P. A. Bobade [1]
O. O. Oduye [1]
H. O. Aghomo [1]

Prevalence of antibodies against *Babesia canis* in dogs in an endemic area

BOBADE (P. A.), ODUYE (O. O.), AGHOMO (H. O.). Prévalence des anticorps contre *Babesia canis* chez les chiens dans une zone endémique. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (2) : 211-217.

Une recherche des anticorps contre *Babesia canis*, par le test ELISA, portant sur 287 chiens d'une zone endémique, a révélé une prévalence de 43 p. 100. On a trouvé des anticorps dans toutes les classes d'âge avec une prévalence significativement plus basse chez les chiens âgés de 1 à 6 mois que chez les animaux plus âgés. Aucune différence entre les chiens nigérians indigènes et les chiens « exotiques » ou étrangers, ni entre les sexes, n'a été remarquée dans la prévalence des anticorps. Les anticorps étaient plus fréquents chez les chiens parasités par *B. canis* et chez ceux à haut risque d'infection. De plus, des anticorps ont été détectés chez quelques chiots nés de chiennes séropositives. Le test ELISA n'a pas détecté d'anticorps chez 36,1 p. 100 de chiens atteints de babésiose à *B. canis*. *Mots clés* : Chien - Babésiose - *Babesia canis* - Anticorps - Sérologie - Test ELISA - Nigeria.

INTRODUCTION

Canine babesiosis caused by *Babesia canis* (Piana and Galli Vallerio, 1895) is endemic in Nigeria (1, 2, 5, 7, 11). In Ibadan, the prevalence of the infection ranges from about 10 per cent to 26 per cent (7, 11). The infection occurs throughout the year with peak periods occurring at the peak of the rainy season (June and July) and the beginning of the dry season (October and November) (2). The parasite is believed to be transmitted in this area by two ticks of dogs, *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* (11).

Though canine babesiosis has been reported in different parts of the world (12) information regarding the prevalence of antibodies against *B. canis* is scanty (9). The enzyme-linked immunosorbent assay (ELISA) is being increasingly used for the detection of antibodies against *B. canis* (3, 4, 8, 9, 17, 18).

This study was conducted to determine the prevalence of antibodies against *B. canis* in an endemic area and to investigate the usefulness of ELISA in the diagnosis of the infection in this area.

1. Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

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MATERIALS AND METHODS

This study was carried out in Ibadan area, which is situated in South-western Nigeria (latitude 7 °N and longitude 3 °E). Dogs were sampled over a period of one year.

The dogs used were those presented at the University of Ibadan Veterinary Teaching Hospital, dogs in some selected households, and dogs in a holding kennel. All the dogs were subjected to detailed clinical and haematological examinations. Some of the dogs were ill at the initial examination, while others were clinically normal (healthy). Attempts were made to monitor as many of these dogs as possible at intervals of 2 weeks during the first two months after the initial examination and thereafter at intervals of 2 to 4 weeks for 6 to 9 months.

Blood samples for parasitological examination and serological studies were obtained by venipuncture. Blood smears on clean grease-free glass slides were stained with Giemsa (13), the smears being stained for 45 minutes. The smears were examined in bright field with oil immersion objective.

ELISA

The serum was separated from each blood sample within 24 hours and stored at - 20 °C until assayed.

The ELISA test was performed as previously described (3). The antigen, which was obtained from Laboratoire IFFA (Lyon, France), was prepared from an *in vitro* culture of *B. canis* and lyophylised (10). The test sera were diluted 1:160, this being the dilution that gave the maximal separation between the control positive serum and the control negative serum in a checkerboard titration (16). Rabbit anti-canine immunoglobulin (IgG) conjugated with horseradish peroxidase (Miles Laboratories, U.K.) was reacted with the enzyme substrate, orthophenylene diamine, diluted with citrate buffer, pH 5.0 with hydrogen peroxide. The optical density (O.D.) was read by a spectrophotometer at 492 nm. The adjusted mean O.D. for each sample was read off a curve constructed as previously described (3) to obtain the antibody titre. Positive threshold for

the ELISA test was fixed at the O.D. value of the control positive serum at 1:160.

Data analysis

The results obtained in this study were subjected to statistical analysis, using the Chi-square (X^2) test with $P \leq 0.05$ as the acceptable level of significance.

