

# PARASITES OF SMALL MAMMALS IN GRAND TETON NATIONAL PARK *BABESIA* AND *HEPATOZOON*

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## ♦ INTRODUCTION

The role of parasitism, whether macro- or microparasites, and whether endo- or ectoparasites, in the demographic machinery of microtines is poorly understood. In a review of the parasites of *Microtus*, Timm (1985) lists no protozoan endoparasites whatsoever for this genus and observes that one of the most challenging and fruitful directions of future research with *Microtus* will be the statistical quantification of the cost of parasitism.

*Babesia microti*, a parasitic protozoan, is transmitted by a tick vector and reproduces in the erythrocytes of its mammalian host. Initially, *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 by *B. microti*. As humans insert themselves into places where they have historically been present only occasionally, they often contract new diseases.

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. Like *Babesia*, *Hepatozoon* is a two-host parasite. 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 swallow the vector.

In our 1995 studies, we sought more data on these two parasites. The objectives for 1995 were: to sample specific populations of *M. montanus*, in which we have previously documented *Hepatozoon* infections, to determine whether there were differences 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. Our long-term objectives are to document 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. Upon removal from the traps, they were killed by overanesthesia, 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, 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. The spleen and liver were weighed and measured. 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. Extra slides were made and fixed in methanol and stained as with the peripheral blood smears. The remaining portions were stored in 10% buffered formalin until preparation for histological examination.

Fleas were collected from the live-trapped animals, both before and after overanesthesia, as soon as they were detected. Fleas were either squashed and examined with a light microscope for the presence of oocysts, or they were placed in 70% ethanol and later sent to Dr. Robert E. Lewis to be identified.

## ◆ RESULTS AND DISCUSSION

Table 1 shows the results of trapping for 1988 through 1995. The extremes of infection rate for *Babesia microti* occurred in 1991 when there was a low of 25.7% in the summer and in 1995 when the high of 100% was recorded in the spring. Each year the spring rate of infection was higher than that of the summer, a finding which is consistent with *Ixodes eastonis*' 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. The infection rate in the voles was consistently high, but reached 100% for the first time in 1995 sampling. The infections appeared to be recently established, with high parasitemias commonplace and one individual's infection rampant at 20.6% infected red blood cells in blood smears.

*Hepatozoon* sp. is the other endoparasite of *M. montanus* we continued to study in 1995. The infection rates for 1995 and previous collection years are displayed in Table 1. Rates of infection of *M. montanus* with *Hepatozoon* were characteristically lower than with *B. microti*; in 1988, 1990, 1994, and 1995 we found no *Hepatozoon* infections in the spring and none in the summer in 1994 and 1995.

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

Year	Season	Number caught	Number positive		Per cent positive	
			<i>Babesia</i>	<i>Hepatozoon</i>	<i>Babesia</i>	<i>Hepatozoon</i>
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

*Hepatozoon* sp. infections have been found in *M. montanus* from 8 study sites. Previously we observed all of the known forms found in vertebrate hosts and learned that schizogony is typically in the lungs of these voles. One of the goals of the 1995 study was to confirm these findings and to seek additional data on the life cycle in both the vertebrate and invertebrate host. Unfortunately, for the second consecutive year we found no animals infected with *Hepatozoon*.

Another of our goals for several years has been to identify an invertebrate host. We had hypothesized initially that a flea was an invertebrate host and vector of *Hepatozoon* sp. because fleas are established vectors for European small mammals. After finding no oocysts in flea squashes from 1989-1991, we began planning to expand our hypothesis to include the possibility that the tick, *I. eastoni*, which is a frequent ectoparasite of *M. montanus*, serves as a vector. In 1994, however, our search yielded 2 fleas containing *Hepatozoon* oocysts and corroborated our original hypothesis (Fig. 1).

The fleas containing oocysts were squashed and many anatomic features disrupted or destroyed, which prompted us to seek help again from Dr. Robert E. Lewis of Iowa State University. Five species, collected as intact specimens from 1989 through 1995 from *M. montanus*, have been

identified by Dr. Lewis: *Megabothris abantis* (Fig. 2, 3), *Me. asio megacolpus*, *Aetheca wagneri*, *Peromyscopsylla selenis*, and *Hystrichopsylla dippiei*. He has now identified one of the two host fleas as *Me. abantis*.

