

## Leukocytes and Reticulocytes Counts in Acute Infection of Dogs with *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888

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**ABSTRACT.** The authors studied alterations in the leukocytes, erythrocytes and reticulocytes counts observed in 14 stray dogs experimentally infected with *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888. Dogs were evaluated at days 0, 3, 6, 9 and 12 after infection. At the same time, 5 non-infected stray dogs were submitted to the same analysis. Only in the infected group changes were observed where erythrocytes count decreased from  $5.6 \times 10^6/\text{mm}^3$  to  $3.9 \times 10^6/\text{mm}^3$ . The acute infection did not cause any other significant alterations ( $p > 0.05$ ), but a slight increase in lymphocytes number was observed, probably due to hosts reaction against parasites.

**Key words:** *Trypanosoma evansi*, leukocytes, erythrocytes and reticulocytes counts.

**RESUMEN.** Fueron evaluadas las alteraciones producidas en 14 perros por la infección experimental de *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888 en el recuento de leucocitos, eritrocitos e reticulocitos. Los animales fueron evaluados en los días 0, 3, 6, 9 e 12 pos-infección. Al mismo tiempo, 5 perros no infectados fueron sometidos a los mismos análisis. Solamente los perros infectados presentaron una reducción significativa en el número de eritrocitos, que disminuyó de  $5.6 \times 10^6/\text{mm}^3$  a  $3.9 \times 10^6/\text{mm}^3$ . Los otros parámetros analizados no cambiaron significativamente ( $p > 0.05$ ). Una discreta linfocitosis (estadísticamente no significativa) fue observada, y que puede ser la respuesta del organismo frente a la infección.

**Palabras clave:** *Trypanosoma evansi*, cuentas de leucocitos, eritrocitos y reticulocitos.

### INTRODUCTION

Spanish settlers probably introduced *Trypanosoma evansi* in South America during the XVI century. Apparently, the disease entered in the Pantanal Region in the 1850s and received the name of Mal de Caderas when horses are infected. In the Old World is known as surra.<sup>8,21</sup> The Pantanal (Fig 1.) is a seasonal floodplain of about 140,000 km<sup>2</sup> ranging in altitude from 80 to 120 m above the sea level, located in the center of South America. Extensive cattle ranches varying from 10,000 to 200,000 hectares occupy most of this wetland. It is populated by 4,000,000 cattle, 4,966 buffaloes and 139,760 horses.<sup>4</sup> The traditional cattle-breeding system is based on calves and yearling production and its commercialization involves taking the animals to market-places, river ports or railway station. Usually, six cowboys herd the animals and each man has one extra horse. Overtime dogs follow the group and play a very important role in the life cycle and epidemiology of this tripanosomiasis.<sup>3</sup> The prevalence of the

disease in horses as well as the infections in dogs and free-ranging capybaras is 4.1% in horses, 7.1% in dogs and 22% in capybaras using an enzyme-linked immunosorbent assay for detection of *T. evansi* antigen (ag-ELISA).<sup>6</sup> This way, dogs and wild animals, like capybaras have an important function as natural reservoirs for the parasite. The transmission, due to *Tabanids* is the most effective manner to maintain the cycle among dogs or wild animals and horses, producing economic losses and difficulties to herd cattles.<sup>8,13</sup>

Pathogenesis of the disease caused by *T. evansi* is characterized by rapid loss of weight and a variety of anemia degrees, including dogs.<sup>23</sup> Some of the lesions observed in the acute syndrome e.g. the urticarial plaques and ophthalmitis are transitory and may be recurrent. In the chronic stages, the animals become weak, the mucous membranes are pale and superficial lymph nodes are enlarged.<sup>14</sup>