The geometric mean antibody titres (GMT) (for seropositive dogs only) were calculated by dividing the titre by 10 and expressing the result as a code titre before taking logarithms to base 2 (15).

RESULTS

Two hundred and eighty-seven dogs made up of 202 indigenous (Nigerian) dogs and 85 exotic (foreign) dogs were examined. There were 186 males and 101 females. The ages of the dogs ranged from 4 weeks to 14 years. Ninety-nine of the 287 dogs were clinically normal (healthy). Fifty-nine of all the dogs were monitored for periods varying from 2 to 12 months.

Antibodies against *B. canis* were detected in 125 (43.6 per cent) of the dogs examined. Antibodies were detected in all age groups (Table I). Forty one of the seropositive dogs were healthy. There was no significant difference ($X^2 = 0.16$, $P > 0.50$) between the prevalence of antibodies against *B. canis* in the healthy dogs and the ill dogs.

The proportions of seropositive dogs aged 2-3 months, and 3 to 6 months were significantly lower ($X^2 = 5.15$, $P < 0.05$; and $X^2 = 4.20$, $P < 0.05$ respectively) than those of the other age groups (Table I). When all dogs aged 1 to 6 months were compared with those older than 6 months, the percentage of seropositive dogs in the former group (27.9 per cent) was significantly lower ($X^2 = 16.95$, $P < 0.001$) than in the latter group (53.4 per cent). From 6 months of age upwards, there were no significant differences in the proportion of seropositive dogs in the different age groups. The age prevalence of antibodies against *B. canis* declined initially in both indigenous and exotic breeds of dogs, reaching the lowest level in dogs aged 2 to 3 months. It then rose and reached a peak in indigenous dogs between the ages of 4 to 6 years. Among exotic dogs, the prevalence increased with age after 3 months and reached peaks in dogs aged 6 to 12 months and those older than 6 years (Fig. 1).

TABLE I Age distribution of dogs with antibodies against **Babesia canis** *infection.*

Age	Number examined	Seropositive dogs		$P \leq 0.05$
		Number	Percentage	
1.0-2.0 months	28	10	35.7	N.S.
2.1-3.0 months	20	3	15.0	Significant
3.1-6.0 months	63	18	28.6	Significant
6.1-11.9 months	52	27	51.9	N.S.
1.0-1.9 years	39	19	48.7	N.S.
2.0-2.9 years	29	14	48.3	N.S.
3.0-3.9 years	12	6	50.0	N.S.
4.0-4.9 years	10	7	70.0	N.S.
5.0-5.9 years	9	5	55.6	N.S.
6.0-7.0 years	12	7	58.3	N.S.
> 7 years	13	9	69.2	N.S.

N.S. = Not significant.

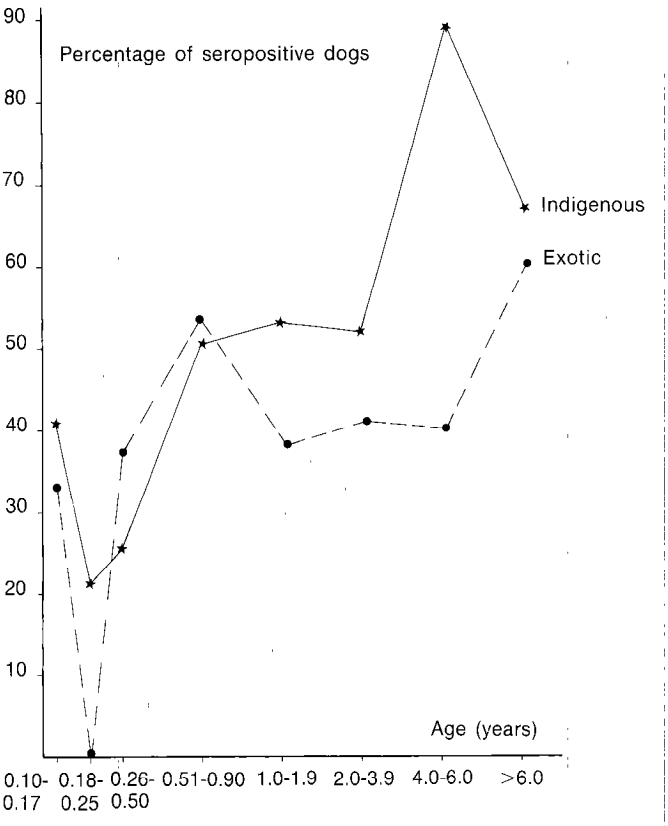

Fig. 1 : Age-breed distribution of dogs with antibodies against B. canis.

