EXPERIMENTAL INFECTION WITH *TRYPANOSOMA EVANSI* IN HORSES: CLINICAL AND HAEMATOLOGICAL OBSERVATIONS

L.C. MARQUES*; R.Z. MACHADO; A.C. ALESSI; L.P.C.T. AQUINO & G.T. PEREIRA

Faculdade de Ciências Agrárias e Veterinárias, Unesp, Rodovia Paulo Donato Castelani, km 05, 14884-900, Jaboticabal, SP, Brasil.
(*) Corresponding author. Fax: (016) 322 4275. E-mail: lmarques@fcav.unesp.br

SUMMARY: This study was conducted to evaluate clinical and haematological features of the disease in horses. Six adult horses were experimentally infected with tripomastigota forms (10^6 parasites) of *Trypanosoma evansi* strain isolated from a naturally infected dog. Three other adult horses served as negative controls. Clinical signs were recorded twice a day, during 11 consecutive weeks and blood analysis was performed once a week. Hyperthermia was recorded as early as the 2nd day, but the highest individual rectal temperature values were more often observed between the 2nd and 3rd weeks post inoculation. It was characterized by intermittent fever in the inoculated animals through the observation period. Visible mucous membranes were pale or yellowish, and petechial hemorrhages were seen in conjunctivae. Edema, enlargement of lymph nodes, weight loss, progressive weakness, cough and incoordination affecting the hind limbs, were other additional signs observed in the inoculated horses. Red blood cell counts, packed cell volume and hemoglobin values decreased until the 3rd week post inoculation. After that, small variations on these values were observed and anemia was persistent. No defined pattern was detected for white blood cell count values, but leukocytosis with neutrophilia and relative lymphopenia was demonstrated by some horses during the experimental period. Icterus index values increased until the 7th week post inoculation and decreased after the 9th week post inoculation. Total protein values for the infected group of horses did not differ significantly from the non-infected group.

KEY WORDS: *Trypanosoma evansi*; trypanosomiasis; horses; clinical signs.

INTRODUCTION

Trypanosoma evansi is widely distributed in the Americas (WELLS, 1984). In South America, the parasite causes a chronic disease in horses known as "mal das cadeiras", "murina", "derrengadera", "peste boba", and "quebra bunda" (CLARK & DUNN, 1933; HOARE, 1972).

In Brazil, the geographical and ecological characteristics enhance the spread of trypanosomiosis to endemic proportion in the Pantanal of Mato Grosso, where it remains a major problem in horses. The parasite is also found in capybaras, coatis and dogs (NUNES & OSHIRO, 1990; NUNES *et alii*, 1993; SILVA *et alii*, 1995).

The course of the disease lasts one week to six months (WOO, 1977) and usually results in emaciation and death. The pre-patent period varies from 4 to 13 days (RAMIREZ *et alii*, 1979) and parasitaemia displays an undulating course. Main clinical signs include fever, paleness of mucous membrane, icterus, enlargement of lymph nodes, weight loss and edema of the lower parts of the body (HÖRCHNER *et alli*, 1983; MONZON *et alli*, 1984). In chronic stages of the disease, animals manifest progressive weakness and motorial disturbances (MONZON *et alli*, 1984). Anemia is the most consistent pathological feature of the infection in horses (SILVA *et alli*, 1995) while WBC have not shown a defined trend. Bilirubinemia and hypoglycemia are commonly reported (BREM *et alli*, 1984; MANOHAR *et alli*, 1984b; MONZON *et alli*, 1990). Most studies on equine disease demonstrates normal total serum protein concentration, even though increased values have been reported by some investigators (KILLICK-KENDRICK, 1964; RAZA *et alli*., 1981).

This research aimed to study clinical, parasitaemic and haematological changes throughout the course of *T. evansi* experimental infection.

MATERIALS AND METHODS

Animals and infection

Nine adult male and female healthy cross-bred horses, 3-10 years old were used. Three of them were used as control animals

(horses 7, 8 and 9) and the other six (horses 1, 2, 3, 4, 5 and 6) were experimentally infected with *T. evansi*. Horses were inoculated intravenously with a tripomastigota forms (10^6 parasites) of *T. evansi* isolated from a naturally infected dog by MOREIRA & MACHADO (1985). After inoculation, the tripomastigota forms were standardized in Neubauer chamber. Sera of the horses was tested by the Indirect Fluorescent Antibody Test (IFAT) and none of the horse utilized presented any anti-*Trypanosoma evansi* antibodies before inoculation.

