ANTI-*EHRLICHIA CANIS* ANTIBODIES DETECTION BY "DOT-ELISA" IN NATURALLY INFECTED DOGS

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SUMMARY: Humoral immune response in naturally infected dogs by *Ehrlichia canis* was evaluated through Dot-ELISA. Main clinical signs and symptoms observed in the dogs (n=52) consisted of pale mucous membranes, inappetence, apathy, vomit, fever, lymphadenopathy and melena. Hemogram analysis evidenced reduction in red blood cells and hemoglobin in 78.85% and 76.92% of the dogs, respectively. Thrombocytes were reduced in 69.23% of the animals. Total white blood cells were bellow normal limits for the species in 32.69% of dogs. Monocytes, lymphocytes and eosinophils were reduced in 80.77%, 42.31% and 55.77% of the animals, respectively. Antibody levels of diagnostic value were detected in 92.31% of dogs (n=48) and only 7.69% (n=4) were negative. DOT-ELISA was efficient in detecting anti-*E. canis* antibodies in sera from naturally infected dogs presenting symptoms.

KEY WORDS: *Ehrlichia canis*, diagnosis, DOT-ELISA

INTRODUCTION

Canine ehrlichiosis was first described in 1935 by DONATIEN & LESTOQUARD in Algeria. In Brazil it was first diagnosed in Belo Horizonte, Minas Gerais by COSTA *et alii*, in 1973. The causative agent occurs worldwide in tropical and subtropical areas (BROUQUI *et alii*, 1991). Epidemiological data about the disease have not been determinated in Brazil; part of the difficult may lie on lack of conclusive diagnostic methods.

Ehrlichia canis is an obligatory intracytoplasmatic parasite (GREENE & HARVEY, 1984; PETERSEN *et alii*, 1989) occurring essentially in domestic dogs (EWING, 1969; BUHLES *et alii*, 1974). It is transmitted by the brown tick *Ripicephalus sanguineus* (GREENE & HARVEY, 1984; PETERSEN *et alii*, 1989) which inoculate contamined saliva into susceptible animals (EWING, 1969; GROOVES *et alii*, 1975). The biological vector infects itself ingesting blood with the rickettsia, which multiplies on salivary glands, mesogastrium and haemocytes (SMITH *et alii*, 1975).

The rickettsia *E. canis* presents a zoonotic potential and several clinical cases in children and adults have been reported (MAEDA *et alii*, 1987; MAGNARELLI, 1990).

Clinical signs observed in acute phase of the disease include fever, anorexia, vomiting, weight loss, hepatosplenomegaly, enlarged lymph nodes and, rarely, epistaxis and hemorrhage. The chronic phase is marked by epistaxis, haematuria, petechiae, equimoses distributed over skin surface and ocular alteration (TROY & FORRESTER, 1990).

The diagnosis of ehrlichiosis is generally based on history, clinical signs and hematological data (GREENE & HARVEY, 1984). Platelet counting is also a good presumptive diagnostic in sub clinical chronic disease (DAVOUST *et alii*, 1991).

The direct detection of *E. canis* morulae in Giemsa's stained blood smears is difficulty, and therefore, it rarely provides a precise diagnosis; the absence of the parasite cannot exclude a positive diagnosis of ehrlichiosis (EWING, 1969; HOSKINS, 1991; CAMPBELL, 1994). Serological detection of anti-*E. canis* antibodies can be done through indirect immunofluorescence antibody assay (IFA), accordingly to WEISER *et alii* (1991), or using DOT-ELISA (CASTRO, 1997). Recently, direct detection of *E. canis* in tissues and blood can be performed using the polymerase chain reaction (PCR) (TROY & FORRESTER, 1990).

The objective of this work was to study the humoral immune response in sera of naturally infected dogs presented at the Veterinary Hospital " Governador Laudo Natel" at FCAV – UNESP, Jaboticabal, SP, Brazil, using "DOT-ELISA".

MATERIALS AND METHODS

Experimental animals

Fifty and two cases of dogs suspected of naturally acquired ehrlichiosis, were selected at the Veterinary Hospital "Governador Laudo Natel" at FCAV-UNESP, Jaboticabal, SP, Brazil, from January 1998 to January 1999. Data referring to history, physical and laboratorial exams were obtained from their individual promptuary.