Antibodies were detected in 44.6 per cent of the males and 41.6 per cent of the females, there being no significant difference ($X^2 = 0.14$, $P > 0.50$) between the sexes. Also there were no significant differences between the proportions of seropositive males and females in each age group.

The seropositive dogs consisted of 91 indigenous and 34 exotic dogs, giving prevalence percentages of 45.1 and 40.0. The prevalence percentages were not significantly different ($X^2 = 0.43$, $P > 0.50$). The proportions of seropositive indigenous and exotic dogs in each age group were also not significantly different.

B. canis parasitaemia was detected by Giemsa staining in 36 of the dogs examined and these were made up of 23 indigenous and 13 exotic dogs. Antibodies against *B. canis* were detected by ELISA in 23 (63.9 per cent) of these while the 13 others had no detectable antibody at initial examination thus giving a false negative result in 36.1 per cent of the parasitaemic dogs. This represented 4.5 per cent of all the dogs examined. Figure 2 shows the proportions of dogs with *B. canis* parasitaemia among the seropositive ones in each age group. The proportion of seropositive parasitaemic dogs was significantly ($X^2 = 6.01$, $P < 0.05$) higher than the proportion of seropositive non-parasitaemic dogs.

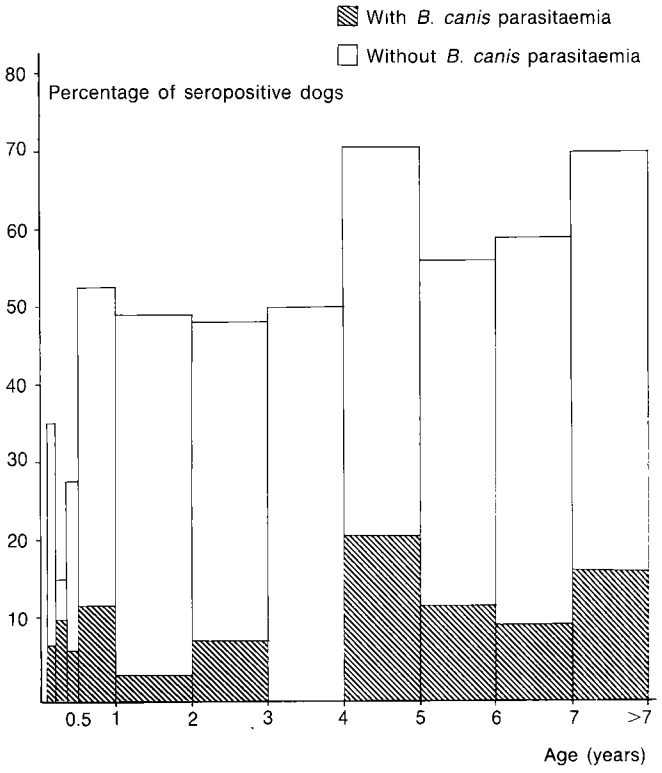

Fig. 2 : Age distribution of seropositive dogs with B. canis *parasitaemia and without parasitaemia.*

Table II shows the age distribution of the dogs that gave false negative ELISA results. Twelve of them were aged 7 weeks to 10 months while the other was a 4 1/2 year old German Shepherd dog. There was no significant difference ($X^2 = 2.68$, $P > 0.10$) between

TABLE II Age distribution of dogs with **B. canis** *parasitaemia and the proportions of these detected by ELISA at initial examination.*

Age	Number with *B. canis* parasitaemia	Number detected by ELISA	False negative	
			Number	As percentage of infected dogs
1.0-2.0 months	5	3	2	40.0
2.1-3.0 months	3	2	1	33.3
3.1-6.0 months	12	4	8	66.7
6.0-11.9 months	7	6	1	14.3
1.0-1.9 years	1	1	—	
2.0-2.9 years	2	2	—	
3.0-3.9 years	—	—	—	
4.0-4.9 years	2	1	1	50.0
5.0-5.9 years	1	1	—	
6.0-7.0 years	1	1	—	
> 7 years	2	2	—	

the proportions of parasitemic dogs that gave false negative results among the dogs less than one year old and those older than one year of age. Also there was no significant difference ($X^2 = 0.02$, $P > 0.50$) between the proportions of seronegative parasitemic indigenous (8/23) and exotic (5/13) dogs ; neither was there any significant difference between the sexes ($X^2 = 0.04$, $P > 0.50$).

