BABESIA CANIS IN STRAY DOGS OF THE CITY OF SÃO PAULO. COMPARATIVE STUDIES BETWEEN THE CLINICAL AND HEMATOLOGICAL ASPECTS AND THE INDIRECT FLUORESCENT ANTIBODY TEST.

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SUMMARY:Hematologic studies, observation of clinical signs and the Indirect Fluorescent Antibody Test (IFAT) were conducted in 106 stray dogs in the city of São Paulo, Brazil, for the diagnosis of *Babesia canis* infection. Hematologic data comprised blood smears, red blood cell counts, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell counts and leukocyte differential counts. Rectal temperature, color of the conjuntivae and the presence of ticks were also registered. Stained blood smears showed only 10.3% of animals infected. Rectal temperature and color of conjuntivae were highly variable and only five out of 45 positive dogs had ticks (*Ripicephalus sanguineus).* Hematologic data were also variable and unreliable for diagnosis. The IFAT showed 42.4% of dogs infected by *B. canis* and is suggested as the best method for diagnosis.

KEY WORDS: *Babesia canis,* canine babesiosis, blood protozoan, serological test, fluorescent antibody test, diagnosis.

INTRODUCTION

Canine babesiosis has been observed in domestic and wild canids in all five continents. Its causal agent, *Babesia canis*, is a parasite of red blood cells and is transmitted by tick bites. Clinically the disease is characterized by hiperthermy, anorexy, anemia, icterus and splenomegaly (BREITSCHWERDT *et alii*, 1983).

This protozoosis constitutes an animal health problem, mainly in pure breed dogs. ARTHUR (1961) commented that in the USA the average mortality in imported adult dogs from parasite-free areas is about 75% occurring at similar rates in young animals. In São Paulo, Brazil, it has been considered a rural disease, however cases in urban areas have also been reported by veterinarians.

The diagnosis of *B. canis* is based on the symptomatology and confirmed by finding the parasites in blood smears (KLINEFELTER, 1982). However, sub clinical cases are not detected by those methods. A study was carried out to verify the usefulness of clinical and hematological data in the diagnosis of sub clinical canine babesiosis, and the use of a serological test.

MATERIALS AND METHODS

One hundred and six stray dogs, adult males and females, from the Center for Zoonosis Control of the city of São Paulo, Brazil, were examined.

Prior to the blood collection, the rectal temperature was measured, conjunctivae were examined and ticks were collected for identification. Thin blood smears were made with peripheral blood from the marginal ear vein, for parasitological investigations. Blood samples were collected from cephalic or saphenous vein by puncture for hematological and serological tests.

The thin blood smears were stained by the Rosenfeld's method (ROSENFELD, 1947). The entire smears were examined.

Two milliliters of blood were collected for hematology, in vials containing 10% di-potassium EDTA. The parameters studied were: red blood cell counts (RBC); hemoglobin (Hb); packed cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); white blood cell counts (WBC) and leukocyte differential counts. The methodology employed was that described in BIRGEL, 1982.

The indirect fluorescent antibody test (IFAT) was used. Ten milliliters of blood were collected in clean test tubes and let to coagulate. Serum was separated and transferred to sterilized vials, mixed with glycerol P.A at 1:1 proportion and maintained at -20°C until use.

For this test crude antigen obtained from one splenectomized pup was used, after inoculation with positive blood to *B. canis*. When parasitaemia reached 4% the blood was collected and the

antigens slides were prepared. The method's procedure was performed according to DELL'PORTO *et alii* (1990). The testing sera were diluted since 1:20 to 1:2560, while both positive and negative sera used as control were diluted at 1:40.

The rabbit anti-canine globulin labeled with fluorescein isotiocyanate was obtained from the Center for Zoonosis Control of the city of São Paulo, and was used at a dilution of 1:80 in PBS (7.2). For statistical analysis the two proportion test with approximation to normal distribution of probabilities, at the significance level of 5% (GOLDSTEIN, 1965) was employed to detect differences between temperature and hematological values from males and females, positive and negative to the IFAT.

RESULTS AND DISCUSSION

Parasitological and serological findings: The stained blood smears showed that 10.3% of the animals were infected with *B. canis*, while the IFAT revealed 42.4% of seropositivity. These results demonstrated that the IFAT has a high sensibility and high specificity, revealing to be a dependable method for epidemiological surveying.

Rectal temperature: Table I depicted the data of arithmetic means, variance, standard deviation and variability coefficient from rectal temperature of positive and negative animals to the IFAT. No significant differences were observed by statistical analysis.

The results showed large variations in the temperature. Normal temperature values of dogs range from 37.5°C to 39°C (CHRISTOPH, 1977). Thirty-five of the 45 positive dogs to IFAT showed values higher than those. The same was registered by FARWELL *et alii* (1982) and by BREITSCHWERDT *et alii* (1983). This fact was explained either by the presence of *B. canis* in the host or by the simultaneous occurrence of other disease agents (MAHONEY, 1977 and PURNELL, 1981).