Serology

Sera samples from the selected dogs were obtained at the Clinical Pathology Laboratory and employed for detection of specific anti-*Ehrlichia canis* IgG antibodies using a commercial Kit Immunocomb™ (Biogal), based on "DOT-BLOT ELISA".

RESULTS

Naturally infected animals

Clinical signs

Clinical signs in naturally infected dogs are shown in Table 1. Clinical alterations observed more frequently were paleness of mucous membranes, loss of appetite, apathy, vomiting, fever, lymphadenopathy and melena. Dogs age varied from 3 months old to 13 years old, with mean age of 4 years old.

Hemogram and platelets counting

Most dog (78.85%) presented erythrocytes counts bellow the normal pattern for the species (5.500 to 8.500 x 10^6/µl) with a mean value of 3281,95 ± 1350,47 x 10^6/µl (Table 2). Hemoglobin content was also reduced below to the normal limit (12 to 18 g/dl) in 76.92% of the animals. Total leucocytes count was below normal values (6,000 to 18,000 x 10^3/µl) in 32,69% of the dogs (Table 2); the mean value found for the studied group was 3664,70 ± 1618,26x 10^3/µl. Mean value for eosinophils count was 12,27 + 28,67x 10^3/µl, and 55,77% of the animals showed eosinophils count below normal range (120 to 1800 x 10^3/µl). Monocytes numbers were bellow normal values (180 to 1800 x10^3/µl) in 80.77% of the animals with mean value of 26,83± 43,68 x 10^3/µl. Lymphocytes values were below normal patterns (1000 to 5400 x 10^3/µl) in 42,31% of the dogs (mean values 535,73 ± 252,88 x 10^3/µl for the group).

Table 1 - Clinical alteration observed in *E. canis* naturally infected dogs (n=52), presented at the Veterinary Hospital "Governador Laudo Natel" at FCAV - UNESP, Jaboticabal, SP, Brazil, during January 1998 to January 1999.

CLINICAL ALTERATION	ANIMALS NUMBER	%
Pale mucous membranes	26	44,07
Loss of appetite	25	42,37
Apathy	18	30,51
Vomiting	17	28,81
Fever	17	28,81
Lymphadenopathy	17	28,81
Melena	15	25,42
Splenomegaly	11	18,64
Epistaxis	10	16,95
Ocular secretion	8	13,56
Emaciation	7	11,86
Renal sensitivity	5	8,47

Platelets count decreased in naturally infected dogs presenting a mean value of 78921.87 ± 49277.29 platelets/mm³, in 69.23% of them it was bellow normal values (200.000 platelets/mm³) for canine specie.

Serology

The results obtained for anti-*E. canis* antibodies titers detected by the Immunocomb™ Kit (Biogal) are shown in Table 2.

A high prevalence of sera positive animals (92.3%) was observed; only four animals were negative (7.7%). Six animals (11.54%) presented titers of 1:1280; 19 animals (36.53%) had 1:60-1:640 titers; 23 animals (44.23%) presented titers bellow 1:160 (13.46% with titers of 1:80; 13.46% with titers between 1:20 and 1:80 and 17.31% with titers of 1:20).

DISCUSSION

The canine ehrlichiosis is getting notability as an important parasitic infectious disease among dogs from northeast region of São Paulo state. The tropical climate of the region favors the development of vector ticks and the transmissions of the hemoparasites. It is not rare to observe dogs with high ticks infestation; though the majority of owners are not aware about the injuries caused by spoliation and transmission of diseases such as ehrlichiosis.

Some of the clinical signs observed in this study as pale of mucous membranes, lymphadenopathy, loss of appetite and weight loss are in agreement with those earlier reported for dogs experimentally infected with *E. canis* (TROY & FORRESTER, 1990; CASTRO, 1997).

It was difficult to determine precisely the onset of febrile period and clinical signs in studied dogs, since the owners were not able to observe the symptoms with correlation to the presence of the vectors ticks, *Rhipicephalus sanguineus*.

Increased renal sensibility was observed, suggesting glomerulonephritis. Glomerulonephritis has been associated to canine ehrlichiosis and can lead to hypoproteinemia, accordingly to CODNER & MASLIN (1992).

All naturally infected animals presented normocytic normochromic anemia, as previously observed by WALKER *et alii* (1970).

