Experimental Infection by *Trypanosoma evansi* in Rabbits: Levels of Sodium, Potassium, Calcium and Phosphorus in Serum

Aleksandro Schafer Da Silva¹, Márcio Machado Costa², Clarissa Marques Moreira³, Régis Adriel Zanette¹, Gustavo Roberto Thomé⁴, Mateus Anderson Otto¹, Érico Marlon de Moraes Flores³, Sonia Terezinha dos Anjos Lopes² & Silvia Gonzalez Monteiro¹

**Background:** *Trypanosoma evansi* is the most widely distributed of the pathogenic African animal trypanosomes, affecting domestic livestock and wildlife in country. The animals presented clinical sings as anemia, emaciation, apathy, recurrent fever, enlarged lymph nodes, edema and abortion. The minerals have different functions in the organism, and an imbalance, either by excess or deficiency, or a pathological condition, causes alterations in the respective serum levels, as well as in trypanosomosis. Therefore, the aim of this study was to evaluate the concentrations of sodium, potassium, calcium and phosphorus in blood serum of rabbits experimentally infected with *Trypanosoma evansi*.

**Materials, Methods & Results:** Twelve adult female *Oryctolagus cuniculus*, weighing average 3.9 kg, were used. Rabbits were divided into two groups, a control group with six animals (rabbits 1-6) and an infected group with six animals (rabbits 7-12). Animals from trypanosome-infected groups were inoculated intraperitoneally with 0.5 mL of rat blood containing 10⁸ trypanosomes (Day 1). Control group received physiological solution by the same route. Parasitemia was estimated daily for 118 days post-inoculation (PI) by microscopic examination of smears. Blood samples for hematology and evaluation of serum minerals were collected at days 1, 5, 20, 35, 50, 80 and 118 PI. Hematocrit was evaluated for monitoring of the disease. Inductively coupled plasma optical emission spectrometry (ICP OES) was used to measure the levels of sodium, potassium, calcium and phosphorus. Hyporexia, edema and fever were clinical signs associated with change in the levels of the minerals. A decrease in the number of red blood cells was only observed at day 5 post-inoculation. Significant differences were observed among groups (*P* < 0.05) in minerals levels. Sodium and calcium were reduced at days 35, 50, 80 and 118 PI. The concentration of potassium was decreased at days 20, 35 and 50 PI, while phosphorus was only at day 35 PI. Interestingly, all minerals were reduced in serum at day 35 PI, where all infected rabbits had edema of the eyelids and ears.

**Discussion:** Changes in the circulating levels of sodium, potassium, calcium and phosphorus where observed when all the rabbits showed clinical signs of the disease. Alteration in the concentrations these minerals were reported in cattle, equines, camels and humans infected with trypanosomatids, although the cause of these changes was not fully elucidated. According to the literature data, differences in concentration of macro minerals have been found among species of trypanosomes and the specific host involved. In other study, researchers reported decreased levels of iron and zinc and increased copper in *T. evansi*-infected cats, which were correlated with anemia, lymphopenia and inflammatory response. This study the reduction in sodium and potassium may have contributed to the edema in the rabbits. As calcium is essential for the life of trypanosomes, these parasites have adapted to store calcium, what might explain the reduction of the mineral in the blood of the rabbits. The reduction of phosphorus might have been due to hyporexia presented by the rabbits, with consequent lower intake of phosphorus. Based on the results, it was concluded that the infection by *Trypanosoma evansi* influences the serum levels of sodium, potassium, calcium and phosphorus in rabbits. The severity of clinical signs can varied among the infected rabbits.

**Keywords:** trypanosomosis, *Trypanosoma evansi*, minerals, rabbit, chronic infection.
INTRODUCTION

Trypanosoma evansi is the most widely distributed of the pathogenic African animal trypanosomes, affecting domestic livestock and wildlife in Asia, Africa and Latin America [17]. T. evansi is mechanically transmitted mainly by biting flies [15,19] and horses, mules, cattle, buffalo, deer, cats, dogs, rodents, rabbits and humans can be affected by T. evansi [2,9,14,16,31,36]. These animals presented clinical sings as anemia, emaciation, apathy, recurrent fever, enlarged lymph nodes, edema and abortion [9,25,34,37].