Positive dogs which presented temperature within the normal range suggest a steady host-parasite relationship.

Conjunctivae: Only five animals showed discolored conjunctivae and all of them were IFAT positive. Thirteen dogs presented hyperemic conjunctivae, from which three were positive to the IFAT. Eighty-eight animals had pink-colored conjunctivae, of which three were IFAT positive.

Although the discoloration of the mucosa and conjunctivae are considered typical sign in acute or chronic *B. canis* infections, sometimes, ill dogs may show hyperemic or pink-colored conjunctivae (KLINEFELTER, 1982 and ROCKEY & RUSSEL, 1961).

The results of this research permit to suggest that the color of conjunctivae is a sign not always related with babesial infection, mainly in chronic cases, but it could be used as a diagnostic help when infection is suspected.

Tick identification: Out of 45 positive dogs, only five showed a few *Rhipicephalus sanguineus* specimens and of the negatives, seven dogs were infested with this species.

R. sanguineus is frequent on dogs with babesiosis (KALRA & SINGH, 1984). Therefore the results lead to suggest that the small number of specimens observed could be related to the biological behavior of this tick, whose instars fall of the host after each meal.

Table 1 - Experimental dogs according to sex and IFAT data. Mean (X), variance (s), standard deviation (s2) and variability coeficient (VC) results of the rectal temperature from experimental dogs.

Statistical measures	Females IFAT Positive	Females IFAT Negative	Males IFAT Positive	Males IFAT Negative
$\overline{X}$	39.1	29.2	38.7	38.7
s^2	0.31	0.23	0.21	0.35
s	0.55	0.48	0.45	0.59
VC(%)	1.44	1.22	1.16	1.52

Table 2 - "Z" values of the two-proportions test to the hematological data itens, according to sex and the IFAT results.

Hematoligical Data	Comparation between sexes F+ x F-	M+ x M-	F+ x M+	F- x M-
RBC	1.55	1.13	2.54	0.44
Hb	0.38	0.38	0.27	0.08
PCV	1.79	0.12	3.28	0.04
MCV	0.38	1.68	1.70	0.11
MCH	0.85	0.12	0.91	0.09
MCHC	5.05	0.05	0.99	12.46
WBC	0.14	1.37	1.03	0.09
Band neutrophils	1.46	1.11	2.71	2.09
Mature neutrophils	0.46	1.31	0.25	0.41
Eosinophils	0.82	1.36	2.08	1.90
Lynfocytes	0.38	1.45	1.87	1.54
Monocytes	0.29	0.05	0.12	2.18

F+ = Positive females to IFAT M+ = Positive males to IFAT
F - = Negative females to IFAT M - = Negative males to IFAT

Hemogram: Table 2 shows the statistical parameters to the hematologic picture of the experimental dogs, according to sex and results to the IFAT. All the blood picture parameters were compared with those estabilished as normal by MIGLIANO (1955) to dogs of the city of São Paulo.

Large variations were observed on RBC, Hb and PCV values of the IFAT positive dogs. Thirty-six of them showed low RBC, 44 low Hb and 31 low PCV values. According to MAEGRAITH *et alii* (1957) the destruction of erythrocytes was caused by the mechanical action of *B. canis* in the cells. In non-parasitized erythrocytes the destruction is a consequence of the recognition by the immune system, once these cells are marked by parasites antigens adsorbed to their membranes.

The low values observed in negative animals could be due to nutritional deficiences or to the action of other parasites, once stray dogs are normally in contact with several sources of infection.

No significant differences were observed between the RBC, Hb and PCV values from positive and negative males and females.

Table 3 - Arithmetic mean (X), variance (s), standard deviation (s2) and variability coeficient (VC) of the hemogram values from experimental dogs, according to sex and to IFAT results.