Clinical and parasitological evaluations

Physical examination included: rectal temperature, heart rate, respiratory frequency, status of visible mucous membrane and superficial lymph nodes intermandibular, retropharyngeal, superfical cervical, subiliac, supramammary and superficial inguinal, and every other clinical alteration observed. These data were recorded twice a day until 67 day after inoculation (DAI) in horse 3 and until 77 DAI in remainder animals. Parasitaemia was estimated daily for the first four day of infection, and after that, at four days intervals or every time infected animals presented hyperthermic. Tripomastigotes were counted in 500 microscopic fields of May-Gruenwald-Giemsa stained blood smears using immersion objective (1000x).

Hemogram and biochemical evaluation

Hemogram, icterus index, and total seric protein values from all experimental animals were determined at weekly intervals. Red cells count, total leukocyte count and haemoglobin determination were performed using an automated blood cell counter (CC-510 - CELM, Barueri, SP) annexed to a haemoglobinometer (HB-520 - CELM, Barueri, SP) and differential white cell were counted in May-Gruenwald-Giemsa stained blood smears. Packed cell volume (PCV) was determined by the microhaematocrit method as described by SCHALM *et alli* (1975) and total protein concentration was determined by biuret reaction according to DITTENBRANDT (1948).

Statistical analysis

Data were analyzed using a randomized design. Tukey test was used to compare data obtained from the two groups at each observation time. Results were considered to be significant at the $p < 0.05$ level.

RESULTS

Statistically significant increase ($p < 0.01$) in mean rectal temperature of infected animals was first observed two day after inoculation. From then on, hyperthermic peaks of short duration (observed in only one of the daily recorded temperatures) and of long duration (observed at least in two consecutive temperature records) occurred in different days for each animal throughout the observation period.

All infected animals displayed progressive emaciation, jaundice, mucosal pallor, enlargement of superficial lymph nodes and submandibular edema. In addition, it could be observed hemorrhage suffusions in the conjunctiva, motor incordination of hind limbs (horses 3, 4 and 6), reduced appetite (horses 1, 2, 3 and 4), lateral recumbency (horses 3 and 4), preputial edema (horse 1, 4 and 6), edema of lower abdominal wall (horse 6) and cough (horse 2). At a later stage of the disease, horses 3, 4 and 6 were reluctant to move and, when guided, they revealed pronounced hind-quarters weakness with ataxia and incoordination of the hindlimbs, demonstrating staggering gait with unsteady and irregular steps. When subjected to physical exertion they readily fell down and assumed a "dog sitting position", and the horse was unable to support weight on the hindlimbs. After some minutes, despite of great difficult they could get up and remain in standing position.

The mean values for haematological parameters of infected horses are shown in the Table 1. No changes in haematological values were observed in control group. Red cells count, packed cell volume and hemoglobin concentration values of infected group decreased about 35% in three weeks of infection and thereafter small variations were observed, but the animals remained anemic until the end of the observation period.

Total white cell counts in infected animals revealed no significant changes, eventhough some of them (horse 1, 3 and 4) demonstrated leukocytosis in variable times during the infection. Statistical significant neutrophilia and relative lymphocytopenia were detected on 7th DAI ($p < 0.05$). Mean icterus indices of infected horses were increased between the 1st and 7th weeks after inoculation ($p < 0.01$). After the 9th week, individual analysis revealed decreases in icterus indices of horses 1, 2, 3 and 4.

Parasitaemia data are shown in Figure 1. Parasites were detected in blood of infected horses on the 4th DAI and highest mean parasitemic peaks were observed along the first seven weeks of infection.

Table 1 - Mean haematological values of horses infected with *Trypanosoma evansi*.