In all study years we have found cytoplasmic inclusions in the white blood cells (Fig. 4, 5) of *M. montanus* that resemble *Theileria* sp. schizonts and the "leucocyte inclusions" reported by Coles (1914) in field voles, *M. agrestis*. Coles wrote, "In the blood of the field vole I have not infrequently met with large granules in the protoplasm of the large uninucleated leucocytes. These granules stain a deep red, almost black, colour with Giemsa. They vary considerably in size. In leucocytes where they are very numerous they are not much larger than eosinophile granules, whilst in others, in which there may be few, they measure as much as 2.5 to 3 u in diameter. They are generally perfectly round, sometimes oval, and rarely elongated. In the blood of one field vole 40% of all the leucocytes contained these granules. They were present also, in smaller numbers, in one of the field voles which showed haemogregarines. I have also found them in other animals, especially in some of the large mononuclear leucocytes of a cow suffering from Redwater Fever. I imagine these are not protozoal, but probably of the nature of secretion products." Unlike Coles, we suspect that they are indeed protozoan parasites. We plan to characterize them further and to consult the literature for additional insight as to their identity. We have so far seen no evidence to indicate that, like *Theileria* sp. they promote division of the host cell. We find no evidence that the inclusions are responsible for illness or mortality, which is also true of *Theileria* sp. infections in some hosts, e.g. the African buffalo (Norval et al., 1991).

## ◆ ACKNOWLEDGMENTS

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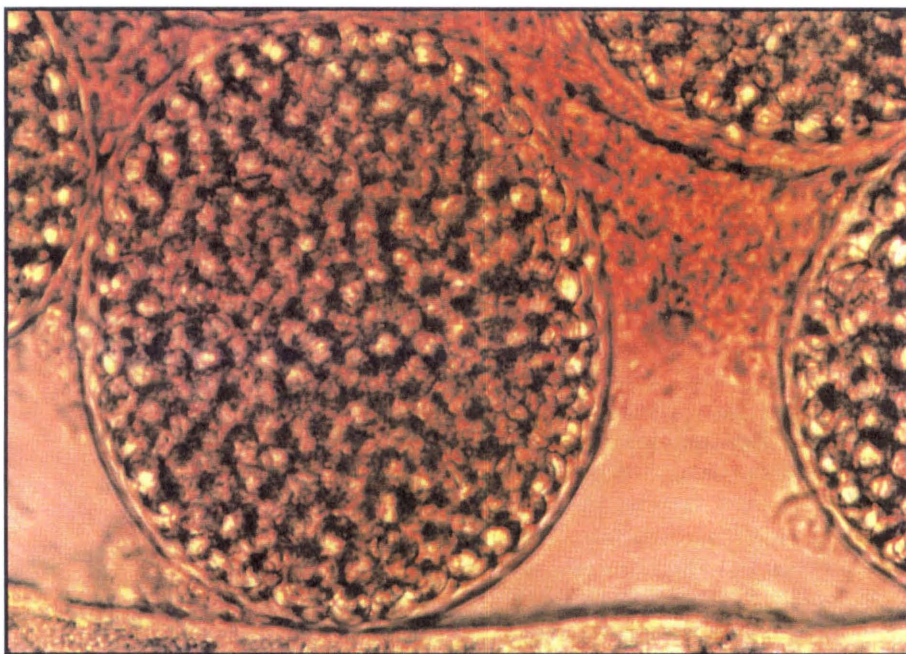


Figure 1. Oocysts of *Hepatozoon* sp. in a squash of *Megabothris abantis*, X 640.