Signs of trypanosomiasis due to *T. evansi* include intermittent fever, urticaria, anemia, edema of the legs and lower parts of the body, progressive weakness, loss of con-



dition and inappetence and another biochemical changes.<sup>13,15,18</sup> The disease is often rapidly fatal for camels, dogs and horses.<sup>15</sup> In naturally infected dogs, a severe anemia was observed with several abnormalities in erythrocytes, which include microespherocytes, acanthocytes and other bizarre shapes. Also adhesion of *Trypanosoma* to erythrocytes and erythrophagocytosis was seen.<sup>23</sup> The origin of these changes is not completely elucidated and several mechanism have been proposed. Several causes of anemia in acute and chronic African trypanosomiasis as increased red cell destruction, extravascular and intravascular hemolysis by the immune system, nonspecific reticuloendothelial system activation, direct traumatic effect of *Trypanosoma* among others.<sup>1,10</sup> The anemia is hemolytic in nature and results primarily from the erythrophagocytosis in the spleen, liver, lungs, bone marrow and even in circulation.<sup>9</sup>

The study of health changes in every animals involved with this trypanosomiasis in Pantanal Region is very important for a better understanding of the disease epidemiology.

## MATERIALS AND METHODS

**Dogs.** 14 stray dogs (about 6 kg each; age between 2 and 4 years) from the city of Corumbá(MS) were intravenously infected with  $3 \times 10^8$  parasites and confine maintained. Venous blood was collected immediately before parasite administration (day 0) and after 3, 6, 9, and 12 days (infected animals). At the same time, another group of 5 uninfected stray dogs were maintained at the same conditions to observe eventual influence of isolation conditions and food in the analyzed parameters (control group). From this group blood was collected at days 0, 6 and 12.

***Trypanosoma evansi*.** The *T. evansi* strain utilized proceeded from Pantanal Matogrossense during a natural outbreak in horses during the year of 1994.<sup>22</sup> To confirm the specie, the distances between posterior end to kinetoplast, from kinetoplast to midnuclear point, from the anterior end to tip of the flagellum and free flagellum length were measured.<sup>11</sup>

**Assays.** Dogs were bled for determination of hematological data using a vacuum system in tubes containing ethylene diaminetetraacetic acid as anticoagulant. The red cell count (RBC) and total white cell count (WBC) were obtained using Neubauer chamber. Thin blood films stained with May-Grünwald-Giemsa were examined by light microscopic (X 1000) for evaluation of the proportion of white blood cells, morphologic abnormalities of erythrocytes and presence of *T. evansi*.

Whole blood was diluted v/v with brilliant cresyl blue (1%) for reticulocytes counts. A thin film smear was stained with May-Grünwald-Giemsa and about 1.000 erythrocytes were counted to find out the proportion of reticulocytes/erythrocytes.<sup>9</sup>

**Statistical analysis.** It was done by regression studies

comparing the relation of each sample with "day 0", for both groups (control and infected animals) with  $p < 0.05$ .

## RESULTS

In the control group, no significant alterations were found in the evaluated parameters. One infected dog died after 9 days, but anatomo-pathological analysis did not revealed whether the "causa-mortis" was due to the infection or not. Every infected animal had after 12 days, at least,  $8.0 \times 10^6$  parasites per milliliter of blood.<sup>7</sup> As expected, erythrocytes count decreased markedly, but reticulocytes proportion hasn't changed from Day "0" until Day "12". It was not significant at statistical levels.

No statistical changes were observed with WBC counts and the proportion of them during the studied period

## DISCUSSION

Experimental infection in dogs with *T. evansi* is very seldom related in literature and a lack of information is observed. The majority of published data are about natural infected animals and chronic cases are discussed. This way, the early stages of this parasitosis were studied here in regard to some hematological changes.

Among the approaches to study pathogenesis caused by *T. evansi*, the determining of changes in tissue metabolism of infected hosts is very important. But, any increase or decrease in the evaluated parameters may be in normal range and does not affect organism function. This was also

Fig. 1. Pantanal localization in South America



Table 1. Erythrocytes count and reticulocytes proportion during acute infection of dogs with *Trypanosoma evansi*.