Minerals can be classified in macro and trace minerals, according to the amount required by the body [20]. Among the macro minerals are calcium, phosphorus, potassium and sodium, which were studied in our experiment. These minerals have different functions in the organism, and an imbalance, either by excess or deficiency, or a pathological condition, causes alterations in the respective serum levels [13,20]. This minerals participates in the formation of bones and teeth, muscle contraction, permeability of cell membranes, blood clotting, enzymatic reactions, secretion of hormones, metabolism of lipids, carbohydrates and proteins, formation of energy, genetic transmission of nucleic acids, responsible for maintaining the osmotic pressure, transmission of nerve impulses, muscle and heart contraction [13,20].

In experimental studies with T. evansi-infected rabbits, changes in hematomatological, biochemical, immunological and pathological parameters, and clinical signs typical of trypanosomosis were reported elsewhere [8-10,34]. Therefore, it was considered appropriate to investigate the levels of macro minerals in the circulation of rabbits experimentally infected with T. evansi.

MATERIALS AND METHODS

Experimental animals

Twelve adult female Oryctolagus cuniculus, weighing average 3.9 kg, were used. The animals were weighed every 30 days. Our research group also chose rabbit as experimental model due to the chronic feature of the infection, similar to what occurs in naturally infected animals. Rats are also susceptible, although the animals die in 5 to 6 days if they are not treated. Animals were kept in individual cages with temperature and humidity controlled at 23°C and 70%, respectively. They were fed with commercial ration and water ad libitum. Hematological (erythrogram, leukogram and platelet count) and biochemical (hepatic and renal function) examinations were performed two times at 15-day intervals. After 30 days (day 0 of the experiment), the evaluated patterns showed normal values [3].

Trypanosome infection

T. evansi was originally isolated from a naturally infected dog [7]. First, a rat was intraperitoneally infected with blood cryopreserved in liquid nitrogen containing 10^6 parasites. This procedure was performed in this study to obtain a large amount of blood parasites for posterior inoculation of the rabbits.

Rabbits were divided into two groups, a control group with six animals (rabbits 1-6) and an infected group with six animals (rabbits 7-12). Animals from trypanosome-infected groups were inoculated intraperitoneally with 0.5 mL of rat blood containing 10^8 trypanosomes (Day 1). Control group received physiological solution by the same route. The number of inoculated flagellates was estimated by using a Neubauer chamber [37].

Estimate of parasitemia

Parasitemia was estimated daily for 118 days post-inoculation (PI) by microscopic examination of smears. Each slide was mounted with blood collected from the ear vein, stained by the panoptic method, and visualized at a magnification of ×1,000.

Blood sampling

Blood samples for hematology and evaluation of serum minerals were collected by cardiac puncture using 5 mL disposable syringes and 25x7 needles at days 1, 5, 20, 35, 50, 80 and 118 PI. For blood collection, the animals were anesthetized with ketamine (0.08 mL kg⁻¹) and xylazine (0.05 mL kg⁻¹), as recommended by the ethics committee. Blood for hematocrit was stored in tubes with anticoagulant and measured using the standard microhematocrit method, according to Schalm et al. [19]. Serum mineral concentrations were determined collecting 3 mL of blood that was stored in tubes without anticoagulant and centrifuged for 10 min. Samples were stored at -20°C until analysis.

Determination of sodium, potassium, calcium and phosphorus

Samples were digested using a Model Multiwave 3000 microwave oven equipped with high-pressure quartz vessels. This procedure was adapted from Cargnelutti et al. [4] and Peixoto et al. [27]. About 250 mg of the sample were weighed and transferred to
digestion quartz vessels. Concentrated HNO₃ was added to each vessel, which was capped and placed into the microwave oven. Digestion was performed at maximum temperature and pressure by 30 min. After digestion, samples were transferred to graduated polypropylene vials and diluted with water to 20 mL for further analysis by inductively coupled plasma optical emission spectrometry (ICP OES). Colorless and clear solutions were obtained after the digestion step.