Thrombocytopenia was frequently observed among dogs naturally infected by *E. canis*. Mechanisms proposed to explain decreasing platelets number include bone marrow aplasia and/or suppression of erythropoiesis, leucopoiesis and

Table 2 - Hemogram related to platelets, serological titers and parasitaemia of naturally infected dogs by *E. canis* presented at the Veterinary Hospital "Governador Laudo Natel" at FCAV – UNESP, Jaboticabal, SP, Brasil, during January 1998 to 1999.

Animals	Erytrocytes x 10^6/ml	Hb g/dl	Leucocytes x 10^3/ml	Basophils x 10^3/ml	Eosinophils x 10^3/ml	Neutrophils		Limphocyte x 10^3/ml	Monocytes x 10^3/ml	Platelets /mm^3	DOT-ELISA	
						Segment x 10^3/ml	Band x 10^3/ml				Serological Titers	Scale[1]
1	5100	11,1	7000	00	140	4200	210	2240	210	R	1:160*	3
2	5900	13,9	27400	00	1096	22468	822	3014	00	N	1:20	1
3	1650	41,2	3100	00	00	2790	62	279	00	30000	1:160	3
4	1850	4,0	7300	00	219	5621	438	1022	00	20000	1:160	3
5	6190	13,9	13400	00	804	11926	00	536	134	60000	1:20	1
6	2960	6,8	3300	00	00	2970	132	165	33	4500	1:320	4
7	1860	7,1	5900	59	118	5251	59	354	59	40000	1:320	4
8	3550	9,6	5900	00	00	5192	177	531	00	50000	1:20	1
9	2900	6,8	7900	00	00	7189	00	711	00	70000	1:640	5
10	2320	4,9	15200	00	00	11400	1520	2280	00	80000	1:20	1
11	7300	15,3	5700	00	00	5415	114	114	57	80000	1:320	4
12	2460	5,8	4100	00	41	3526	82	369	82	20000	1:160	3
13	4700	10,8	5800	00	00	4640	00	1044	116	-	1:20	1
14	4110	10,6	6200	00	124	5146	124	806	00	160000	1:20	1
15	6190	14,2	11800	00	1770	6490	00	3422	118	-	1:20-1:80	1
16	2570	6,8	24300	00	00	21384	00	2916	00	120000	1:80	2
17	5000	9,3	12300	00	00	10578	369	984	369	100000	1:80	2
18	4950	10,5	11800	236	472	6726	00	4366	00	130000	1:80	2
19	5200	10,8	9500	00	00	7885	190	1425	00	130000	1:20	1
20	6750	15,3	18200	00	00	15834	182	2002	182	N	1:20-1:80	1
21	4200	8,2	9800	00	392	6664	98	2646	00	-	1:20	1
22	5700	12,2	6500	00	325	5590	130	390	65	N	1:80	2
23	800	2,8	2200	00	00	1826	22	352	00	-	1:20-1:80	1
24	4650	11,2	13500	00	00	7830	135	5400	135	130000	1:20-1:80	1
25	3300	3,6	13300	00	1862	8113	00	3857	00	R	1:640	5
26	4400	10,1	33000	00	00	29040	1980	1980	00	-	negative	-
27	1140	4,7	2000	00	00	1340	240	420	00	R	1:320	4
28	2200	5,9	18200	00	00	15834	182	1820	364	100000	1:320	4
29	6000	11,9	12900	00	129	11223	258	1032	258	150000	1:640	5
30	4750	10,5	6700	00	201	5360	335	804	00	-	1: 640	5
31	4100	8,3	18900	00	567	17010	189	1134	00	-	negative	-
32	6000	12,4	29200	00	292	14600	4672	9052	584	-	1:640	5
33	2320	4,9	15200	00	00	11400	1520	2280	00	80000	1:80	2
34	5800	12,1	17800	00	00	13884	00	3382	534	-	1:640	5
35	3540	7,2	12000	00	720	8520	240	2280	240	160000	1:80	2
36	4640	6,9	3000	00	90	2130	240	510	30	30000	1:1280**	6
37	3500	10,0	3000	00	00	570	00	2430	00	45000	1:1280	6
38	1900	5,3	21600	00	00	16416	4536	648	00	70000	1:160	3
39	1380	4,8	4200	00	126	2562	00	1512	00	80000	1:1280	6
40	1250	4,4	2400	00	24	1200	72	1056	48	15000	1: 640	5
41	2000	6,9	30000	00	900	21300	2400	5100	300	90000	1:1280	6
42	5050	11,4	5700	00	57	4503	114	912	114	45000	1:20-1:80	1
43	1480	4,5	2600	00	26	910	156	1404	104	-	1:1280	6
44	5410	10,8	13300	00	399	12103	133	665	00	15000	1:80	2
45	3860	5,6	17800	00	00	15842	534	1424	00	190000	1:1280	6
46	4600	-	13690	00	137	12184	684	684	00	40000	1: 640	5
47	1560	4,3	200	00	00	90	2	88	00	R	1: 640	5
48	3200	8,0	3200	00	00	2240	672	576	32	51000	1:20-1:80	1
49	8500	19,0	11100	00	555	9546	111	888	00	N	1:20	1
50	4300	9,6	16200	00	162	12150	648	3240	00	N	1:20-1:80	1
51	7300	16,7	13600	00	544	10472	680	1904	00	N	negative	-
52	3850	7,8	22300	00	446	20070	223	1338	223	140000	negative**	-

