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SHORT COMMUNICATION

Blood gas analyses and other components involved in the acid–base metabolism of rats infected by *Trypanosoma evansi*

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Matheus D. Baldissera [a,b,*], Rodrigo A. Vaucher [a], Camila B. Oliveira [b],
Virginia C. Rech [c], Michele R. Sagrillo [a], Daniel R. Stainki [b], Raqueli T. França [d],
Gustavo Machado [e], Sonia T.A. Lopes [d], Silvia G. Monteiro [b], Lenita M. Stefani [f],
Aleksandro S. Da Silva [f]

[a] *Centro Universitário Franciscano, Santa Maria, RS, Brazil*
[b] *Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil*
[c] *Laboratory of Nanotechnology, Centro Universitário Franciscano, Santa Maria, RS, Brazil*
[d] *Department of Small Animals, Universidade Federal de Santa Maria, Brazil*
[e] *Laboratory of Veterinary Epidemiology, Faculty of Veterinary, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*
[f] *Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil*

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ABSTRACT

The aim of this study was to investigate the effects of *Trypanosoma evansi* infections on arterial blood gases of experimentally infected rats. Two groups with eight animals each were used; group A (uninfected) and group B (infected). Infected animals were daily monitored through blood smears that showed high parasitemia with 30 trypanosomes per field (1000×) on average, 5 days post-infection (PI). Arterial blood was collected at 5 days PI for blood gas analysis using an automated method based on dry-chemistry. Hydrogen potential (pH), partial oxygen pressure (pO_2), oxygen saturation (sO_2), sodium (Na), ionic calcium (Ca ionic), chlorides (Cl), partial dioxide carbon pressure (pCO_2), base excess (BE), base excess in the extracellular fluid (BEecf), bicarbonate ($cHCO_3$), potassium (K), lactate, and blood total dioxide the carbon (tCO_2) were evaluated. The levels of pH, pCO_2, BE, BEecf, $cHCO_3$, and tCO_2 were significantly decreased ($P < 0.05$) in group B compared to group A. Additionally, the same group showed increases in Cl and lactate levels when compared to uninfected group. Therefore, it is possible

* Corresponding author. Tel.: +55 55 32208958.
E-mail address: matheusd.biomed@yahoo.com.br (M.D. Baldissera).
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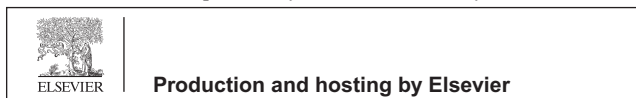
to state that the infection caused by *T. evansi* led to alterations in the acid–base status, findings that are correlated to metabolic acidosis.

Introduction

Trypanosoma evansi, the etiological agent of a disease known as "*Surra*" or "*Mal das Cadeiras*" in horses, is a hemoflagellate with wide geographic distribution in tropical and subtropical regions [1–3]. The parasite is transmitted primarily by blood sucking insects and possibly by vampire bats [4]. In horses, dogs, and camels the disease progresses to death, except in rare cases. The main clinical signs of the disease include: fever, anemia, swollen lymph nodes, jaundice, weight loss, and edema of hind limbs. Horses, cats, and rats show progressive weakness and motor disorders at chronic stages of the disease [5,6]. Rats infected by *T. evansi* without treatment usually die within 4–6 days post-infection (PI), and usually show seizures, few hours before death [7].

One of the main pathological findings in animals infected by *T. evansi* is anemia [3,6,7], which can lead to major changes in blood, as well as acid–base imbalance [8]. Acid–base disturbances are commonly observed in many infections and metabolic disorders, drawing the attention to the need of a precise description of these disorders in humans and animals [8]. The arterial blood gas determination plays an important role in diagnosing acid–base status disturbances, oxygenation, and ventilation [9]. Therefore, the aim of this study was to assess the levels of blood gases and other components involved in the metabolic acid–base status during an acute infection in rats experimentally infected by *T. evansi*.

Material and methods

T. evansi *isolate*

In this experiment, *T. evansi* isolate was obtained from a naturally infected dog [10] kept in liquid nitrogen. One rat (R_1) was inoculated with cryopreserved parasites in order to reactivate the *T. evansi* isolate.

Animal model

Sixteen female rats (Wistar) with mean age of 70 days weighing approximately 200 (± 10 g) were used. They were housed in cages on a light/dark cycle of 12 h in an experimental room with controlled temperature and humidity (25 °C; 70% respectively), fed with commercial feed and water *ad libitum*. All the animals were submitted to a period of 15 days for adaptation. *The procedure was approved by the Animal Welfare Committee of The Federal University of Santa Maria, under protocol number 065/2012.*

Experimental design

Rats were divided into two groups with eight animals each: group A was used as a negative control (uninfected animals), while group B was used as a test group (animals infected by *T. evansi*). The infection was induced intraperitoneally with 0.1 mL of blood from rat (R_1) containing 2.7×10^6 trypanosomes (Day 0).

