

## **Biochemical Changes in Acute Infection of Dogs with *Trypanosoma evansi* (Steel 1885) Balbiani, 1888**

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### **ABSTRACT**

This article reports the biochemical changes observed in 14 stray dogs experimentally infected with *Trypanosoma evansi*. The dogs were evaluated at days 0, 3, 6, 9 and 12 regarding the following parameters: glucose, urea, creatinine, uric acid, albumin, total proteins, alanineamime transferase (ALT), aspartatealanine transferase (AST) and creatinine phosphokinase (CK) employing non-enzymatic commercial kits. At the same time, 5 non-infected stray dogs were submitted to the same analysis. Only in the infected group changes were observed. Very slight increase with glucose and ALT levels were observed and only albumin decreased significantly in the whole analysed parameters. This suggested that edema may be the first clinical sign in chronic disease due to *T. evansi*.

### **INTRODUCTION**

*Trypanosoma evansi* is reported as the agent of a disease known in South America as "mal de caderas" or "derrengadera". One of the main sites to find the parasite is the Pantanal Matogrossense (BRAZIL) (Fig. 1) which has an area of 139,111 km<sup>2</sup>. It is one of the most important livestock production center in Brazil and has a rich fauna with about 658 species of birds, 80 species of mammals, 50 species of reptiles and 230 species of fish (EMBRAPA-CENARGEN 1987). This parasite produces a great number of changes in a lot of mammalian species, like cattle, horses, dogs and rodents (Losos 1980; Mahmoud and Gray 1980; Stevens et al. 1989). Levine (1973) reported that the signs of trypanosomosis due to *T.*

*evansi* include intermittent fever, urticaria, anemia, edema of the legs and lower parts of the body, loss of hair, progressive weakness and loss of condition. Mahmoud and Gray (1980) assert that the disease is often rapidly fatal for camels, dogs and horses. According to Losos (1986) there is a considerable similarity between the pathology of disease caused by *T. brucei*, *T. evansi* and *T. equiperdum* affecting animals which allow to make some comparison among these parasitosis.

Despite a large number of hematological studies in animals infected with *T. evansi*, only a few reports concern the biochemical changes, and most of them are only case reports of naturally infected animals. One of the most common findings in these studies is the decrease of albumin levels, which could explain the edema found in chronic cases (Monzon and Villavicencio 1990). Seed and Hall (1985) listed the changes found in a lot of rodents and cattle, i.e. decrease in serum concentrations of glucose, albumin and creatinine kinase (CK). Other products, as urea, creatinine, aspartateamine transferase (AST), alanineamine transferase (ALT) showed low increases during infection. This results were also reported by Sandoval et al.(1994) in one naturally infected dog but it still remains a lack of information with serial studies which would help to understand the *T. evansi* pathology in acute and chronic infected dogs and horses.

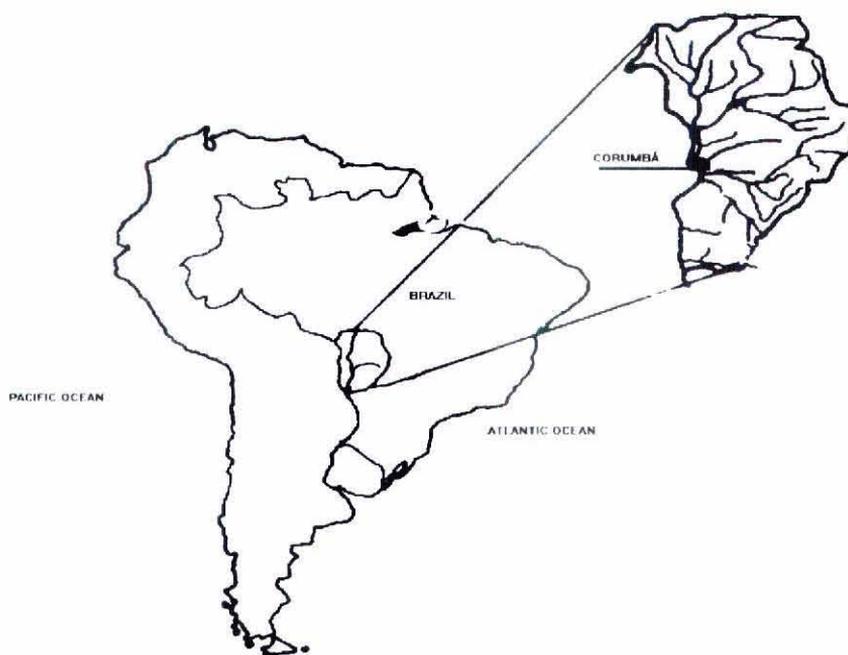


Fig. 1 Pantanal localization in South America.

## MATERIALS AND METHODS

### *Dogs*

Fourteen stray dogs (about 6 kg each; age between 2 and 4 years) from the city of Corumba (MS) were intravenously infected with  $3 \times 10^8$  parasites and confine maintained. Dogs were bled for determination of biochemical data using a vacuum system (Vacuum II, Labnew, Campinas, Brazil). Venous blood was collected immediately (without anticoagulant) 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 was maintained at the same conditions to observe eventual influence of isolate conditions and food in the analysed parameters (control group). In this group blood was collected at days 0, 6 and 12. Every infected animal had after 12 days, at least,  $8.0 \times 10^6$  parasites per milliliter of blood, according to the technique described by Herbert and Lumsden (1976).

### *Trypanosoma evansi*

The *T. evansi* strain utilized proceeded from Pantanal Matogrossense during a natural outbreak in horses during the year of 1994 (Silva et al. 1995a). To confirm the specie, the technique described by John et al. (1992) was employed. Basically, 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.

