COAGULOPATHY IN DOGS INFECTED WITH *Trypanosoma (Trypanozoon) evansi* (STEEL, 1885) BALBIANI, 1888

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ABSTRACT

The authors studied the hematologic alterations of 14 dogs experimentally infected with *Trypanosoma (Trypanozoon) evansi* (Steel, 1885) Balbiani, 1888. The acute phase of parasitemia was characterized by a decrease in erythrocyte and platelet numbers, in mean corpuscular hemoglobin rate and in hematocrit, and by an increase in the rate of partial prothrombin activation time (p < 0.05). The presence of infection did not cause any alterations in mean corpuscular volume, hemoglobin levels, leukocyte numbers, prothrombin time, or plasma fibrinogen (p > 0.05).

Key words: *Trypanosoma (Trypanozoon) evansi*, dogs, platelets, fibrinogen, partial thromboplastin time activation, prothrombin time, coagulopathy.

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INTRODUCTION

*Trypanosoma (Trypanozoon) evansi* (Steel, 1885) Balbiani, 1888 is a protozoan parasite transmitted by a variety of biting flies. In Central and South America, hematophagous bats may act as vectors of this hemoflagellate. Wild and domestic animals may develop clinical disease and also become infected and act as reservoirs. Clinically, the disease is similar to that caused by *Trypanosoma brucei brucei*.

*Trypanosoma evansi* is the agent of "surra", a disease that occurs in the acute or chronic form. Chronic infection of horses in South America attacks the central nervous system and is called "mal das cadeiras". Silva demonstrated the presence of *Trypanosoma evansi* in horses and dogs naturally infected in the region of the Mato Grosso "Pantanal" or "Wetland", Mato Grosso do sul (MS), Brazil (Figura 1).

The Pantanal is a seasonal floodplain of about 140,000 km², ranging in altitude from 80 to 120 m above the sea level, located in the center of South America, between 16° and 21° south and 55° and 58° west. In the Wetland, dogs help cowboys herd cattle through the region and represent the most important reservoir of the parasite in this area.

The tripanosomosis transmitted by *Trypanosoma evansi* has been described in different wild and domestic animals, but the coagulopathy of infected dog has not been...
studied. The objective of the present investigation was to study the different variables of coagulopathy during the acute phase of the protozoosis in dogs experimentally infected with *T. evansi*.

**Fig. 1 Mato Grosso Pantanal**

**MATERIAL AND METHODS**

**Animals:** 14 stray mongrel dogs aged 3 to 5 years and weighing approximately 6 kg, originating from the town of Corumba (MS) were used in the present study.

**Food and water:** the 2 groups received the same amount of ration and water throughout the study.

**Parasites:** the *T. evansi* strain used was isolated from infected dogs during an outbreak in the Mato Grosso Pantanal. The isolated organisms were studied morphologically by the method of John. All infected animals presented $8 \times 10^6$ parasites per ml blood after the 12th day of infection. The dogs were infected intravenously with $3 \times 10^8$ parasites and were then immediately confined.

**Blood collection:** a blood sample was obtained by puncturing the saphena vein before parasite inoculation and on the 6th and 12th days after inoculation.

**Controls:** the control group consisted of 5 noninfected stray mongrel dogs aged 3 to 5 years and weighing approximately 6 kg which were maintained under the same confinement and feeding conditions as the inoculated dogs.

**Variables studied**

**Blood counts:** whole blood was diluted in Hayen fluid and erythrocytes counts were performed in a Neubauer chamber. Hemoglobin was measured by the method of Drabkin and hematocrit by the microhematocrit technique. Mean corpuscular hemoglobin (MCH) is expressed as percent (%), mean corpuscular volume (MCV) as fentoliters (fl), hemoglobin as gram per deciliter (g/dl), and hematocrit as millimetre (mm). Platelet counts were performed by diluting bloodin Rees and Ecker reagent and counting in a Neubauer chamber. The results are reported as cubic millimeter (mm$^3$).

**Fibrinogen:** fibrinogen was measured by comparing unheated plasma to plasma heated to 58ºC for 3 minutes. The concentration is expressed as milligram per deciliter (mg/dl).

**Prothrombin time (PT):** PT was determined by the method of Quick using thromboplastin supplied by Biolab Diagnostica S.A. RJ, Brazil. The values are reported as seconds (s).
Partial thromboplastin activation time (PTAT): PTAT was determined by the method of Bell-Alton using reagents supplied by Biolab Diagnóstica S.A., and the results are reported as seconds (s).

Statistical analysis: Regression studies were performed by adjusting the equations of the variables of the responses as a function of number of days, with the level of significance set at p < 0.05. The normal hematologic values for dogs have been established by Jain.

RESULTS

No significant differences in final weight were observed between the two groups of dogs (p > 0.05). The animals did not show any changes related to the amount of ration or water ingested. All dogs were active, with no apparent symptoms. One dog died suddenly after 9 days but autopsy did not show lesions or histologic changes attributable to the parasite.

The data for the two groups refer to days 0, 6 and 12. Venous blood was collected immediately before infection with T. evansi (time 0) and then on the 6th and 12th days, a period corresponding to the acute phase of infection. The results are presented in Table 1.

