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Diminazene aceturate in the control of *Trypanosoma evansi* infection in cats

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ABSTRACT

The aim of this study was to investigate the efficacy of diminazene aceturate in the control of the infection by *Trypanosoma evansi* in cats. Fourteen animals were infected with 10^8 trypomastigote forms each and six were used as negative control (group A). Seven of the infected cats were used as positive control (group B) and seven were treated with diminazene aceturate (3.5 mg kg^{-1}) for 5 consecutive days (group C). Biochemical and hematological parameters were evaluated during the experiment. Blood with anticoagulant was collected at day 49 post-inoculation and preserved in ethanol for DNA extraction. Samples were analyzed using PCR *T. evansi*-specific to assess the effectiveness of treatment. The treatment with diminazene aceturate had an efficacy of 85.7%. Alanine aminotransferase, gamma-glutamyltransferase, urea, and creatinine values remained within the normal physiological range in the treated cats. Hemogram was normalized in all the cured animals. Therefore, the therapy used is effective in controlling *T. evansi* in cats.

1. Introduction

Among the pathogenic trypanosome species, *Trypanosoma evansi* (Trypanosomatidae, Kinetoplastida) is known to have a large diversity of mammalian hosts and is an important disease producing agent throughout the tropical and subtropical areas of the world (Hoare, 1972; Lun and Desser, 1995; Herrera et al., 2004). Outbreaks of disease caused by *T. evansi* are associated mainly with the rainy season, when tabanids are abundant (Silva et al., 1995; Dávila et al., 1999). *T. evansi* is mechanically transmitted by biting insects including Tabanus, Cryptotylus and Stomoxys species (Vokaty et al., 1996).

The syndromes associated with the infection caused by this parasite vary from chronic to acute and fatal, with clinical signs as progressive weakness, emaciation, fever, anemia, and death (Camargo et al., 2004). In Brazil, two forms of the disease are described due to *T. evansi* infections: acute syndrome that produces early death in horses and dogs if untreated, and chronic, affecting mainly capybaras (*Hydrochaeris hydrochaeris*) and coatis (*Nasua nasua*) (Franke et al., 1994; Silva et al., 1995; Aquino et al., 1999; Herrera et al., 2002). Studies involving felines infected with *T. evansi* are rare, and hematological and pathological alterations due to the parasitism and treatment are not mentioned (Choudhury and Misra, 1972; Dakshinkar et al., 2002; Tarello, 2005).

Absolute control of animal trypanosomosis cannot be achieved with the available current methods, which are inadequate to prevent the enormous socio-economic losses caused by this disease. These methods include treatment with trypanocides, livestock animals that are more resistant to disease, reduction of the proximity of livestock to reservoir hosts, and the control of the population of vectors by spraying insecticides or by trapping (Taylor, 1998; Camargo et al., 2004).

Treatment of trypanosomiasis relies on the use of diminazene aceturate which is effective for the treatment

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of disease in cattle, buffalo, sheep, pigs and camels (Peregrine and Mamman, 1993; Sirivan et al., 1994). However, single doses of medicine are not effective for horses, mules and dogs (Tuntasuvan et al., 2003; Colpo et al., 2005).

Diminazene aceturate either does not cross the blood–brain barrier or does in insufficient doses to control the *T. evansi* infection (Jenings et al., 1977; Spinosa et al., 1999). To address this issue, we increased the dose until a non-toxic protocol was obtained. Thus, the aim of this study was to investigate the efficacy of diminazene aceturate in the infection by *T. evansi* in cats.

2. Materials and methods

Twenty adult female non-breeding *Felis catus*, weighing between 1912 and 2561 g were used. We chose to use cats in our study due to the facility to handle the animals and the lack of studies involving the trypanosomosis in this species. 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*. All animals received a formulation containing pirantel pamoate, praziquantel and fenbendazole, and were submitted to a period of 30 days for adaptation. Hematological and biochemical examinations were performed three times at 14 day intervals. The evaluated parameters were inside the normal range (Bush, 2004).

Cats were divided into three groups, a negative control group (A: six animals), a positive control group (B: seven animals) and a group medicated with diminazene aceturate (C: seven animals). Cats from groups B and C were intraperitoneally inoculated with a strain of *T. evansi* (day 1) that had been obtained from a naturally infected dog (Colpo et al., 2005) and had been kept in liquid nitrogen. The infected groups received 10^8 infectious trypomastigote forms of *T. evansi* whereas the group A received a physiological solution.