Parasitemia evolution and sampling

The rats were observed during 5 days with the evolution of parasitemia monitored daily through blood smears. For this procedure, each slide was prepared with fresh blood collected from the tail vein, stained by the panoptic method, and visualized at a magnification of 1000× according to the methodology described by Da Silva et al. [11]. On day 5 PI the animals were anesthetized in a chamber with isoflurane for blood sampling (an average of 7 mL per animal by intra-cardiac puncture of the left ventricle) using syringes of 0.7×25 mm and 22 gauge needles (BD Preset Eclipse®). A part of the blood was stored in tubes with ethylenediamine tetraacetic acid (EDTA) for hematological analyses and other part was stored in sodium fluoride for lactate and gas analyses. All analyses were immediately performed using fresh samples. After collection, the animals were decapitated as recommended by the Ethics Committee.

Hematological analyzes

The hematocrit was determined by centrifugation of microhematocrit tubes in a microhematocrit centrifuge (Sigma Laborzentrifugen, Osterode am Harz, Germany) for 5 min at 19,720*g*. Erythrocytes count and hemoglobin concentration were determined using an electronic counter (CELM CC-550).

Blood gas analyses and other components involved in the acid–base status

The samples were stored in a cold water bath (0 °C) and they were analyzed within 45 min as recommended by Takada et al. [12]. Initially, the negative logarithm of hydrogen ions (pH) activity in a blood gas analyzer (OMNI C® Roche Diagnostics, Brazil) was performed. Subsequently, the other variables were determined using the Vitros 250 analyzer (Ortho-Clinical Diagnostics) by the method of dry chemistry. The pH (hydrogen potential), pCO_2 (partial dioxide the carbon pressure), pO_2 (partial oxygen pressure), BE (bases excess), BEecf (base excess in the extracellular fluid), $cHCO_3$ (bicarbonate), sO_2 (oxygen saturation), tCO_2 (blood total dioxide the carbon), Na (sodium), K (potassium), Ca ionic (calcium ionic), Cl (chlorides), and lactate were carried out in all blood samples.

Statistical analysis

Data of blood gas analyses and other components involved in the acid–base status were first analyzed descriptively; measures of central tendency and dispersion were computed. Further, all variables were submitted to Shapiro and Wilk's test. Since

most of the data did not meet the assumption of parametric testing, the nonparametric test for two independence groups Mann–Whitney test was used. It was considered statistically different when p-value was < 0.05. The Spearman correlation was also conducted to identify the relation between blood gas levels and hemogram variables.

Results

Hemogram results are shown in Table 1. The animals of group B showed high parasitemia with an average of 30 trypanosomes per field at 1000×. The pre-patent period was 24 h PI with the infection showing a progressive course throughout the experimental period. The parasitemia of the infected animals was on average 1, 3, 8, and 30 trypanosomes per field on days 1, 2, 3, and 4 PI, respectively. Infected animals showed significant decrease in hematocrit, total erythrocytes, and hemoglobin compared to uninfected animals ($P < 0.05$). Infected rats showed no apparent clinical signs, compared to uninfected.

Data of blood gas levels are shown in Table 2. The pH, pCO_2, BE, BEecf, $cHCO_3$, and tCO_2 levels significantly decreased in infected animals ($P < 0.05$) compared to uninfected. On the other hand, chlorides and lactate levels significantly increased ($P < 0.05$) in infected compared uninfected animals. The results of K^+, pO_2, sO_2, Na, and ionic Ca did not differ between groups ($P > 0.05$). The correlation results did not suggest a strong relation among tested parameters. A specific study should be designed to address these matters.

Discussion

T. evansi infection causes various pathological changes in naturally or experimentally infected animals. The pathological findings and clinical signs are due to direct and/or indirect consequences of the parasite [3,6,7,13]. Therefore, our hypothesis is that the infection caused by *T. evansi* alters the acid–base balance, which was demonstrated in the current study by changes in the blood gas parameters. Arterial blood analysis is an effective way to verify oxygenation, same as metabolic parameters (related to acid–base status), a useful tool for clinicians to prescribe proper therapy [14]. In this context, we are able to conclude that changes in the acid–base status could contribute to the disease pathogenesis as a consequence of some disorders such as anemia, where erythrocyte reduction may lead to tissue hypoxia.