The group of infected dogs showed a significant reduction in erythrocytes and CMCH (p < 0.05). The changes in hematocrit were significant (p < 0.05), with an increase in PTAT (p < 0.05) and a decrease in platelet numbers (p < 0.05) in MCV, PT or plasma fibrinogen were observed in the infected group. Although hemoglobin rates did not present significant changes (p > 0.05), the values were close to significance (a = 0.06479). No significant alterations were observed in the control group with respect to any of the parameters analyzed (p > 0.05).

DISCUSSION

The abnormalities of coagulation and fibrinolysis observed in the pathogeny of African trypanosomiases have been studied and established by Didishein and Gododwin. Other procedures such as histologic methods and the determination of the presence of intravascular thrombi in the glomeruli of the brain, liver and lungs have been used by different investigators. The changes in coagulation determined on the basis of PT and PTAT, platelet counts and plasma fibrinogen concentration have complemented the anatomopathologic studies.

On the basis of a joint analysis of these parameters, Jenkins and Facer showed that disseminate intravascular coagulopathy (DIC) occurred in African trypanosomosis when there was a drastic reduction in total platelet number, an increase in PTAT and in the levels of fibrin degradation products (PDF), a
reduction in coagulation factors V and VII-C without modifications in plasma fibrinogen levels or PT.

No reports are available in the literature about the pathogeny of *T. evansi* in dogs, a fact that impairs the understanding of the mechanisms involved in comparative studies with *T. brucei brucei*.

There is divergence among investigators about the number of parasites needed to produce experimental infection with *Trypanosoma* spp. Srivastava used 1x10⁹ parasites in experimental infections of dogs, whereas Monzon and Willavicencio used 50 parasites to infect a horse. In the present study we inoculated 3x10⁸ parasites to produce the acute phase, despite the possibility that this amount was not correlated with natural infection of dogs. Confinement and feeding did not produce any changes in the parameters studied. In the infected group there was a sizable reduction in total platelet number (a mean of 0.37x10⁵/ml after 12 days) and in PTAT (a mean a 25.1 s after 12 days). According to Jain, the normal platelet levels of dogs range from 2 to 5x10⁵ mm³, and normal PTAT ranges from 12 to 20 s. No aggregation or changes in platelet size or morphology were observed. The acute phase of the parasitosis was characterized by a reduction of erythrocytes, of hematocrit and of hemoglobin rate, with the last parameter almost reaching significance. The calculation of hematimetric indices showed a significant reduction in CMCH values. On the basis of this result, we may conclude that the anemia was normocytic and hypochromic.

In endemic areas it is difficult to evaluate the significance of anemia in human and animal trypanosomosis due to the presence of nutritional and parasitic problems. Studies conducted in Africa on cattle revealed that the trypanosomiasis produces more severe anemia compared to hematozoa and gastrointestinal helminths.

In a study of dogs naturally infected with *T. evansi* in the Lowlands, Silva diagnosed microcytic and hypochromic anemia. In our study, anemia was attributed to the presence of the hematozoon, with all parameters being evaluated during the acute phase, whereas in Silva’s study the same parameters were determined during the chronic phase of the parasitosis.

Plasma fibrinogen levels and PT values did not change, probably because of the close relationship existing between these two variables, since thrombin cleaves fibrinogen, producing fibrin 21 monomers. Consequently, when PT does not increase there will be no decrease in plasma fibrinogen.

Hoare stated that there is evidence that *T. evansi* may have originated from *T. brucei brucei*, and Losos, in a study comparing the pathogenic action of these two parasites, showed the existence of a close relationship between the two flagellates. This statement permitted us to compare our data with those obtained for *T. brucei brucei*.

The alterations in *T. brucei* infection associated with DIC were the same as those detected in the present study on dogs infected with *T. evansi*. Thus, alterations in the intrinsic mechanism of coagulation determined by the increase in PTAT and by a decrease in platelet number permitted us to conclude that disseminated DIC occurs in dogs infected with *T. evansi*.

RESUMEN

Los autores estudiaron las alteraciones hematólogicas de 14 perros experimentalmente infectados con *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888. LA fase aguda se caracterizó por la disminución del número de eritrocitos, plaquetas y de la tasa de hemoglobina corpuscular media (CHCM), del
hematocrito y por un aumento del índice de activación del tiempo de tromboplastina parcial (ATTP) \( (p < 0.05) \). La presencia de infección no causó alteraciones en el volumen corpuscular medio (VCM), en la tasa de hemoglobina, en el número de leucocitos, en el tiempo de protrombina (TP) y en el fibrinogeno plasmático \( (p > 0.05) \).

**RESUMO**

Os autores estudaram as alterações hematológicas de 14 caes experimentalmente infectados com *Trypanosoma (Trypanozoon) evansi* (Steel, 1885) Balbiani, 1888. A fase aguda da parasitemia caracterizou-se pela diminuição do número de eritrocitos, das plaquetas, da taxa de hemoglobina corpuscular média (CHCM), do hematocrito, e pelo aumento do índice de ativacao do tempo de tromboplastina parcial (ATTP) \( (p < 0.05) \). A presença da infeccao nao ocasionou alteracoes no volume corpuscular médio (VCM), na taxa de hemoglobina, no número de leucócitos, no tempo de protrombina (TP) e no fibrinogenio plamático \( (p > 0.05) \).

**REFERENCES**


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