Cats of group C were treated with intramuscular diminazene aceturate at a dose of 3.5 mg kg^{-1} for 5 consecutive days, at 24-h intervals (Da Silva et al., 2008a). The treatment started at day 8, when the infected animals showed parasitemia (1–3 trypanosomes per microscopic field at 1000× magnification) and mild anemia. Parasitemia was estimated daily by microscopic examination of smears. Each slide was mounted with blood collected from the tail vein, stained by the panoptic method and visualized at a magnification of 1000×.

Hematological and biochemical examinations were performed to monitor the toxicity of the drug. Blood samples were collected at days 0, 7, 10, 14, 35 and 49 post-inoculation (PI) by jugular puncture after anesthesia with ketamine (0.08 mL kg^{-1}) and xylazine (0.05 mL kg^{-1}). Samples were kept in tubes containing ethylenediamine tetraacetic acid (EDTA) and in tubes without anticoagulant. The hematological parameters evaluated were hematocrit (Ht), total red blood cell count (RBC), hemoglobin (Hb), and white blood cells (WBC). Alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), urea, and creatinine tests were performed to assess toxicity of the diminazene aceturate.

The efficacy of the treatment was evaluated by collecting blood samples at day 49 PI. Samples were collected with anticoagulant and preserved in ethanol (v/v) for DNA extraction and posterior analysis using PCR *T. evansi*-specific (Ventura et al., 2002). Felines were monitored for 70 days after the end of the experiment.

The data were submitted for the analysis of variance (ANOVA) followed by the Tukey's test ($P < 0.05$) (Silva and Azevedo, 2002). The procedure was approved by the Animal Welfare Committee of Federal University de Santa Maria (UFSM), number 23081.002891/2008-47, in accordance to Brazilian laws and ethical principles published by the Colégio Brasileiro de Experimentação Animal (COBEA).

3. Results

Examination of the peripheral blood smears showed a prepatency period between 24 and 48 h in the infected cats, with the peak of parasitemia recorded at day 5 PI (10–15 trypanosomes per microscopic). Thereafter, irregular waves of parasitemia were observed, ranging from zero to three trypomastigotes per microscopic field. Ht, RBC, Hb showed a drop in all of the infected animals from the seventh day PI (Table 1). Clinical alterations such as apathy, hyporexia, and vomiting were observed in all of the infected cats; diarrhea was observed in nine animals of groups B and C.

The diminazene aceturate was effective 24 h after the beginning of the therapy, due to the absence of trypanosomes in peripheral blood smears of the treated cats, in opposite to the positive control group. After the treatment and until the end of the experiment, blood smear examination of the animals from group C resulted negative for *T. evansi* and characteristics of clinical signs of the disease were not observed anymore. However, clinical alterations such as anorexia, dehydration, lymphadenopathy, pale and congested mucosa, and cachexia were observed in all cats of group B. Conjunctivitis, unilateral corneal opacity, abdominal breath, edema of the face tissues, eyelid and limbs, dry fur, alopecic areas in the face, external and internal abscesses and instability of the hind limbs were observed in two or three of the felines from group B. Moreover, three animals of this group died during the experimental period (days 40 and 56).

A mild anemia was observed at day 7 in all of the parasitized animals (groups B and C) ($P < 0.001$). Animals from group B kept low hematocrit levels (23.5–25%) during all the experiment, whereas felines from the treated group (group C) recovered from anemia on days 35 and 49 PI (Table 1). WBC were increased at day 49 in groups B and C. Signs of intoxication due to the treatment were not observed. This hypothesis was confirmed by ALT, GGT, urea and creatinine tests, which remained normal in all groups (A–C).

Six (85.7%) of the treated animals were PCR negative for *T. evansi*. One of the cats (14.3%) was PCR positive, which was also evidenced by the presence of flagellates in peripheral blood smears and clinical signs such as apathy, alopecia, edema of the members and tail, loss of weight, jaundice and anemia. Hematocrit showed a drop from 31 to 22% 70 days after treatment, when she was in period of observation.