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DECREASED ERYTHROCYTE OSMOTIC FRAGILITY DURING CANINE LEPTOSPIROSIS

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SUMMARY

Erythrocyte osmotic fragility (EOF) was carried out in nineteen dogs naturally infected by *Leptospira interrogans* serovar *icterohaemorrhagiae/copenhagi*. A decreased EOF was observed, suggesting a modification of erythrocyte components secondary to disturbances that occur during canine leptospirosis, such as renal damage and hepatic disease.

KEYWORDS: Dog; Osmotic resistance; Red blood cell.

INTRODUCTION

Leptospirosis is the most widespread contemporary zoonosis; it affects human beings and a variety of domestic and wild mammals. Although the majority of canine infections is not clinically apparent, acute systemic disease can occur in infections caused by *Leptospira interrogans* belonging to serovars *canicola* or *icterohaemorrhagiae.*

There are several reports in literature showing that hemolysis and anemia may be found as a consequence of leptospiral infection, e.g., serovar *pomona* in cows and sheep [19], and serovar *icterohaemorragiae* in dogs [10]. However, in Brazil, it was observed that dogs naturally infected by serovars *icterohaemorragiae* or *canicola* did not present signs of anemia and hemolysis [12].

Erythrocyte osmotic fragility (EOF) is a test introduced in the beginning of this century and further standardized [14]; it measures the capacity of erythrocytes to resist hemoglobin leakage in solutions of decreasing NaCl concentration. In fact, it is a good estimate of the erythrocyte deformability in blood stream, and is a valuable tool to detect increased erythrocyte fragility associated with hemolysis.

We observed that dogs with clinical and laboratorial diagnosis of leptospirosis showed decreased EOF. The results are reported herein.

MATERIAL AND METHODS

Nineteen dogs with clinical, laboratorial and serologic diagnosis of leptospirosis (for serovar *icterohaemorrhagiae/copenhagi*), admitted at the College of Veterinary Medicine from University of São Paulo, were analyzed. These dogs ranged between 2 months and 6 years old. Thirteen clinically normal dogs served as controls (4 months up to 8 years old).

For erythron and EOF evaluation blood was collected in Na_2 EDTA. Red blood cell counts were performed on a Coulter Counter model DN-VET;

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microhematocrit and hemoglobin concentration determinations were performed by standard techniques.

Erythrocyte osmotic fragility was performed as described elsewhere [10]. Briefly, 20 µl of blood was aspirated in Sahli pipet and added into each of 16 tubes containing 5.0 ml of buffered saline solutions, with NaCl concentration ranging from 8.5 to 0.0 g/l. The tubes were homogenized and allowed to stand undisturbed for 30 minutes at room temperature; they were then centrifuged at 800 *g* for 10 minutes. The supernatant was removed, and its optical density read at 540 nm in a Coleman Junior II spectrophotometer.

NaCl concentration was plotted against percent hemolysis, and cumulative and derivative curves [18] were obtained for each animal. NaCl concentrations at 5 percent ($H_{5\%}$), 50 percent ($H_{50\%}$ or mean corpuscular fragility), and 90 percent ($H_{90\%}$) hemolysis were read directly on cumulative graph, and used as parameters to compare values between control and *L. interrogans*-infected dogs.

Serum was obtained from dogs with leptospirosis by centrifuging blood immediately after collection at 1500 *g* during 10 minutes. Alanine amino transferase (ALT), alkaline phosphatase (AP), blood urea (BUN), creatinine (CRT), and bilirrubin levels were determined by standard techniques described elsewhere. Antibody titers against *Leptospira* serovars were also verified in these samples, utilizing a microscopic serum agglutination technique [16]. Urinalysis was performed by routine techniques.

The difference between control and leptospirosis groups was analyzed by means of non-paired Student's t test.

RESULTS

All *L. interrogans*-infected dogs had serum antibody titer against serovar *icterohaemorrhagiae* or *copenhagi*, as well as signs of renal and hepatic damage. Small to medium amounts of protein (30 up to 100 mg/dl), granular casts, and bilirrubinuria were observed in urine from affected dogs. Serum biochemistry (Table 1) revealed increased levels of total bilirrubin, direct bilirrubin, AP, ALT, BUN and CRT.