Twenty-one of the seropositive parasitemic dogs and 11 of the seronegative parasitemic dogs were ill at initial examination. The proportion of ill dogs in the two groups were not significantly different ($X^2 = 0.0038$, $P > 0.95$).

Two of the seronegative parasitemic dogs, a 3-month old indigenous dog and the 4 1/2-year old German Shepherd dog, seroconverted when re-examined two weeks after the initial examination. The others were still seronegative 85 days after the initial examination. Also 3 non-parasitemic dogs, 2 indigenous dogs aged 1 and 3 months and a 2-month old German Shepherd dog seroconverted two weeks after the initial examination.

Multidog households

The results of a study conducted in 8 households that had more than one dog, and a holding kennel are presented in table III. All the dogs had a history of previous tick infestation. In household 5, there was a strict program of tick control involving the use of acaricides on the dogs and their quarters every fortnight. There were seropositive dogs in all the households except household 5. Dogs in households in which a dog or more had current *B. canis* infection

TABLE III Occurrence of **B. canis** *antibodies in multi-dog households.*

Household	Case No.	Breed	Age (years)	Sex	Antibody titre (reciprocal)	*B. canis* parasitaemia
1	1	Indigenous	6.5	M	640	—
	2	German shepherd	1.5	M	640	—
2	3	Indigenous	0.5	M	640	—
	4	Indigenous	0.5	M	640	—
3	5	Indigenous	1.5	F	320	—
	6	Indigenous	1.5	F	640	—
4	7	German shepherd	2.5	M	160	—
	8	German shepherd	2.5	F	320	—
5	9	German shepherd	4.0	M	40*	—
	10	Chow	1.5	M	40*	—
	11	Indigenous	3.0	F	40*	—
6	12 +	Indigenous	0.2	M	40*	—
	13 +	Indigenous	0.2	M	160	—
7	14	Great Dane	6.5	M	> 5 120	Positive
	15	Indigenous	13.0	M	1 280	—
	16	Indigenous	14.0	M	> 5 120	Positive
8	17	Indigenous	6.0	M	> 5 120	—
	18	Indigenous	2.0	M	1 280	Positive
9	19	Indigenous	0.5	F	1 280	—
(Kennel)	20	Indigenous	1.0	M	> 5 120	—
	21	Indigenous	0.8	F	> 5 120	Positive
	22	Indigenous	1.0	M	> 5 120	—
	23	Indigenous	1.0	M	> 5 120	Positive
	24	Indigenous	1.0	M	> 5 120	—

(+ Same litter), * = Negative.

TABLE IV Antibodies against **B. canis** *in offsprings of seropositive bitches.*

Litter	Serial No.	Breed	Age	Sex	Antibody titre (reciprocal)
1	1 (Dam)	Indigenous	2.5 years	F	160
	2 (Puppy)	Indigenous	7.0 days	F	< 40*
	3 (Puppy)	Indigenous	7.0 days	M	< 40*
	4 (Puppy)	Indigenous	7.0 days	F	160
	5 (Puppy)	Indigenous	7.0 days	F	160
	6 (Puppy)	Indigenous	7.0 days	F	80*
	7 (Puppy)	Indigenous	7.0 days	F	160
2	8 (Dam)	German shepherd	3.0 years	F	320
	9 (Puppy)	German shepherd	9.0 days	F	40*
	10 (Puppy)	German shepherd	9.0 days	F	80*
	11 (Puppy)	German shepherd	9.0 days	F	80*
	12 (Puppy)	German shepherd	9.0 days	M	40*
	13 (Puppy)	German shepherd	9.0 days	M	40*
3	14 (Dam)	Indigenous	2.0 years	F	1 280
	15 (Puppy)	Indigenous	6.0 weeks	M	40*
	16 (Puppy)	Indigenous	6.0 weeks	M	40*
	17 (Puppy)	Indigenous	6.0 weeks	F	80*
	18 (Puppy)	Indigenous	6.0 weeks	M	< 40*
4	19 (Dam)	German shepherd	1.5 years	F	160
	20 (Puppy)	German shepherd	7.0 weeks	M	80*
	21 (Puppy)	German shepherd		F	160

* = Negative (Positive antibody titre threshold = 160).

had high antibody levels even when such dogs did not have a current infection.