### *Assays*

For glucose, urea, creatinine, uric acid, albumin, total proteins, alanineaminotransferase (ALT) (formerly known as GPT), aspartatealanine transferase (AST) (formerly known as GOT) and creatinine phosphokinase (CK) analysis were employed commercial kits (Biolab Diagnostica S. A., Rio de Janeiro, Brazil) and concentration was determined colorimetrically (Micronal digital spectrophotometer, B-34212, Sao Paulo, Brazil).

### *Statistical analysis*

Statistical analysis 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

During the experiment, dogs did not show significant alterations in food and water ingestion. One infected dog died after 9 days, but anatomo-pathological

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analysis did not revealed whether the "causa-mortis" was due to the infection or not.

Table 1 shows the results with control group (c) and infected group (i) of dogs during 12 days of analysis. Medium values found in control group did not change significantly in the analysed parameters, and in infected animals, only albumin decreased significantly ( $p<0.05$ ). There was a slight increase in AST level, although within normal range for dogs (For normal ranges in dogs see Kaneko 1989).

Table 1. Biochemical mean values found in control and infected dogs with *T. evansi*.

	Days after infection				
	0	3	6	9	12
Glucose (c) (mg/dl)	101	-	76	-	84
Glucose (i) (mg/dl)	83	66	71	62	65
Urea (c) (mg/dl)	33	-	38	-	39
Urea (i) (mg/dl)	39	37	39	41	40
Creatinine (c) (mg/dl)	0.8	-	0.8	-	0.9
Creatinine (i) (mg/dl)	0.8	1.0	1.0	1.0	0.9
Uric acid (c) (mg/dl)	2.3	-	3.5	-	2.5
Uric acid (i) (mg/dl)	2.5	2.8	2.8	2.6	2.7
Total protein (c) (g/dl)	8.1	-	7.3	-	5.6
Total protein(i) (g/dl)	7.4	6.5	5.8	5.6	5.6
Albumin (c) (g/dl)	3.8	-	2.8	-	3.6
Albumin (i) (g/dl)	3.7	2.8	3.2	1.9	1.8
ALT (c) (U/l)	13	-	18	-	19
ALT (i) (U/l)	15	18	17	19	19
AST (c) (U/l)	8	-	34	-	25
AST (i) (U/l)	14	32	31	31	38
CK (c) (U/l)	11	-	10	-	10
CK (i) (U/l)	12	8	8	7	11

c: controls, i: infected, mg/dl: milligrams per deciliter, g/dl: grams per deciliter, U/l: Unit per liter.

## 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 biochemical alterations.

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 parameter may be in normal range and does not affect organism function. This was also found in this work with a large variation of values, but the majority of them within normal range for dogs, according to Kaneko (1989).

There is no uniformity among authors about the exact *Trypanosoma* number necessary to produce an experimental infection (Dwivedi et al. 1977; Moulton and Sollod 1976). Srivastava et al. (1967) used 10,000 to produce an experimental infection in dogs. In the other hand, Monzon and Villavicencio (1990) used only 50 parasites to infect a horse. This way, was decided to infect dogs with a huge number of parasites ( $3 \times 10^8$ ) to produce a very quick acute infection.

The lack of alteration in many metabolic products analysed in this work may be due to the study of acute phase in which the parasite could not have produced marked cellular lesion. No alteration in glucose levels was observed during the experiment, possibly because the study was performed in few days. Glucose abnormalities were observed in chronic phase of disease in dogs (Sandoval et al. 1994) and terminal phase in rodents infected with *Trypanosoma* (Ashman and Seed 1973; Sandoval et al. 1994). Rodents were more susceptible to *T. evansi* infection than horses and dogs and die in a few days (Losos 1980). No significant alteration was found in this work for uric acid, which can show any disorder in purin's metabolism and is produced in liver. Also the kidney function was probably not affected. Urea and creatinine levels did not change significantly. These results were also found by Sandoval et al. (1994) in rodents. However, Srivastava et al. (1967) revealed that in chronic disease a subacute interstitial nephritis characterized by intense mononuclear cell infiltrations of the periglomerular regions may occur. It would be possible to find other abnormalities if our study prolonged until the chronic phase.

The total protein levels did not change during the experiment, but albumin decreased significantly. Probably, no changes were found in total protein levels, because organism infected with *T. evansi* increases globulins synthesis to avoid an alteration in osmotic pressure due to constant decrease in albumin during

infection. In chronic stages of trypanosomiasis, low albumin levels may be the causative agent of edema and possible indicate a great liver damage (Monzon and Villavicencio 1990; Silva et al. 1995b; Srivastava et al. 1967).

According to Kramer (1989), many seric enzymes could be analysed for their diagnostic and prognostic values. The analysis of ALT and AST is the most common. ALT is a specific liver enzyme found in the cell cytoplasm and there is a steep rises in its level in cell membrane damage. AST is found mostly in cell organelles and rises when there is a great damage to the heart, kidney, skeletal muscles and liver. The results found in this work showed a very slight rise in ALT, within normal range for dogs (23-66 U/l) and not statistically significant. AST levels did not change during the examinations. These values are similar to the results of Dwivedi et al. (1977) which infected dogs with *T. evansi* in India. This may indicate a slight hepatocyte membrane damage due to the parasite and probably without heart damage because AST did not change in this work, as CK.

CK is an enzyme found in muscles and increases its levels when occur a cell damage (Kramer 1989). Seed and Hall (1985) indicated a decrease in CK levels in infected rabbits with *T. brucei*. This difference might be due to different species of *Trypanosoma* used and the susceptible host.

As no significant biochemical changes were observed, except albumin, it could be suggested that among clinical signs in dogs infected with *T. evansi*, edema may be one of the first observed changes, in the beginning of the chronic phase of infection.

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