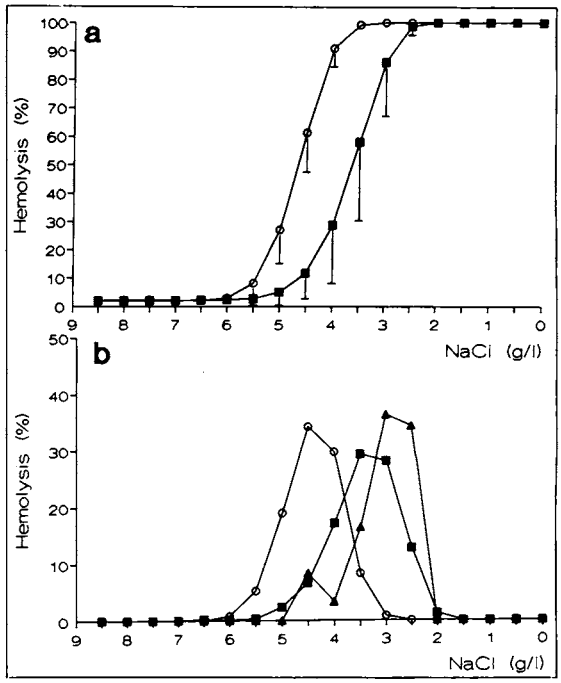

Fig. 1. **a**- Cumulative curves of EOF from control (n=13) (○) and *L. interrogans*-infected (serovar *icterhaemorrhagiae/copenhagi*) (n=19) (■) dogs. Each bar represents 1 s.d. **b**- Derivative curves of EOF from control (n=13) (○) and *L. interrogans*-infected (n=19) (■) dogs. An illustrative example (▲) is also depicted of a curve from one of the 6 dogs with leptospirosis that presented two erythrocyte populations.

Table 1

Biochemical data from dogs naturally infected by *L. interrogans* (serovar *icterohaemorrhagiae/copenhagi*)

Group	RBC[1] (. 10^{12}/l)	PCV[2]	Hb[3] (g/l)	MCV[4] (fl)	MCH[5] (pg)	MCHC[6] (g/l)
Leptospirosis Group						
Mean ± s. e. m.	6.45 ± 1.66	0.42 ± 0.11	141 ± 40	66.0 ± 5.0	21.9 ± 1.9	332 ± 25
Range	2.8 - 8.7	0.20 - 0.59	65 - 212	57.5 - 75.0	18.1 - 24.6	303 - 400
n	18	18	18	18	18	18
Normal Range*	6.0 - 8.0	0.42 - 0.55	140 - 180	67.4 - 81.0	22.2 - 27.1	290 - 364

[1]Red blood cells; [2]Packed cell volume; [3]Hemoglobin concentration; [4]Mean cell volume; [5]Mean cell hemoglobin; [6]Mean cell hemoglobin concentration; *Reference 03.

As shown in figure 1a, EOF showed a marked decrease in dogs with leptospirosis. Table 2 depicts data concerning EOF values.

Table 2

Mean erythrocyte osmotic fragility values from control and *L. interrogans*-infected (serovar *icterohaemorrhagiae/copenhagi*) dogs.

	NaCl (g/l)		
	$H_{5\%}$	$H_{50\%}$	$H_{90\%}$
Control	5.71	4.67	4.00
	±0.08	±0.06	±0.05
	(13)*	(13)	(13)
Leptospirosis	4.85	3.62	3.04
	±0.15	±0.09	±0.08
	(19)	(19)	(19)
p	<0.001	<0.001	<0.001

Date are expressed as mean ± s. e. m. *Number of observations.

Six out of 19 dogs with leptospirosis presented two erythrocyte populations in derivative graph (fig. 1b). The largest population comprised more resistant erythrocytes, and the smallest, erythrocytes with normal fragility. In no instance was observed an erythrocyte population of increased EOF.

Regarding the erythron data from *L. interrogans*-infected dogs, all average values were within normal range for Brazilian dogs (Table 3). Moreover, no signs of hemolytic anemia - such as increased polychromatic staining or poikilocytosis - was observed in blood smears. Only 2 dogs (2 and 6 months old) among those with leptospirosis presented normocytic normochromic anemia, probably attributed to an overall poor nutritional condition. The pattern of EOF curves from these two dogs was not different from other infected dogs.

DISCUSSION

Our data show that dogs infected by serovar *icterohaemorrhagiae/copenhagi* presented no signs of hemolytic anemia, in accordance with a previous paper [12].