Dam-offspring study

Table IV shows the results of a study carried out on the litters of 4 seropositive bitches. These samples were tested twice and each test was run with the positive and negative control sera to detect any technical error. The results of both tests were similar.

Litter 1 was monitored weekly for the first 9 weeks of life and thereafter at intervals of 4 weeks for 16 weeks. One of the seronegative puppies in this litter (No. 3) seroconverted at 3 weeks of age and was seropositive for 6 weeks thereafter. Puppy Nos. 2 and 6 remained seronegative throughout the 6 months of this study. Puppy Nos 4, 5 and 7 became seronegative at 5 weeks of age. Two of them (Nos. 5 and 7) remained seronegative till the end of the study while No. 4 became seropositive again at 17 weeks of age.

Antibodies against *B. canis* in dogs with *Babesia gibsoni* infection

Babesia gibsoni (Patton, 1910) infection was detected in 20 of the dogs examined and antibodies against *B. canis* was detected in 7 (35 per cent) of them (Table V). They were all indigenous dogs aged 2 months to 8 years. The antibody titres ranged from 1:160 in a 2 month old dog to 1:1280 in a 2 year old. The 2-year old dog still had *B. gibsoni* parasitaemia 133 days after the initial examination but antibodies against *B. canis* could not be detected on this occasion (< 1:40).

TABLE V Antibodies against **B. canis** *in dogs with current* **B. gibsoni** *infection.*

Age (months)	Number infected with *B. gibsoni*	No. with antibodies against *B. canis*	G.M.T. (reciprocal)
1.0-3.0	4	1	160
3.1-6.0	8	2	453
6.1-12.0	2	1	320
> 12.0	6	3	640
Total	20	7	431 (± 19.7)

DISCUSSION

This study has confirmed the findings of earlier studies in France (8, 9) that in endemic areas, antibodies against *B. canis* are more prevalent in dogs than are current *B. canis* infections. However, while up to 85 per cent of the dogs sampled in an endemic area of France had antibodies against *B. canis* (9) the percentage was much lower in Ibadan, Nigeria despite the similarity in the prevalence of *B. canis* infections in the two areas. The reasons for this are not understood. It may however be due to climatic factors and the management of the breeds of dogs involved. It is pertinent to draw attention to the fact that more than 70 per cent of the dogs in this study were indigenous Nigerian breeds, while some of the breeds sampled in France were included in the remaining 30 per cent.

This investigation has also confirmed earlier reports (2, 9, 14) that dogs from different parts of the world, as well as both sexes are equally susceptible to *B. canis* infection. In addition, it has shown that both indigenous and exotic breeds of dogs, and both males and females, are equally capable of responding to *B. canis* infection by producing antibodies against the parasites.

The age-related differences in the prevalence of antibodies against *B. canis* is reflected in the higher prevalence of *B. canis* parasitaemia in young dogs (1 to 6 months old) than in older dogs in this study, a situation that has been reported in other studies in Nigeria (2, 5) and elsewhere (14). Dogs without antibodies against *B. canis* have been shown to be more susceptible to the infection than those with antibodies (9).

The reasons why some dogs which were clinically infected with *B. canis* failed to produce antibodies against the parasite are not known. A similar situation was reported in an endemic area in France (9). One reason that can be adduced from this study is that these dogs were probably being infected for the first time and needed some latent period to produce antibodies against the parasite. This is borne out by the facts that most of the seronegative dogs were less than a year old, and that two of the seronegative dogs seroconverted two weeks after the initial examination. In a study with dogs experimentally infected with *B. canis*, specific antibodies against the parasite were not detected until 6 to 14 days post-infection (17). Another reason for the seronegativity of infected dogs could be that some dogs require a booster challenge to produce specific antibodies against the parasite. In such cases, there would be an anamnestic response to the parasite. The explanations proposed are only speculative and need to be substantiated by further studies in view of the complexity of the host-parasite relationship especially in naturally-occurring infections.