	Control group			Infected group		
	Days					
	0	6	12	0	6	12
Erythrocytes (10 <sup>6</sup> /mm <sup>3</sup> )	5,2±1,9	4,8 ± 1.6	4,5±1,6	5,6±1,5	3,0 ±1,8	3,9 ±1,7
Reticulocytes (%)	0,4 ± 0,4	0,3 ± 0,4	0,6 ± 0,8	0,3 ± 0,4	0,7 ±0,9	0,5 ± 0,9

Medium values of triplicates ( $\pm$ : standard deviation)

Table 2. Leukocytes (WBC) count during acute infection of dogs with *Trypanosoma evansi*.

	Control group			Infected group		
	Days					
	0	6	12	0	6	12
Leukocytes/mm <sup>3</sup> (X 1,000)	8.87±7.9	7.88±6.6	576±2.5	8.5±6.4	12.1±5.3	8.4±1.9
Neutrophiles (%)	73,2±10,9	69,4 ± 6,1	70,4 ±8,2	72,1 ± 9,6	70,0 ± 8,0	65,8 ± 5,8
Band neutroph. (%)	0,0 ± 0,0	0,0 ± 0,0	1,4± 1,9	0,2 ± 0,8	1,2 ± 2,0	0,4 ±1,3
Eosinophiles (%)	5,2 ± 2,8	10,6 ± 4,3	7,0 ± 1,4	7,1 ± 7,1	4,7 ±2,9	5,3 ±2,6
Basophiles (%)	0,0 ± 0,0	0,0± 0,0	0,0 ± 0,0	0,0, ±0,0	0,0 ±0,0	0,1 ± 0,3
Lymphocytes (%)	19,2 ± 11,3	14,8 ± 6,8	18,4 ± 8,3	15,6 ±9,7	19,3 ±5,8	20,1 ± 4,1
Monocytes (%)	2,0 ± 0,7	4,2 ± 1,3	2,8 ±2,7	5,3 ± 3,9	3,0 ±2,6	4,8 ±2,2

Medium values of triplicates( $\pm$ : standard deviation)

found in this work with a large variation of values, but the majority of them within normal range for dogs.<sup>12</sup>

Anemia is one of the most consistent findings in animal tripanosomosis and its nature is not completely elucidated.<sup>1,2,19</sup> The significance of anemia on the overall morbidity of African tripanosomosis, both in man and livestock, is often difficult to assess since many other cause (nutritional and parasitic) are concurrently present in endemic areas. However, the bone marrow response, in front of hemolytic stimulus was not studied. Table1 shows a decrease in erythrocytes but reticulocytes count maintained stable ( $p>0.05$ ). In *T. vivax* infection in calves, only after 28 days reticulocytes counts can reach a measurable peak.<sup>24</sup> The production of this cell may be depressed in the bone marrow due to *Trypanosoma*<sup>9</sup> and also an alteration in erythrocytes production due to macrophage's iron sequestration<sup>5</sup> carrying to an anemia without cells production in bone marrow. It would be possible to find another abnormalities if this study prolonged until the chronic phase. Leukocytes counts and the proportion of this cells may vary in cases of infection with *T. evansi*. Some authors

found leukopenia<sup>20,23</sup> while others found leukocytosis.<sup>16</sup> In this study, neither the total count of leukocytes nor the proportion of them changed significantly (Table 2). Only a very slight increase in the proportion of lymphocytes was observed. It may be an attempt from the infected host to fight against infection and it is common in endemic areas where animals are used to the parasite and are more capable to kill it. This is based on the fact that tolerant animals develop leukocytosis and non-tolerant develop leukopenia.<sup>17</sup> All the studied animals here proceeded from the endemic zone and should bring immunologic memory from anterior infections or genetic inheritance, which could be transmitted through generations and, therefore will react against parasite.

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