SEX	Statistical Parameters	RBC ($x10^6/mm^3$)	HB (g%)	PCV (%)	MCV (m3)	HCV (gg)	MCHC (%)	WBC ($x10^3/mm^3$)	Band Neutroph. (%)	Mature Neutroph. (%)	Eosinophils (%)	Lynfocytes (%)	Monocytes (%)
POSITIVE FEMALES	X	4.15	9.43	35.37	87.60	24.39	27.09	11.25	3.75	64.50	4.37	21.81	4.00
	s^2	0.90	18.26	23.90	157.55	230.68	147.72	33.14	2.92	71.86	15.89	89.02	9.25
	s	0.94	4.27	4.88	12.55	15.18	12.23	5.75	1.71	8.47	3.98	9.43	3.04
	VC(%)	22.60	45.28	13.79	14.32	62.23	45.17	51.11	45.60	13.13	91.07	43.23	96.00
NEGATIVE FEMALES	X	4.94	9.91	40.58	85.72	20.99	10.97	11.01	6.08	60.08	6.33	19.91	4.25
	s^2	2.43	5.36	83.73	163.12	17.70	10.39	10.39	6.73	84.80	32.60	83.67	13.84
	s	1.55	2.31	9.15	12.77	4.20	3.22	3.22	2.59	9.18	5.70	9.14	3.72
	VC(%)	31.37	23.30	22.54	14.89	20.00	29.35	29.24	42.59	15.27	90.04	45.90	87.52
POSITIVE MALES	$\bar{X}$	5.29	9.73	40.55	80.22	19.24	24.03	13.80	10.86	59.00	9.46	15.86	6.44
	s^2	4.19	1.55	28.93	256.06	14.66	3.19	117.35	68.41	173.03	45.54	73.46	7.40
	s	2.04	1.24	5.37	16.00	3.82	1.78	10.83	8.27	13.15	6.74	8.57	2.72
	VC(%)	38.56	12.74	13.24	19.94	19.85	7.40	78.00	76.15	22.28	71.24	54.03	42.23
NEGATIVE MALES	$\bar{X}$	4.73	9.85	40.71	86.18	20.87	24.06	10.91	11.00	53.83	11.04	14.53	6.93
	s^2	1.12	2.26	32.68	178.22	13.48	11.66	17.45	84.38	196.83	62.05	48.25	21.06
	s	1.05	1.50	5.71	13.34	3.67	3.41	4.17	9.18	14.02	7.87	6.94	4.58
	VC(%)	22.19	15.22	14.02	15.47	17.58	14.17	38.22	83.45	26.04	71.28	47.76	66.06

In normal dogs there were also no difference in the erythrogram values concerning to sex (SCHALM *et alii*, 1975).

Among the positive animals, 21 showed higher MCV. This increase could be a consequence of the medular hyperactivity to compensate blood loss. Macrocytosis was observed when babesias were found in the circulating blood (HAGIWARA, 1983), and high erythrocyte production is commonly seen when blood sucking parasites are present and when there are nutritional deficiences. These last observations could explain the higher levels of MCV that occurred in negative dogs.

Among the positive animals, 14 presented normal values for MCV. These data were similar to those found by HAGIWARA (1983).

Relatively to the MCH values it was observed that 31 positive and 48 negative dogs to the IFAT, presented low values. The MCHC data from 44 positive and 59 negative animals showed a decrease as compared to the normal values.Those findings reveal hypochromic anemia, commonly seen in canine babesiosis (YONAMINE *et alii*, 1984).

Leukocyte counts were variable among the 45 positive dogs to the IFAT, from which 13 showed leukocytosis and 22 leukopenia. Statistical analysis showed no significative difference between leukocyte numbers in both groups, positive and negative to the IFAT. Despite this, it is not possible to affirm whether there is any difference in the number of these cells in infected or non-infected dog populations. The mean values of the blood picture of positive and negative animals to the IFAT,were within the normal range (Table 2). However considering positive males and females, it was observed a significant difference associated to band neutrophils and eosinophils, where females showed lower values. The same was ascertained in negative females, which presented in addition a significant decrease on monocyte values. These results suggest that differences could be related to the higher degree of excitement observed in males upon restraining.

The present results lead to conclude that the data on hematology and clinical signs were very variable and therefore proved to be unreliable for clinical diagnosis, mainly in chronic or subclinical babesiosis. The serological test employed, the IFAT, seems to be the best method for routine diagnosis.

SUMÁRIO

Estudos hematológicos, exames clínicos e o Teste de Imunofluorescência Indireta (TIFI) foram conduzidos em 106 cães de rua, na cidade de São Paulo, Brasil, para o diagnóstico de infecção por *Babesia canis*. Os dados hematológicos abrangeram esfregaços sanguíneos, contagens de glóbulos vermelhos, determinação da percentagem de hemoglobina, hematócrito, volume corpuscular médio, concentração média de hemoglobina corpuscular, contagens de glóbulos brancos e contagens diferenciais de leucócitos. A temperatura retal, coloração da conjuntiva e presença de carrapatos foram também registradas. Os esfregaços de sangue mostraram somente 10,3% de animais infectados. Tanto a temperatura retal, como a cor da conjuntiva foram muito variáveis e apenas cinco, dentre 45 cães positivos à sorologia, tinham carrapatos (*Ripicephalus sanguineus*). Os dados hematológicos também variaram muito, não sendo confiáveis. O TIFI revelou 42,4% de cães infectados por *B. canis* sendo sugerido como o melhor método diagnóstico.

PALAVRAS-CHAVE: *Babesia canis*, babesiose canina, hematozoário, teste de imunofluorescência, diagnóstico.

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