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Antibody titres in naturally occurring *Babesia canis* infections in dogs

Titres des anticorps dans les infections naturelles à *Babesia canis* chez le chien — Les anticorps ont été titrés par la technique ELISA (TIE test, immunoenzymatique), chez 2 groupes de chiens naturellement infectés par *Babesia canis*. L'un des groupes a reçu un traitement contre la babésiose avec du dipropionate de carbamide, l'autre a servi de témoin. Chez les animaux de moins de 1 an, les titres d'anticorps ont atteint un maximum suivi d'une chute, que les animaux aient été traités ou non. Chez les animaux plus âgés, les titres ont augmenté pour les non-traités et diminué chez les témoins. Dans les zones d'endémie, les auteurs conseillent de ne traiter que les chiens âgés qui présentent une babésiose clinique, cependant que la chimioprophylaxie à l'aide du dipropionate de carbamide pourra être pratiquée chez les chiens de moins de 1 an. *Mots clés :* Chien - Babésiose - *Babesia canis* - Anticorps - Test ELISA - Prophylaxie - Nigeria.

INTRODUCTION

Infection of the domestic dog with *Babesia canis* often results in overt disease (11). Though the disease can manifest in many different ways, the most constant clinical signs of canine babesiosis are pyrexia, anaemia and icterus (5). However, during a survey of *Babesia* infections of dogs in Ibadan, Nigeria, it was observed that some dogs were infected by *B. canis* but did not show any sign of clinical babesiosis. On the other hand, there were other infected dogs that were clinically ill, showing signs of babesiosis, and required specific treatment for babesiosis. It was then decided not to give any treatment against babesiosis in those dogs that were not clinically ill and had no concurrent infection, with a view to studying the antibody responses in them. These were compared with the antibody responses in the dogs that received specific treatment for babesiosis.

Also chemoprophylaxis with imidocarb has been suggested for prevention of canine babesiosis (2, 3, 4). Therefore imidocarb was used in the treatment of the clinically ill dogs with the aim of assessing its effect on the antibody responses of the infected dogs.

MATERIALS AND METHODS

The dogs used for this study were those presented at the Small Animal Clinic of the University of Ibadan Veterinary Teaching Hospital. The dogs were subjected to detailed clinical examination, and blood examination for *B. canis* using Giemsa staining procedure. Only those dogs that had uncomplicated *B. canis* infection were included in this study.

Those that were clinically ill among the dogs selected were treated with imidocarb dipropionate (Imizol*) at the rate of 6 mg/kg body weight intramuscularly. Those that were not ill were not treated until they showed signs of illness. Detailed clinical and haematological examinations, as well as antibody titre determination by the enzyme linked immunosorbent assay (ELISA) were carried out on both the treated and untreated dogs on a two weekly basis for 12 to 24 weeks.

Enzyme-linked immunosorbent assay (ELISA)

The assay was carried out as follows. The antigen used was obtained from Laboratoire IFFA (Lyon, France). The antigen was diluted 1 : 240 in 0.05 M carbonate buffer, pH 9.6. The diluted antigen was allowed to adsorb to the wells of polyvinyl microtitration plates (Titertek, Flow Laboratories. N° 76-212-05) overnight at 4 °C. The plates were washed with phosphate buffered saline (PBS) containing 0.05 p. 100 Tween 20 (PBS/Tween). Then the test sera, diluted 1 : 160 in PBS/Tween, were incubated in the wells for two hours at room temperature. The plates were washed as before and incubated for one hour at 37 °C with diluted (1 : 20 000) rabbit anti-canine immunoglobulin (IgG) conjugated with horseradish peroxidase (Miles Laboratories, Slough, U.K.). The plates were washed again and incubated in a dark humid chamber at room temperature with the enzyme substrate, orthophenylene diamine (OPD), diluted to 0.3 mg/ml with citrate buffer, pH 5.0 with hydrogen

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* Imizol®, manufactured by The Wellcome Foundation Ltd, London, England.

peroxide. The reaction was stopped after 30 minutes by the addition of 2 M sulphuric acid. The colour produced by the action of the enzyme on its substrate was determined spectrophotometrically at 492 nm, using an ELISA reader (Titertek Multiskan, Flow Laboratories).