A microwave sample preparation system (Multiwave 3000) equipped with eight high-pressure quartz vessels (internal volume of 80 mL, maximum temperature and pressure of 280°C and 80 bar, respectively). A Spectro CIROS CCD simultaneous inductively coupled plasma optical emission spectrometer, with axial view configuration was used for calcium, phosphorus, potassium and sodium determination. A cross flow nebulizer coupled to a double pass-Scott type nebulization spray chamber was used. Calcium, phosphorus, potassium and sodium were determined at 396.847, 177.495, 766.491 and 589.592 nm, respectively. The operating plasma conditions of the ICP OES equipment were adapted from Moraes et al. [21] and the radiofrequency power was set at 1400 W. Argon (99.996%) was used for plasma generation and plasma gas flow rates were set at 14, 1.00, and 0.90 L min⁻¹ for principal, auxiliary and nebulizer gas, respectively. Calibration standards were prepared by sequential dilution of a multielemental standard solution in 5% (v/v) nitric acid. The calibration for the four evaluated elements ranged from 0.01 to 0.5 mg L⁻¹. All samples were analyzed in triplicate and the serum levels of sodium, potassium, calcium and phosphorus were expressed in µg g⁻¹.

**Statistical analysis**

Data were statically analyzed by the Kolmogorov-Smirnov normality test. Thereafter, data were submitted to analysis of variance (ANOVA) followed by the Tukey’s test (P < 0.05).

**RESULTS**

**Parasitemia and clinical course of infection**

Examination of the peripheral blood smears showed a prepatency period between 24 and 72 h in the infected rabbits. Irregular waves of parasitemia, ranging from zero to one trypomastigote per microscopic field, were observed until 35 days PI. Parasites were no longer observed in blood smears from the 37th day onwards (Figure 1).

Clinical signs as hyporexia, fever and weight loss (8.7%) were observed in the first 30 days PI in all infected rabbits (acute phase and peak of parasitemia). Edema of

![Figure 1](image.png)

*Figure 1.* Parasitemia of *Trypanosoma evansi* in infected rabbits was estimated for 118 days post-inoculation by microscopic examination of smears. Each slide was mounted with blood collected from the ear vein, stained by the panoptic method, and visualized at a magnification of × 1,000.
the eyelids, ears and vulva was observed in all infected animals at determined periods of the experiment. This clinical signs disappeared in a few moments, but relapsed after a period that varied among individuals. At days 60, 90 and 118 PI the infected rabbits gained weight. The non-infected rabbits showed no clinical changes and gained weight throughout the study.

Anemia was not observed in the *T. evansi*-infected rabbits. However, a significant reduction in the hematocrit values was observed in these animals at day 5 PI (*P < 0.01*), when compared to the control group. This feature was not observed in the other evaluated days (Figure 2).

Anemia was not observed in the *T. evansi*-infected rabbits. However, a significant reduction in the hematocrit values was observed in these animals at day 5 PI (*P < 0.01*), when compared to the control group. This feature was not observed in the other evaluated days (Figure 2).

![Figure 2. Mean hematocrit values of *Trypanosoma evansi*-infected and non-infected rabbits. Asterisk indicate statistical difference (*P <0.05*) between groups (*ANOVA, Tukey test).](image)

Concentration of sodium, potassium, calcium and phosphorus

A significant reduction in the levels of sodium, potassium, calcium and phosphorus was observed in the serum of the *T. evansi*-infected animals (*P < 0.05*), during some periods of the experiment (Figure 3). Sodium (Figure 3a) and calcium (Figure 3c) were reduced at days 35, 50, 80 and 118 PI. The concentration of potassium was decreased at days 20, 35 and 50 PI (Figure 3b), while phosphorus was only at day 35 PI (Figure 3d). Interestingly, all minerals were reduced in serum at day 35 PI, where all infected rabbits had edema of the eyelids and ears.

**DISCUSSION**

This study seems to be the first investigation on the concentration of sodium, potassium, calcium and phosphorus in animals infected by *T. evansi*. Changes in the circulating levels of these minerals were observed when all the rabbits showed clinical signs of the disease. These four electrolytes are extremely important in various body functions and, therefore, changes in serum concentrations of the infected rabbits are related to trypanosomosis by *T. evansi*. Moreover, it was already observed decreased levels of iron and zinc and increased copper in *T. evansi*-infected cats, which were correlated with anemia, lymphopenia and inflammatory response [11].