The seroconversion observed in 3 non-parasitemic dogs could probably have been due to a subclinical infection, that did not produce patent parasitaemia or

which had been eliminated by the host. It has been recognized that in endemic areas, dogs are able to synthesize antibodies against *B. canis* even in the absence of clinical signs of infection (9).

The results of the limited study of multidog households confirm an earlier report that there is a positive correlation between infection rate, and the level of antibodies against *B. canis* (9). Thus the higher the risk of the disease, the higher the antibody level. In this study, the antibody titres were higher in the dogs within households in which a dog or more had current *B. canis* infection than those in which there were no current infections.

Very little is known about the transfer of antibodies against *B. canis* from dam to offspring. The finding of antibodies in some offsprings of seropositive bitches in this study and the higher prevalence of antibodies against *B. canis* in puppies aged 1 to 2 months than in those aged 2 to 6 months, suggest the possibility of such a transfer. This may probably be through colostrum since not all the puppies in the two litters had antibodies against *B. canis*. In this environment some puppies are unable to feed during the first few hours of life and may not be able to obtain colostrum. The presence of maternally transmitted resistance to *Babesia* infection in puppies has been suggested by the demonstration of resistance to *Babesia* infection in puppies born to a bitch which had recovered from the infection (6).

The antibodies against *B. canis* detected in some dogs with *B. gibsoni* infection were probably due to previous infections with *B. canis* and not cross-reactivity. The fact that the antibody level declined in a 2 year old dog with persistent *B. gibsoni* parasitaemia suggests this. If there had been cross-reactivity the antibody level would have increased. It has been shown that antibody levels would rise in the presence of persistent *B. canis* parasitaemia in dogs older than one year (3).

This study has shown that while ELISA is a useful technique for detecting and measuring levels of antibodies against *B. canis* it has limitations in the diagnosis of current infections. It is therefore suggested that in endemic areas, demonstration of the parasite in the blood and/or clinical observations should be used in diagnosing individual cases while for surveys, these can be combined with serological tests in order to obtain a complete picture of the prevalence of the infection and the groups of animals at risk.

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BOBADE (P. A.), ODUYE (O. O.), AGHOMO (H. O.). Prevalence of antibodies against *Babesia canis* in dogs in an endemic area. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (2) : 211-217.

A survey of 287 dogs for antibodies against *Babesia canis* in dogs in an endemic area, using ELISA, produced a prevalence of 43 per cent. Antibodies occurred in dogs of all age groups, the prevalence being significantly lower in dogs aged 1 to 6 months than in older dogs. There were no differences between indigenous Nigerian dogs and exotic (foreign) dogs ; and between the sexes in the prevalence of antibodies. Antibodies were more prevalent in dogs with *B. canis* parasitaemia and in those with a higher risk of infection. Also antibodies were detected in some puppies born to seropositive bitches. The ELISA test failed to detect antibodies in 36.1 per cent of dogs with *B. canis* parasitaemia. *Key words* : Dog - Babesiosis - *Babesia canis* - Antibody - Serology - ELISA test - Nigeria.

BOBADE (P. A.), ODUYE (O. O.), AGHOMO (H. O.). Prevalencia de los anticuerpos contra *Babesia canis* en perros en una zona endemica. *Revue Élev. Méd. vét. Pays trop.*, 1989, **42** (2) : 211-217.

Una encuesta en 287 perros de una zona endemica, al utilizar la prueba ELISA, demostró una prevalencia de 43 p. 100 de los anticuerpos contra *Babesia canis*. Se observaron anticuerpos en todos los grupos de edad con una prevalencia significativamente más baja en los perros de 1 a 6 meses de edad que en los animales más viejos. No se notó en la prevalencia de los anticuerpos ninguna diferencia entre los perros de Nigeria y los « exóticos » (extranjeros), lo mismo entre los sexos. Los anticuerpos eran más frecuentes en los perros parasitados por *B. canis* y en los teniendo un riesgo elevado de infección. Además, se evidenciaron anticuerpos en cachorros nacidos de perras seropositovas. La prueba ELISA no evidencia anticuerpos en 36,1 p. 100 de perros parasitados por *B. canis*. *Palabras claves* : Perro - Babesiosis - *Babesia canis* - Anticuerpo - Serologia - Prueba ELISA - Nigeria.

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