Positive reference serum was obtained from a natural case of *B. canis* infection while the negative reference serum was obtained from a 12 week old puppy that had never been exposed to *B. canis* infection. The positive and negative reference sera were serially diluted from 1 : 40 to 1 : 5 120. These were assayed and the adjusted mean absorbance (optical density) for each dilution of the positive reference serum was obtained. The value for each dilution was plotted against the reciprocal of the dilution. A smooth curve was constructed through all the points obtained. The adjusted mean absorbance value for each test serum was read off the curve constructed to obtain the titre of the serum.

RESULTS

Forty-two dogs were selected for this study. However, only 20 were available for subsequent examinations for periods ranging from 12 to 24 weeks. The ages of the dogs ranged from 3 months to 7 years and a half. They consisted of 10 indigenous Nigerian dogs, 7 exotic dogs and 3 crossbreeds between indigenous and exotic dogs (mixed). Ten of the dogs were treated for babesiosis while the other 10 were not.

The antibody titres in the untreated dogs are shown in table I. In this group, the antibody titres in most of the dogs less than one year old declined after the initial examination, despite the persistence of *B. canis* parasitaemia. On the other hand, in dogs aged one year and above, the antibody titres increased after the initial examination. *B. canis* was demonstrable in dogs n^{os} 7, 8 and 10 throughout the period of study while in n^{os} 6 and 9, they could not be found from days 70 and 42 respectively.

Among the untreated dogs, 3 of those less than one year old (n^{os} 2, 4 and 5) and a one year old dog (n° 7) became clinically ill and required treatment on days 175, 126, 158 and 126 after the initial examinations respectively. None of the other dogs developed clinical babesiosis during the 6 months following the completion of the study.

B. canis could not be detected in the blood by day 14 post-treatment in treated dogs. The antibody titres of all the dogs declined following treatment (Tabl. II). In 5 of the dogs (n^{os} 11, 12, 14, 15 and 18) the antibody titres declined below detectable levels between 8 and 16 weeks after treatment. Two of the dogs (n^{os} 12 and 15) had relapses of clinical babesiosis on days 76 and 110 post-treatment respectively and *B. canis* was demonstrable in their blood. However, none of the other dogs developed clinical babesiosis during the 6 months following the completion of this study.

TABLE I Antibody titres measured by ELISA in dogs with untreated natural **B. canis** *infection.*

Serial n°	Age (in years)	Breed	Days after initial examination												
			0	14	28	42	56	70	84	98	112	126	140	154	168
1	0.25	IND	1 : 40	N.D.	1 : 80	1 : 160	1 : 160	1 : 80	1 : 80						
2	0.40	IND	1 : 640	1 : 640	1 : 640	1 : 320	1 : 160	1 : 160	1 : 160	1 : 160	1 : 80	1 : 40	1 : 40	1 : 40	1 : 40
3	0.50	Mixed	1 : 320	1 : 160	1 : 80	< 1 : 40	< 1 : 40	< 1 : 40	< 1 : 40	< 1 : 40	< 1 : 40	< 1 : 40			
4	0.50	IND	1 : 320	1 : 320	1 : 320	1 : 160	1 : 80	1 : 40	N.D.	N.D.	< 1 : 40	< 1 : 40			
5	0.70	IND	1 : 2 560	1 : 1 280	1 : 1 280	1 : 1 280	1 : 640	1 : 640	1 : 320	1 : 80	1 : 40	< 1 : 40	< 1 : 40	< 1 : 40	
6	1.00	IND	1 : 320	1 : 320	1 : 320	1 : 320	1 : 640	1 : 640	1 : 640	1 : 640					
7	1.00	IND	1 : 160	1 : 160	1 : 160	1 : 160	1 : 320	1 : 320	1 : 640	N.D.	1 : 640	1 : 1 280			
8	4.50	Exotic	1 : 80	1 : 160	1 : 160	N.D.	1 : 160	1 : 160	1 : 320	1 : 320					
9	5.50	IND	1 : 320	1 : 320	1 : 640	1 : 640	1 : 640	1 : 1 280	1 : 1 280						
10	6.50	Exotic	1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120	> 1 : 5 120		

IND = Indigenous dogs ; N.D. = Not done.

