DECREASED ERYTHROCYTE OSMOTIC FRAGILITY DURING CANINE LEPTOSPIROSI

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SUMMARY

Erythrocyte osmotic fragility (EOF) was carried out in nineteen dogs naturally infected by
Leptospira interrogans serovar icterohaemorrhagiae/copenhagen. 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 sys-
temic 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 icterohaemorrhagiae in dogs 10.
However, in Brazil, it was observed that dogs naturally
infected by serovars icterohaemorrhagiae or canicola did
not present signs of anemia and hemolysis 12.

Erythrocyte osmotic fragility (EOF) is a test intro-
duced in the beginning of this century and further stan-
dardized 41; 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 valu-
able tool to detect increased erythrocyte fragility asso-
ciated with hemolysis.

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

MATERIAL AND METHODS

Nineteen dogs with clinical, laboratorial and sero-
logic diagnosis of leptospirosis (for serovar icterohaem-
orrhagiae/copenhagen), admitted at the College of Vet-
erinary 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 erythrocyte and EOF evaluation blood was col-
lected in Na2 EDTA. Red blood cell counts were per-
formed 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. 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 were obtained for each animal. NaCl concentrations at 5 percent (H₅₀), 50 percent (H₅₀₀₀₀ or mean corpuscular fragility), and 90 percent (H₄₀₀₀₀₀) 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 bilirubin 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. 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 copenhageni, as well as signs of renal and hepatic damage. Small to medium amounts of protein (30 up to 100 mg/dl), granular casts, and bilirubinuria were observed in urine from affected dogs. Serum biochemistry (Table 1) revealed increased levels of total bilirubin, direct bilirubin, AP, ALT, BUN and CRT.

![Graph showing cumulative curves of EOF from control and L. interrogans-infected dogs.]

Fig. 1. a- Cumulative curves of EOF from control (n=13) and L. interrogans-infected (serovar icterohaemorrhagiae/copenhageni) (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

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC* (10⁶/µl)</th>
<th>PCV*</th>
<th>Hb* (g/l)</th>
<th>MCV* (fl)</th>
<th>MCH* (pg)</th>
<th>MCHC* (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis Group</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± s.e. m.</td>
<td>6.45 ± 1.66</td>
<td>0.42 ± 0.11</td>
<td>141 ± 40</td>
<td>66.0 ± 5.0</td>
<td>21.9 ± 1.9</td>
<td>332 ± 25</td>
</tr>
<tr>
<td>Range</td>
<td>2.8 - 8.7</td>
<td>0.20 - 0.59</td>
<td>65 - 212</td>
<td>57.5 - 75.0</td>
<td>18.1 - 24.6</td>
<td>303 - 400</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Normal Range</td>
<td>6.0 - 8.0</td>
<td>0.42 - 0.55</td>
<td>140 - 180</td>
<td>67.4 - 81.0</td>
<td>22.2 - 27.1</td>
<td>290 - 364</td>
</tr>
</tbody>
</table>

*Red blood cells; **Packed cell volume; ***Hemoglobin concentration; ****Mean cell volume; *****Mean cell hemoglobin; ******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.

**DISCUSSION**

Our data show that dogs infected by serovar *icterohaemorrhagiae/copenhaeni* 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 5,11,12. 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 species 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 3. This increase in the surface/volume ratio has been ascribed to the intercalation of cholesterol in erythrocyte cytoplasmic membrane (causing leucocyte and target cell development) and to a decrease of serum LCAT (lecithin cholesterol acyltransferase) activity 5,11,15,12. 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 2**

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

<table>
<thead>
<tr>
<th></th>
<th>NaCl (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H∞</td>
</tr>
<tr>
<td>Control</td>
<td>5.71</td>
</tr>
<tr>
<td></td>
<td>±0.08</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>±0.15</td>
</tr>
<tr>
<td></td>
<td>(19)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data 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 polychromatric 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.

**Table 3**

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

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>CRT (mg/dl)</th>
<th>ALT (U/l)</th>
<th>ALP (U/l)</th>
<th>Total Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptospirosis</td>
<td>Mean ± s. e. m.</td>
<td>491.0 ± 128.9</td>
<td>8.7 ± 3.8</td>
<td>51.1 ± 86.2</td>
<td>222.2 ± 214.9</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>164.1 - 683.6</td>
<td>3.8 - 16.7</td>
<td>0.0 - 340.0</td>
<td>57.9 - 964.9</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>19</td>
<td>16</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Normal Range*</td>
<td>10.0 - 30.0</td>
<td>1.0 - 2.0</td>
<td>4.8 - 24.0</td>
<td>29.0 - 137.0</td>
<td>0.0 - 0.30</td>
</tr>
</tbody>
</table>

*Blood urea nitrogen; CRT: Creatinine; ALT: Alanine amino transferease; ALP: 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. During canine leptospirosis, metabolic acidosis develops due to renal failure. 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 Ca²⁺ concentration.

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/copenhagen*.

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 etiopathology of hemolysis in human or animal leptospirosis.

**RESUMO**

**Diminuição da fragilidade osmótica eritrocítica na leptospirose canina**

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

**REFERENCES**


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