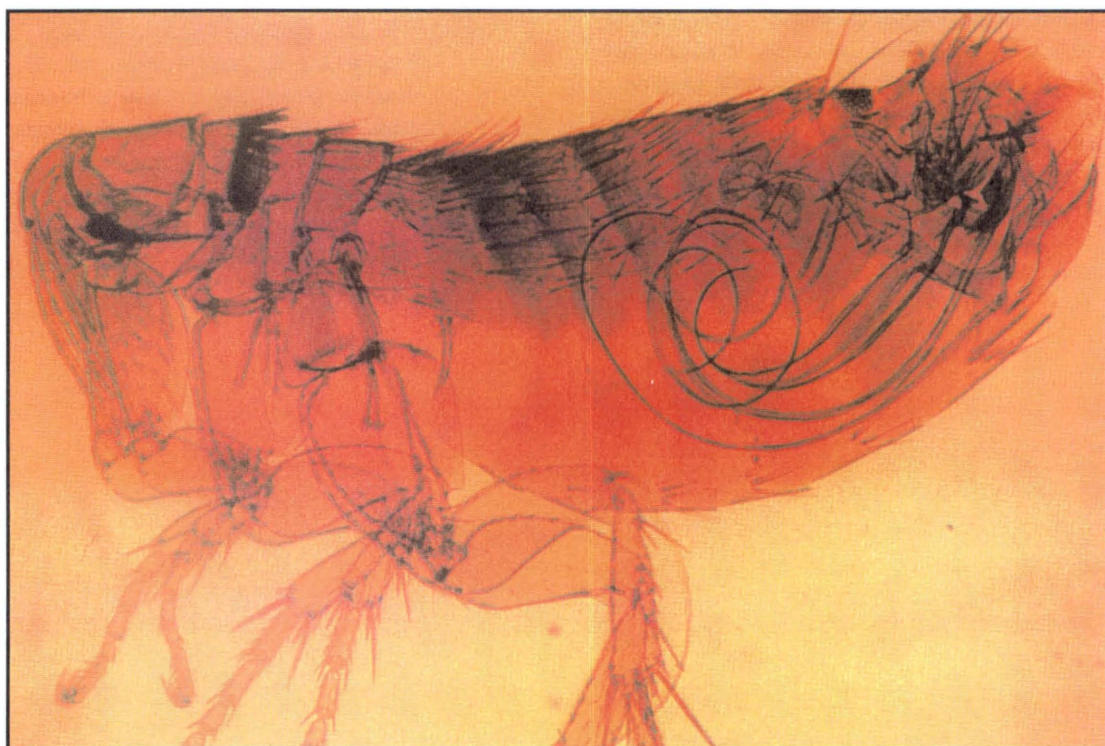


Figure 2. *Megabothris abantis*, male, X 75. (Cleared mounted, and identified by R.E. Lewis.)

