in 1994 and 1995 we found no *Hepatozoon* infections at all in either the spring or the summer.

Hepatozoon sp. infections have been found in *M. montanus* from 8 study sites. Since some sites appeared to have a higher infection rate than other sites, one of the goals for 1996 was to determine whether this observation could be more thoroughly documented with more data. Unfortunately, for the third consecutive year we found too few animals infected with *Hepatozoon* to accomplish this goal.

Since the inception of our studies, we have photographed as many stages in the life cycle of *Hepatozoon* as possible. This year we report the discovery in *M. montanus* of monozoic and dizoic cysts, two little known features of the life cycle of *Hepatozoon*. First described in reptiles by Landau et al.in 1972 (Desser, 1990), these cysts are latent forms that are infective when the host in ingested by a predator. Thus, they constitute a second mode of transmission to a vertebrate besides the better known route by ingestion of an infected invertebrate. *Microtus montanus* may harbor monozoic cysts, dizoic cysts, and schizonts. Figures 1-3 show dizoic cysts; Figure 4 shows a monozoic cyst. For comparison, Figures 7 and 8 show several merozoites from a ruptured schizont. They are not as slender as merozoites previously encountered. We do not know whether this difference reflects their developmental stage, the length of time free from the schizont, species differences, or some other factor. Figures 5 and 6 show vermicules from a preparation in which their motility was observed prior to fixation and staining. They, too, are less slender than vermicules observed previously.

From 1989 through 1995, five flea species, collected as intact specimens from *M. montanus*, were identified by Dr. Robert Lewis: *Megabothris abantis*, *Me. asio megacolpus*, *Aetheca wagneri*, *Peromyscopsylla selenis* and *Hystrichopsylla dippiei dippiei*. He also identified one of the two host fleas as *Me. abantis*. In 1996 a sixth species, *Peromyscopsylla hesperomys*, was collected from 2 different voles and identified by Dr. Lewis.

We presented many of our recent findings at the annual meeting of the American Society of Parasitologists in June, 1997, in Nashville, Tennessee. The title of our paper was "*Hepatozoon* sp. Infections in Three Newly Recognized North American Mammalian Hosts (*Microtus montanus*, *Mi. pennsylvanicus*, *Thomomys talpoides*) and the flea *Megabothris abantis* Identified as an Invertebrate Host in *Mi. montanus* Infections."

✦ ACKNOWLEDGMENTS

We are grateful to Dr. Robert E. Lewis, Department of Entomology, Iowa State University, for identifying fleas. Thanks to Elisabeth Miller for able assistance in both the field and the laboratory. We are grateful for the use of the University of Wyoming-National Park Service Research Center facilities, where field work was conducted.

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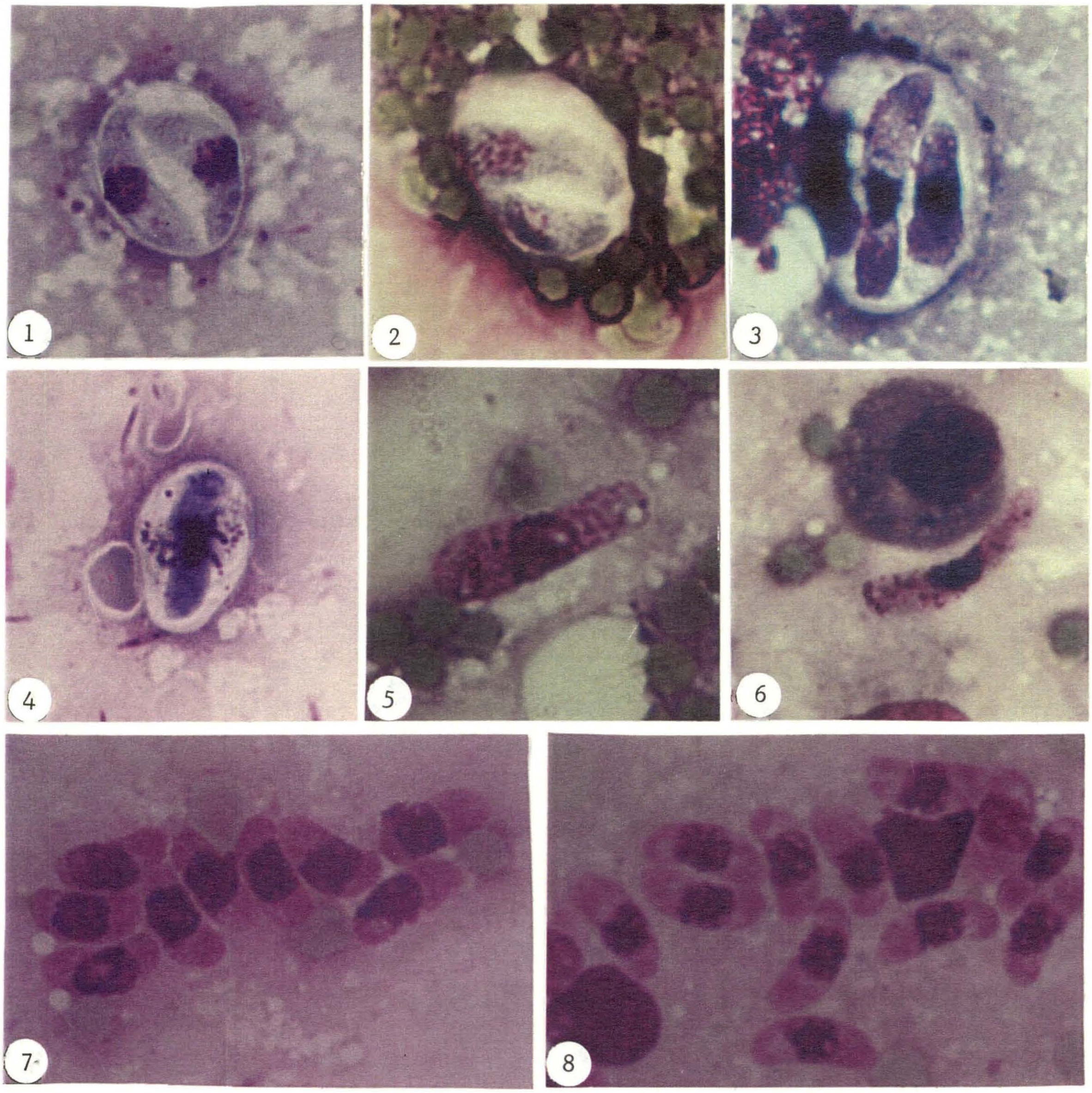

Figures 1-3. Dizoic cysts in lung squashes. Figure 4. Monozoic cyst from a lung squash. Figures 5-6. Vermicules from lung squashes. Figures 7-8. Merozoites from a ruptured schizont in a lung squash. All preparations were stained with Wrights-Giemsa stain. X 1,600.