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Citation	Japanese Journal of Veterinary Research, 33(1-2), 77-77
Issue Date	1985-04-30
Doc URL	<a href="http://hdl.handle.net/2115/2332">http://hdl.handle.net/2115/2332</a>
Type	bulletin (article)
File Information	KJ00002374304.pdf



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MITOGEN INDUCED TRANSFORMATION OF LYMPHOCYTES IN CANINE  
BABESIOSIS WITH SPECIAL REFERENCE TO THE EFFECTS  
OF THE SPLEEN ON IT

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In order to clarify the mechanisms of immune-responses in canine babesiosis, mitogen-induced transformation of lymphocytes from peripheral blood and its relation to parasitemia were chiefly examined in both splenectomized and non-splenectomized dogs suffering from *Babesia gibsoni*. In addition, the role of the spleen in canine babesiosis was also investigated. The results obtained were as follows.

(1) Dog lymphocytes required for the transformation test were collected at a high recovery rate (average of 76.3%) and purity (average of 73.9%) from approximately 3ml of peripheral blood centrifuged over Ficoll-Conray with a specific gravity of 1.082. The optimum condition of cell suspension of the isolated lymphocytes was  $5 \times 10^5$ /ml. The optimum concentration of PHA-P, Con A and PWM used as mitogens, was determined as follow: PHA-P 0.1% of stock solution, Con A was 0.05% and PWM was 1% (final concentration, respectively).

(2) Although marked individual variation of the lymphocyte response to the mitogens was observed, the response was almost stable in all of the dogs examined, even in repeated examinations.

(3) The lymphocyte response to each of the mitogens in dogs infected with *B. gibsoni* was extremely depressed as the parasite increased in their peripheral blood.

(4) Percentage of the erythrocytes parasitized with *B. gibsoni* (parasitemia) in the peripheral blood of the splenectomized dogs ranged from 20 to 30%, average of 23%, whereas it ranged from 2 to 10%, average of 5% in intact dogs infected with *B. gibsoni*.

(5) The lymphocyte response to the mitogens greatly decreased after splenectomy in both groups infected and non-infected with *B. gibsoni*. Furthermore, severe relapse of parasitemia appeared in the infected dogs after splenectomy.

(6) Mild parasitemia (0.4–1.8%) was observed in spite of a high serum IFA titer to the parasite in splenectomized dogs, while it almost disappeared following the rise of the IFA titer in intact dogs.

From the results mentioned above, it was concluded that *B. gibsoni* may suppress the activity of lymphocytes in canine peripheral blood, but an increase of the parasites in the blood may also be inhibited by the functions of the spleen and the humoral antibody to the parasite.