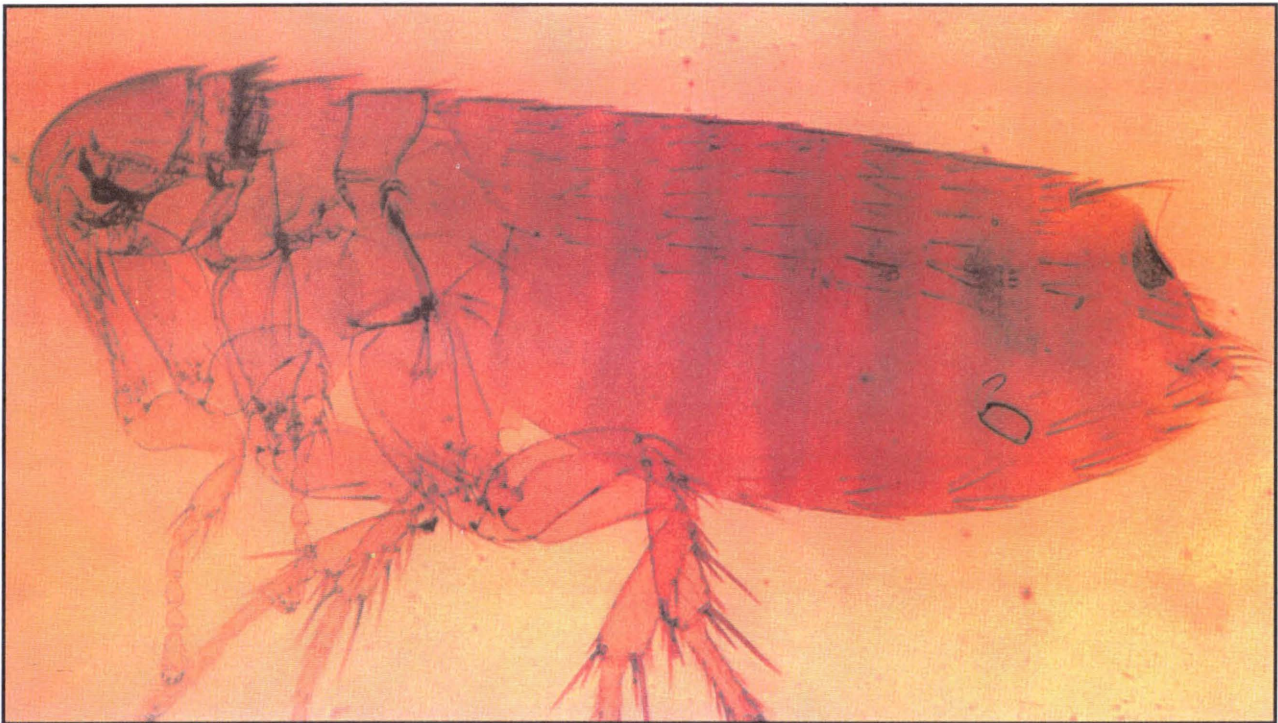


Figure 3. *Megabothris abantis*, female, X 75. (Cleared, mounted and identified by R.E. Lewis.)

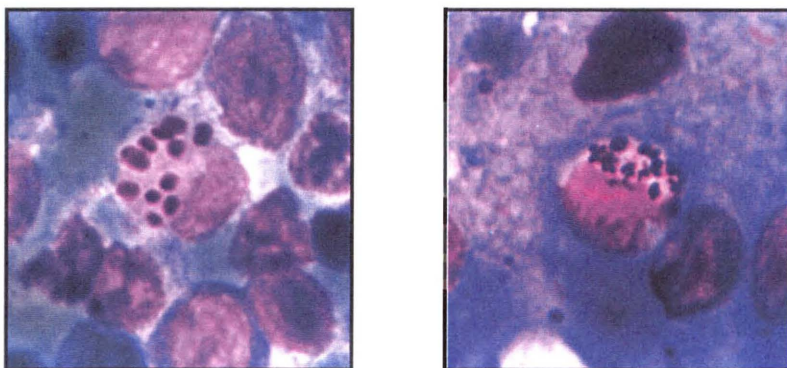


Figure 4. Leukocytic inclusions, spleen smears, X 1,600.

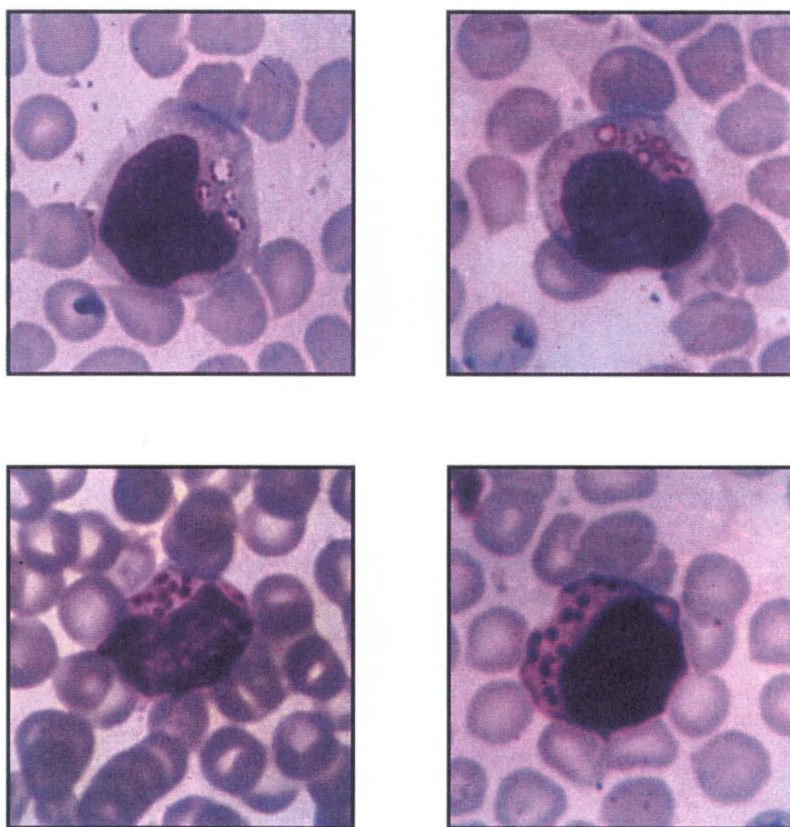


Figure 5. Leukocytic inclusions, blood smears, X 1,600.