Changes in the concentrations of sodium, potassium, calcium and phosphorus were reported in cattle, equines, camels and humans infected with trypanosomatids [1,5,23,24], although the cause of these changes was not fully elucidated. According to the literature data, differences in concentration of macro minerals have been found among species of trypanosomes and the specific host involved. For example, the concentration of sodium increased and of potassium decreased in sheep infected with *T. congolense* [33]. Researchers also reported in this specie a reduction in the levels of potassium in *T. brucei* infection, with no mention on the sodium level [24]. No changes in these minerals were observed in *T. brucei*-infected boars, when
compared to non-infected animals [26]. Levels of calcium did not change in camels infected by the protozoan [6], although sheep infected with *T. congolense* showed increased levels of calcium and phosphorus [23]. Calcium and phosphorus concentrations remained unaltered in cattle infected with *T. vivax* [30].

Both sodium and potassium concentrations were reduced in the blood of the infected rabbits in this study. The levels of sodium decreased from the 35th day PI onwards (Figure 3a). Low levels of potassium were seen since the beginning of the experiment, although they became statistically significant only at days 20, 35 and 50 PI, when compared to the control group (Figure 3b). Some of the clinical signs presented by the *T. evansi*-infected animals (e.g., edema, disorientation, instability and paralysis of hind limbs) might be related to the change in concentrations of sodium and potassium [11,14,18], since these minerals are involved in the regulation of the acid-base balance and in the volume of body fluid, participating also in the transmission of nerve impulses and in muscle contraction [20]. Therefore, the reduction in both minerals may have contributed to the edema in the rabbits. Renal function was not investigated in this study, but our research group already observed that urea and creatinine levels remained within the reference values in *T. evansi*-infected rabbits [8]. Thus, we believe that the low levels of sodium and potassium observed are not in consequence of the elimination of electrolytes in urine due to kidney impairment.

No apparent clinical changes were showed by the infected rabbits in consequence of the reduction in calcium stock. But, since calcium is directly involved in muscle contraction, low levels of calcium observed in
our study may be involved in the etiology of the clinical signs often reported in the infections by *T. evansi*, as incoordination and instability of hind limbs, atrophy of the large muscles of the limbs, difficulty to stand up and muscle weakness [14,18]. Moreover, low calcium concentrations might be involved in clotting disturbances that are often identified in trypanosomosis [12]. Thus, in an outbreak of *T. evansi* with clinical signs typical of the cachectizing form and/or coagulopathies, clinicians should investigate the levels of calcium stock to prove the suggested hypothesis.

Calcium plays an important role in the regulation of multiple cellular activities in different trypanosomatids [32]. These parasites possess a calcium transport system in the endoplasmic reticulum that is involved in calcium-homeostasis. As calcium is essential for the life of trypanosomes [5], these parasites have adapted to store calcium [22], what might explain the reduction of the mineral in the blood of the rabbits. Since the reduction of calcium occurred when none or few parasites were found in the blood, we suspect that *T. evansi* has migrated to the muscle and invaded the muscle cells generating an inflammatory process, as previously described by Quiñones et al. [28]. The affinity of the parasite by the muscle can be easily explained by its need for the calcium involved in muscle contraction.

The concentration of phosphorus was only reduced in one of the evaluated periods (day 35). The reduction of this mineral might have been due to hyporexia presented by the rabbits at days 20 and 35 PI, with consequent lower intake of phosphorus. We believe that the clinical signs showed by the infected animals were not related with the reduction in phosphorus levels.

In this study, the trypanosomosis in adult rabbits was characterized by a chronic infection with low parasitemia, similar to others studies [9,34]. The small number of bloodstream parasites is explained by the fact that these animals have antibodies (IgG, IgM and IgA) against *T. evansi* that can control the infection [34]. In young rabbits infected with *T. evansi*, we previously observed anemia [9], contrasting to the adult rabbits of this study. The reduction of hematocrit possibly this relationship’s the peak of parasitemia in this study. In both studies, no statistical difference in the hematological parameters was observed between the infected and non-infected group, showing that rabbits have the ability to recover from the clinical disease. As observed in this study, edema of eyelids and ears in *T. evansi*-infected a rabbit has been described elsewhere [10,35].

Based on the results, it was concluded that the infection by *Trypanosoma evansi* influences the serum levels of sodium, potassium, calcium and phosphorus in rabbits. The severity of clinical signs can varied among the infected rabbits.

REFERENCES