The phospholipasic activity of hemolysins, produced by certain serovars, could account hypothetically for increasing EOF in leptospirosis [2, 11, 20]. In fact, two factors seem responsible for triggering hemolysis during leptospirosis: hemolytic activity of different serovars [1], and sensitivity of host erythrocytes to hemolysins [2, 11]. It was observed that canine erythrocytes manifest a reduced EOF during leptospirosis, suggesting that erythrocyte membranes are more resistant to hemolysis, despite reports of hemolytic anemia in this specie [10]. Our data provide evidence that during canine leptospirosis, hemolysis due to an increased EOF is not likely to occur.

In canine leptospirosis there are at least two important events causing EOF modification: first, obstructive jaundice is a common finding during leptospirosis. COOPER & JANDL reported that human erythrocytes demonstrated a decreased EOF during obstructive hepatopathies, due to an increase of surface/volume ratio of erythrocytes [5]. This increase in the surface/volume ratio has been ascribed to the intercalation of cholesterol in erythrocyte cytoplasmic membrane (causing leptocyte and target cell development) and to a decrease of serum LCAT (lecithin cholesterol acyltransferase) activity [5, 13, 15, 22]. LCAT esterifies free cholesterol in plasma, i.e., decreases the amount of free cholesterol within erythrocyte cytoplasmic membrane and its activity is decreased during hepatic disorders [13]. However, serum LCAT activity was not monitored in *L. interrogans*-infected dogs.

Table 3

Hematological data from dogs naturally infected by *L. interrogans* (serovar *icterohaemorrhagiae/copenhagi*)

Group	BUN[1] (mg/dl)	CRT[2] (mg/dl)	ALT[3] (U/l)	ALP[4] (U/l)	Bilirrubin Total (mg/dl)	Bilirrubin Direct (mg/dl)
Leptospirosis group						
Mean ± s. e. m.	401.0 ± 128.9	8.7 ± 3.8	51.1 ± 86.2	222.2 ± 214.9	29.9 ± 14.6	24.7 ± 10.6
Range	164.4 - 683.6	3.8 - 16.7	0.0 - 340.0	57.9 - 964.9	8.7 - 49.6	8.5 - 37.2
n	19	16	14	17	11	11
Normal Range*	10.0 - 30.0	1.0 - 2.0	4.8 - 24.0	29.0 - 137.0	0.0 - 0.30	0.0 - 0.14

[1]Blood urea nitrogen; [2]Creatinine; [3]Alanine amino transferase; [4]Alkaline phosphatase; *Reference 03.

Second, canine erythrocytes are unique compared with other mammalian erythrocytes: they show an increased fragility at alkaline pH (*in vitro* and *in vivo*), and intracellular Na^+ and K^+ concentrations are similar to plasma [9, 21]. During canine leptospirosis, metabolic acidosis develops due to renal failure [7]. Furthermore, canine erythrocytes show a reduced EOF in renal failure (ML Santoro, unpublished data). Thus, one possible reason for the decreased EOF during canine leptospirosis might be explained by the acid pH that erythrocytes face in circulation. On the other hand, human erythrocytes show an increased EOF during renal disorders; it has been attributed to the decrease of cholesterol concentration in cytoplasmatic membranes of erythrocytes, or to an increased intracellular Ca^{2+} concentration [4, 6, 8, 9, 17].

In conclusion, canine erythrocytes showed a decreased osmotic fragility that might be ascribed to the hepatic and renal damage, which occurs during infection by *L. interrogans* serovar *icterohaemorrhagiae/copenhagi*. Further studies on EOF in hepatic or renal disease, without any other organ involvement, will provide information about the mechanisms that lead to the decreased EOF in dogs with leptospirosis. Moreover, the erythrocyte membrane lipid alteration could account in part for the low activity of hemolysins on erythrocytes; this study may provide more details for the comprehension of the etiopathogeny of hemolysis in human or animal leptospirosis.

RESUMO

Diminuição da fragilidade osmótica eritrocitária na leptospirose canina

A fragilidade osmótica eritrocitária foi estudada em dezenove cães infectados naturalmente pela *Leptospira interrogans* serovar *icterohaemorrhagiae/copenhagi*. Observou-se uma redução da fragilidade osmótica eritrocitária, sem a presença de anemia, possivelmente relacionada aos distúrbios hepato-renais que ocorrem nesta patologia.

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