TABLE II Antibody titres measured by ELISA in dogs with natural **B. canis** *infection before (Day 0) and after treatment.*

Serial n°	Age (in years)	Breed	Days after initial examination												
			0	14	28	42	56	70	84	98	112	126	140	154	168
11	0.25	Exotic	1 : 640	1 : 320	1 : 160	1 : 80	1 : 40	1 : 40	<1 : 40	<1 : 40					
12	0.30	IND	1 : 80	1 : 80	1 : 80	1 : 40	<1 : 40	<1 : 40	<1 : 40						
13	0.40	Mixed	1 : 640	1 : 640	1 : 640	1 : 320	1 : 320	1 : 160	N.D.	1 : 80					
14	0.60	IND	1 : 640	1 : 320	1 : 320	1 : 160	1 : 80	1 : 80	1 : 40	<1 : 40	<1 : 40	<1 : 40	<1 : 40		
15	1.25	Mixed	1 : 320	1 : 320	1 : 320	1 : 160	1 : 80	1 : 40	1 : 40	1 : 40	<1 : 40				
16	1.50	Exotic	1 : 5 120	1 : 2 560	1 : 1 280	1 : 1 280	1 : 640	1 : 320	1 : 320	1 : 320	1 : 160	N.D.	1 : 80	1 : 40	1 .40
17	4.50	Exotic	1 : 2 560	1 : 2 560	1 : 1 280	1 : 1 280	1 : 640	1 : 320	1 : 320	1 : 160					
18	5.50	Exotic	1 : 80	1 : 80	1 . 80	1 : 80	1 : 40	<1 : 40	<1 : 40	<1 : 40					
19	6.00	Exotic	1 : 5 120	1 : 5 120	1 : 2 560	1 : 1 280	1 : 640	1 : 320	1 : 320	1 . 1 320	1 : 320	1 : 160	1 : 160	1 : 80	1 :40
20	7.50	IND	1 : 5 120	1 : 5 120	N.D.	1 : 5 120	1 : 2 560	1 : 1 280	1 : 1 280	1 : 1 280	1 : 640	1 : 640	1 : 640	1 : 640	1 :640

IND = Indigenous dogs ; N.D. = Not done.

DISCUSSION

The protective activity of antibody in *B. canis* infection in dogs has been demonstrated (6, 9). The antibody titre has also been shown to be a measure of immunity in *B. canis* infection (7). Therefore the changes in the antibody titres recorded in this study are indicative of changes in the immune status of the dogs.

In dogs less than one year of age, it seems the antibody titre rises to a peak following *B. canis* infection and then declines, whether the dog is treated or not. This decline in antibody level is indicative of a lowering of the dog's immunity and this might have been responsible for the clinical babesiosis observed later in 3 of the 5 untreated dogs that were less than one year old. This may also be the reason for the higher frequency of *B. canis* infection and subsequent clinical babesiosis observed in dogs in this age group (1, 8).

In dogs older than one year, the increase in the antibody titres in untreated dogs is indicative of an active immune response to the infection and probably an attempt by the host to eliminate the parasite. Thus the disappearance of the parasite from the blood of 2 of the 5 untreated dogs that were over one year of age might have been due to elimination of the parasite.

The differences observed between the responses of the untreated dogs less than one year old and those above one year of age may be due to the fact that while the former were probably being infected for the first time, the latter probably had previous infections, since *B. canis* is endemic in Ibadan (1). Thus the humoral antibody responses observed in the latter were most likely anamnestic. It has been suggested that the immunity developed by dogs against babesiosis is poor and requires the continuous stimulation (through re-infection) of the immune system to maintain itself (10).

Chemotherapy with imidocarb does not seem to have an adverse effect on the immune status of dogs less than one year old. Therefore such dogs would benefit from chemoprophylaxis with this drug. On the other hand, in the older dogs, chemotherapy with imidocarb seems to decrease the immunity of the dogs to *B. canis* infection. In an area where *B. canis* is endemic such as Ibadan (1), chemoprophylaxis with imidocarb will greatly reduce the capability of such dogs to develop resistance to *B. canis* infection. It may therefore be beneficial to wait until these older dogs develop clinical babesiosis before treating them.

ACKNOWLEDGEMENTS

We are grateful to Dr. Y. MOREAU of Laboratoire IFFA, Lyon, for supplying the *B. canis* antigen. This work was supported by funds from the University of Ibadan Senate Research Grant. ■

BOBADE (P. A.), ODUYE (O. O.). Antibody titres in naturally occurring *Babesia canis* infections in dogs. *Rev. Elev. Méd. vét. Pays trop.*, 1986, **39** (2) : 185-188.