WAI	RBC count (X 10^6 ml)	PCV (%)	Hb (g/dl)	WBC count (X 10^3 ml)	TP (g dl)	I (Units)
0	6.12^{ns}	31.66^{ns}	12.29^{ns}	10.15^{ns}	6.88^{ns}	13.34^{ns}
1	5.65^{ns}	26.66^{a}	10.43^{ns}	9.86^{ns}	6.88^{ns}	24.96^{a}
2	4.49^{ns}	21.33^{b}	9.35^{ns}	9.05^{ns}	6.48^{ns}	32.37^{b}
3	4.03^{b}	20.50^{b}	8.20^{a}	10.60^{ns}	6.91^{ns}	31.10^{b}
4	3.53^{b}	21.66^{b}	8.25^{b}	10.13^{ns}	7.36^{ns}	24.85^{b}
5	3.24^{b}	18.50^{b}	7.91^{b}	10.28^{ns}	6.68^{ns}	21.17^{ns}
6	3.44^{b}	19.50^{b}	7.63^{b}	10.43^{ns}	6.86^{ns}	21.18^{b}
7	3.43^{b}	20.66^{b}	7.81^{b}	10.63^{ns}	7.20^{ns}	25.85^{b}
8	3.86^{b}	19.83^{b}	8.20^{a}	11.45^{ns}	7.53^{ns}	13.26^{b}
9	3.50^{b}	19.16^{b}	7.48^{b}	10.25^{ns}	7.85^{ns}	6.34
10	2.77^{b}	18.60^{b}	6.54^{b}	9.52^{ns}	7.81^{ns}	8.39^{ns}
11	3.48^{b}	18.80^{b}	6.74^{b}	10.54^{ns}	7.58^{ns}	6.70^{ns}

WAI: week after inoculation, RBC: red blood cell, PCV: packed cell volume, Hb: hemoglobin, WBC: white blood cell, TP: total protein, II: icterus index, ns: not significant difference; a: $p < 0.05$, b: $p < 0.01$.

