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# Letter to the Editor

## Roles of the Maltese Cross Form of *Babesia microti* in the Development of Parasitemia in *B. microti* Infection

Studies of parasite infection involving animal hosts other than humans have contributed significantly to our understanding of the disease process, including possible pathogenic mechanisms of parasites and immunological responses of the hosts.

In dogs, it was traditionally assumed that the only *Babesia* species causing disease were *Babesia canis* and *Babesia gibsoni*, but recently published reports (1, 5, 7) demonstrate that a different (*Babesia microti*-like) piroplasm can parasitize dogs.

In the first report (7), molecular phylogenetic analysis indicated that the infecting agent was in fact more closely related to *B. microti*, a Holarctic rodent parasite and the cause of human babesiosis in eastern North America, than to other *Babesia* species. In an extensive series of case studies from Galicia, Spain (1, 5), the agent was also definitively identified by molecular phylogenetic methods as the *B. microti*-like parasite. Analysis of the rDNA sequences indicates that the Spanish isolate is closely related, but not identical, to the reference *B. microti* strains isolated in Japan and the United States. Whether the parasite should be referred to as a geographical variant of *B. microti* or a new species (or subspecies) remains unresolved.

This newly recognized piroplasm appears to be hyperendemic in Galicia, northwest Spain, and associated with the tick *Ixodes hexagonus* (2). *B. microti* infection in dogs is frequently associated with pathology. Intense regenerative anemia and thrombocytopenia are common findings among infected dogs, many of which also develop serum biochemistry abnormalities compatible with renal disease (3).

In the January 2003 issue of *Infection and Immunity*, Yokoyama et al. (6) published an important paper that gives clues to a possible protective role of the Maltese cross form of the parasite in the course of *B. microti* infection in mice and in the development of parasitemia.

After this interesting work, between October and December 2003, 945 dogs had blood samples submitted for hematological analysis to a diagnostic laboratory located in Vigo, northwest Spain. All the samples were analyzed by bright field microscopy of Giemsa-stained thin blood smears, and small ring-shaped piroplasms (*B. microti* like) were detected in 41 of the samples, with parasitemia within 1 to 30%. The identity of the infecting agents was confirmed by PCR sequencing. The results show the following conclusions.

(i) The Maltese cross form is often described as characteristic of *B. microti*, as well as *Babesia* (*Theileria*) *equi* and *Theileria parva*. In the present study, it was also observed among *B. microti*-like piroplasm-infected dogs (<1%).

(ii) In splenectomized dogs with the *B. microti*-like piroplasm (4), the Maltese cross form represented approximately 2% of the total merozoite forms. In this case, during the acute febrile phase, the main laboratory finding was the presence of multiple intraerythrocytic merozoites of a small piroplasm in Giemsa-stained thin blood smears observed microscopically. The typical features of ring, binary, and tetrad forms were observed.

(iii) According to the results of these investigations, the severity of the disease is not believed to be associated with the level of parasitemia (1). In general, the levels of parasitemia of the sick dogs were low (1 to 2%).

I would like to know whether Dr. Yokoyama and coauthors know of any human cases that show changes similar to those observed in their experiments. Since the pathophysiology of *B. microti* infection remains enigmatic, the opinion of Yokoyama et al. on this issue would be greatly appreciated.

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**Tomás Camacho**
*Laboratorio Lema & Bandín*
*C/ Lepanto 5, bajo*
*36201 Vigo, Spain*

Phone: 33-986437400
Fax: 33-986431145
E-mail: atcamacho@teleline.es

### Author's Reply

The correspondence of Dr. Camacho has raised an interesting issue pertaining to the recent study by Yokoyama et al. (2), that is, the importance of roles of the Maltese cross form of the parasite in the development of parasitemia and protective response to *Babesia microti* infection in mice. Dr. Camacho asks whether a similar observation has been made in human cases. To examine roles of the Maltese cross form in human cases seems very interesting for understanding the pathophysiology of *B. microti* infection. Although many human cases of *B. microti* infection have been reported, development of the Maltese cross form or a relationship between development of parasitemia and the Maltese cross form has not been observed during infection (1). When human cases are identified, the parasitemia has already developed to some extent and treatment of patients is the first priority. Therefore, it is almost

impossible to examine the changes of the Maltese cross form or the relationship of the Maltese cross form and pathophysiology in human cases of *B. microti* infection. Based on the reasons above mentioned, I do not know of any human cases that showed changes similar to those observed in our experiments.

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**Ikuo Igarashi**
*National Research Center for Protozoan Diseases*
*Obihiro University of Agriculture and Veterinary Medicine*
*Obihiro, Hokkaido 080-8555, Japan*

Phone: 81 155 49 5641
Fax: 81 155 49 5643
E-mail: igarcpmi@obihiro.ac.jp