[1]Scale used for the colorimetric lecture and obtainment of the correspondent antibodies titers
N=normal number of platelets (200 to 400 x 10^3/mm^3) - = not valued
R=decreased number of platelets (not quantified)
* positive parasitaemia to *E. canis* ** positive parasitaemia to *Babesia canis*

thrombopoiesis, as well as destruction of platelets by immunomediate mechanisms (HIBBLER *et alii*, 1986; WEISER *et alii*, 1991). Leukopenia, eosinopenia and neutrophilia detected in the studied dogs were compatible to experimental results described by CASTRO (1997) during acute phase of the disease; In a retrospective study of 27 cases of natural ehrlichiosis, WADDLE & LITTMAN (1987) found neutropenia, lymphopenia, eosinopenia and monocytopenia in 22%, 48%, 63% and 51% of experimental animals, respectively.

Immunocomb® Kit showed to be sensitive in detecting specific *E. canis* IgG antibodies. Positive titers were observed in 92.32%, of the studied dogs; just four dogs were negative.

CADMAN *et alii* (1994) demonstrated the superiority of DOT ELISA when compared to IFAT, when considering the material used, easiness in lecture that can be performed by not trained people and possibility to keep the results as permanent files.

Direct detection of the intracytoplasmatic parasite morulae in blood smears was possible in only one of the 48 sera positive dogs, what denotes the difficulty in finding *E. canis* in infected animals, a fact also noticed by MATTHEWMAN *et alii* (1993).

Two dogs (3.85%) presented *Babesia spp* in blood smear. One of them also presented high antibody titer for *E. canis* by DOT BLOT ELISA, while the other animal was negative. These results were also reported by MATTHEWMAN *et alii* (1993).

It can be concluded that the Immunocomb® Kit can be used as one important tool, in association to clinical and laboratorial findings to provide a more precise and safe diagnosis of canine ehrlichiosis.

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SUMÁRIO

A resposta imune humoral de cães naturalmente infectados pela *Ehrlichia canis* foi avaliada através do Dot-Elisa. Dentre os sinais e sintomas apresentados pelos cães destacavam-se palidez das mucosas, inapetência, apatia, vômitos, febre, linfadenopatia e melena. As avaliações eritroleucocitárias, dos cães (n=52), evidenciavam redução nas contagens de eritrócitos e hemoglobina em 78,85% e 76,92%, respectivamente. Os trombócitos estavam reduzidos em 69,23% dos animais. Leucócitos totais estavam abaixo dos limites normais da espécie em 32,69% dos cães. Monócitos, linfócitos e eosinófilos mostraram-se reduzidos em 80,77%; 42,31%; 55,77% dos animais, respectivamente. Títulos de anticorpos de valor diagnóstico foram detectados em 92,31% dos cães (n=48) e apenas 7,69% (n=4) não demonstraram reatividade. O Dot-Elisa mostrou-se eficiente para detecção de anticorpos anti-*Ehrlichia canis* em soros de cães naturalmente infectados e com sintomatologia compatível.

PALAVRAS-CHAVE: *Ehrlichia canis*, diagnóstico, DOT-ELISA.

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