It was observed that rats infected by *T. evansi* had a decrease in base excess (BE) in the extracellular fluid and an increase in the lactate levels ($P < 0.05$). An imbalance between oxygen supply and consumption may lead to an anaerobic metabolism and, as a consequence, lactic acidosis [15]. The lactate level is useful in the early detection of tissue hypoxia, preventing progressive organ dysfunction and even death [16]. Persistent high levels of lactate are considered better predictors of mortality than other variables that measure the oxygen transport [17]. The increase of lactate in this study corroborates with findings from other publications that have described a situation of oxidative stress in rats infected by *T. evansi*, which also contributed to anemia [18,19].

Table 1 Median and range (minimum and maximum values) of hematocrit, total erythrocytes count, and hemoglobin concentration in rats experimentally infected by *T. evansi*.

Variables	Group A (uninfected)	Group B (infected)	P-value
Hematocrit (%)	38.0 (37–40)	31.0 (24.8–34)	0.002
Total erythrocytes (×10^6/μL)	7.3 (7–7.6)	6.3 (4.4–6.5)	<0.001
Hemoglobin (g/dL)	12.9 (12.8–13.4)	11.6 (7.9–12.4)	0.002

Table 2 Distribution of studied parameters represented by its median as central tendency measure and range (minimum and maximum values) for dispersion of the blood gas levels in rats experimentally infected by *T. evansi*: pH (hydrogen potential), pCO_2 (partial dioxide the carbon pressure), pO_2 (partial oxygen pressure), BE (bases excess), BEecf (base excess in the extracellular fluid), $cHCO_3$ (bicarbonate), sO_2 (oxygen saturation), tCO_2 (blood total dioxide the carbon), Na (sodium), K (potassium), Ca ionic (calcium ionic), Cl (chlorides) and lactate.

Variables	Group A ($n = 8$; uninfected)	Group B ($n = 8$; infected)	P-value
pH	7.9 (7.6–7.8)	7.7 (7.7–8.1)	0.001
pCO_2 (mmHg)	21.2 (17.6–25.6)	9.8 (6.8–17.0)	<0.001
pO_2 (mmHg)	199.2 (184–213.1)	193.6 (186.7–205.4)	0.56
BE (mmol/L)	7.7 (2.8–10.3)	3.6 (0.2–6.5)	0.01
BEecf (mmol/L)	5.5 (3.8–7.6)	−0.4 (−3.9 to 2.6)	<0.001
$cHCO_3$ (mmol/L)	26.0 (18–29.1)	16.7 (13.3–19.8)	0.02
sO_2 (%)	99.9 (99.8–99.9)	99.9 (99.9–99.9)	0.07
tCO_2 (mmol/L)	26.7 (23.1–29.4)	17.0 (13.5–20.2)	<0.001
Na (mmol/L)	138.7 (137.1–143.6)	143.1 (137–150.2)	0.37
K^+ (mmol/L)	4.9 (3.9–5.5)	5.6 (4.5–7.9)	0.06
Ca ionic (mmol/L)	1.0 (0.8–1.1)	0.9 (0.7–1.1)	0.13
Cl (mmol/L)	98.9 (97.9–103.8)	104.9 (99.7–109.6)	0.002
Lactate (mmol/L)	6.3 (5.0–8.5)	10.3 (7.8–14.2)	<0.001

According to Rose [20], the accumulation of lactate, is the most common cause of metabolic acidosis. Animals of the group B (infected) showed decrease in cHCO₃ levels, and this bicarbonate reduction may lead to metabolic acidosis [20]. Metabolic acidosis is a pathological process characterized by an increased acid concentration in the extracellular fluid, evidenced in this study by decreasing the base excess in the extracellular fluid, leading to bicarbonate, pH, and pCO$_2$. Drops in pH stimulate the respiratory center to increase alveolar ventilation, reducing to pCO$_2$, and attenuating the acidosis. While this is a positive physiological response, maintaining this prolonged hyperventilation causes muscle fatigue, which can be related to one of the symptoms of the disease, weakness of the lower limbs, which gives the name of the disease ("*mal das cadeiras*" or bad hind limbs). The main consequences of acidemia are weakness, inhibition of brain metabolism, and liver and kidney damage [21], observed in the *T. evansi* infection [22,23].

Conclusions

Based on these facts, *T. evansi* infection was able to cause alteration in the acid–base balance in rats, a condition correlated with the respiratory alkalosis. However, it was difficult to clarify whether these changes were a cause or a consequence of *T. evansi* pathogenesis. Therefore, more studies are needed focusing mainly on these variables and in animals chronically infected by *T. evansi*.

Conflict of Interest

The authors have declared no conflict of interest.

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