Antibody titres were measured by enzyme linked immunosorbent assay (ELISA) in two groups of dogs naturally infected with *Babesia canis*. One group was treated for babesiosis with imidocarb dipropionate, while the other was not. In dogs less than one year old, the antibody titres reached a peak and declined in both treated and untreated dogs. In older dogs, the antibody titres increased in the untreated dogs while they declined in the treated ones. It is suggested that in endemic areas, while chemoprophylaxis against *B. canis* using imidocarb, can be practised in dogs less than one year old, older dogs should only be treated when they develop clinical babesiosis. *Key words :* Dog - Babesiosis - *Babesia canis* - Antibody - ELISA test - Prophylaxis - Imidocarb dipropionate - Nigeria.

BOBADE (P. A.), ODUYE (O. O.). Título de los anticuerpos en las babesiosis naturales con *B. canis* en los perros. *Rev. Elev. Méd. vét. Pays trop.*, 1986, **39** (2) : 185-188.

Se determinó el título de los anticuerpos por medio de la prueba ELISA en dos grupos de perros naturalmente infectados por *Babesia bovis* (*B. canis*). Un grupo recibió un tratamiento con dipropionato de Imidocarb mientras que otro, no tratado, fué el grupo testigo. En los perros de menos de 1 año de edad, el título de los anticuerpos llegaba a un máximo y disminuía en los dos grupos. En perros más viejos, el título aumentaba en los perros testigos mientras que disminuía en los perros tratados. Se sugiere que, en región endémica, se puede preveer una profilaxia con imidocarb en los perros de menos de 1 año de edad ; en cambio se necesita tratar los perros más viejos sólo cuando muestran una babesiosis clínica. *Parabras claves :* Perro - Babesiosis - *Babesia canis* - Anticuerpos - Prueba ELISA - Profilaxia - Imidocarb - Nigeria.

REFERENCES

1. BOBADE (P. A.). Studies on canine anaemia with particular reference to *Babesia* infections. Ph. D. Thesis. University of Ibadan, Nigeria, 1985.
2. COLL (J. L.), DESCAMPS (H.), FAYETTE (J. P.), FERAUD (J. P.), VILLEMIN (P.). Essai de chimio-prévention de la babesiose canine avec l'imidocarbe. *Recl Méd. vét.*, 1982, **158** : 791-798.
3. EUZEBY (J.), MOREAU (Y.), GAUTHEY (M.), DUBOR (M.). Expérimentation des propriétés antipiroplasmiques de l'imidocarbe sur *B. divergens* et *B. canis*. *Bull. Soc. Sci. vét. Méd. comp. Lyon,* 1981, **83** : 129-134.
4. GUELFI (J. F.). Utilisation du dipropionate d'imidocarbe pour le traitement de la babesiose canine. Essais de chimio-prévention. *Rev. Méd. vét.*, 1982, **133** : 617-621.
5. MALHERBE (W. D.). The manifestations and diagnosis of *Babesia* infections. *N. Y. Acad. Sci.*, 1956, **64** : 128-146.
6. NOCARD (E.), MOTAS (C. S.). Contribution à l'étude de la piroplasmose canine. *Annls Inst. Pasteur,* 1902, **16** : 257-290.
7. RISTIC (M.), SMITH (R. D.), KAKOMA (I.). Characterization of *Babesia* antigens derived from cell cultures and ticks. *In* : Babesiosis, RISTIC (M.), KREIER (J. P.) eds, New York, Academic Press, 1981. pp. 337-380.
8. SHORTT (H. E.). *Babesia canis* : the life cycle and laboratory maintenance in its arthropod and mammalian hosts. *Int. J. Parasit.*, 1973, **3** : 119-148.
9. SIBINOVIC (K. H.), SIBINOVIC (S.), RISTIC (M.), COS (H. W.). Immunogenic properties of babesial serum antigens. *J. Parasit.*, 1967, **53** : 112-129.
10. STEWART (C. G.). A comparison of the efficacy of isometamidium, amicarbalide and diminazine against *Babesia canis* in dogs and the effect on subsequent immunity. *J. S. Afr. vet. Ass.*, 1983, **54** : 47-51.
11. VAN HEERDEN (J.). The transmission of *Babesia canis* to the wild dog *Lycaon pictus* (Temminck) and black-backed jackal *Canis mesomelas* Schreber. *J. S. Afr. vet. Ass.*, 1980, **51** : 119-120.