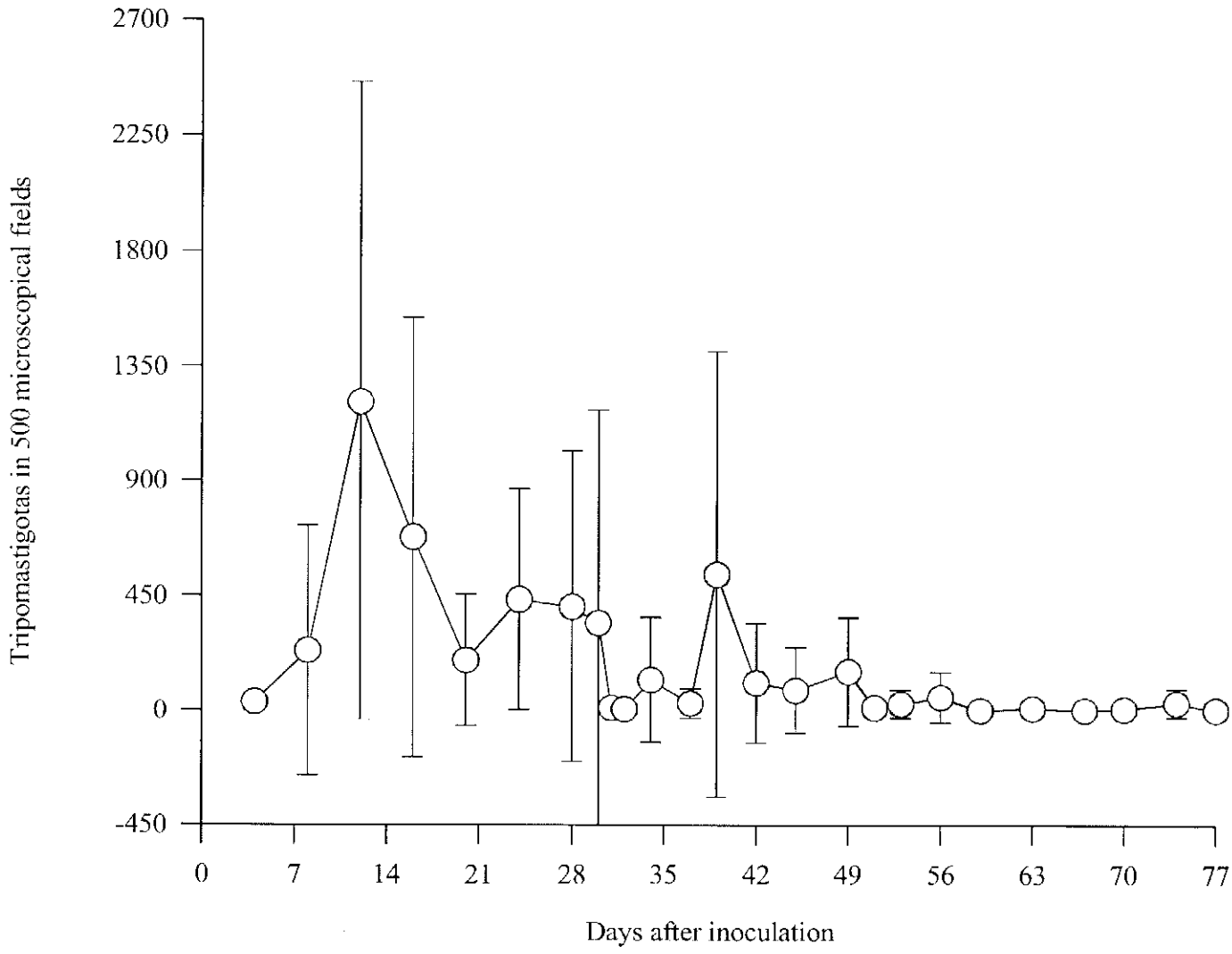

Fig. 1. Mean parasitemic outbreaks (± SE) examined in blood smears of horses infected with *Trypanosoma evansi* (tripomastigotas number in 500 microscopical fields using immersion objetive 1000X).

DISCUSSION

Intravenous or subcutaneous inoculation of 50 to 100000000 tripomastigotas per animal have been successfully used in experimental infection of horses with *T. evansi* (HÖRCHNER *et alli*, 1983; BENITEZ *et alli*, 1984; MANOHAR *et alli*, 1984ab; MONZON *et alli*, 1984, OSHIRO *et alli*, 1989; MONZON *et alli*, 1991). The inoculum of 1000000 parasites was able to infect horses in this experiment. It can be evidenced by the presence of parasitaemic peaks and complex development of clinical changes that evolved to the presence of nervous symptoms in 50% of inoculated animals. The presence of tripomastigotes in the blood was observed within four day of infection in five inoculated horses, corroborating with the prepatent period reported in naturally (RAMIREZ *et alli*, 1979) and experimentally infected horses (BENITEZ *et alli*, 1984).

Peaks of fever (41.5°C) in individual animals were more frequently noticed between weeks one and three after experimental inoculation. Periods of fever and normothermia with remission phases happened in turns characterizing an intermittent fever in all infected horses. This finding is in agreement with those reported by OSHIRO *et alli* (1989). The correlation between high temperatures and intensity of parasitaemia was not evident in this experiment, particularly in chronic stages of the disease, similarly as reported by MONZON *et alli* (1984).

Increases in heart and respiratory rates of inoculated horses were noticed within the 2nd and 3rd DAI, respectively. Comparative analysis of mean rectal temperature, heart rate and respiratory frequency curves in experimental group demonstrated to be higher than in control horses. Compensatory tachycardia and tachypnea have been reported in infected horses (BOERO,

1974; HÖRCHNER *et alli,* 1983). Pale or yellowish mucous membrane, edemas, enlargement of lymph nodes, emaciation and progressive weakness were consistent clinical signs evidenced in all infected animals in this research and corroborate with those previously reported in infected horses (HÖRCHNER *et alli,* 1983; MONZON *et alli,* 1984; OSHIRO *et alli,* 1989; SILVA *et alli,* 1995). Nervous symptoms affecting mainly the hindquarters were observed in 50% of inoculated horses after 11 weeks of infection. Motorial disturbances affecting hindlimbs of *T. evansi* infected horses have been firstly described by ELMASSIAN (1902). However, more detailed studies aiming to elucidate the origin of such disturbs have not been conducted up to now. According to KRANEVELD & DJAENOEDIN (1949), it is likely that *T. evansi* invade CNS in an early stage of infection without necessarily causing immediate nervous symptoms. In experimentally infected horses, HÖRCHNER *et alli,* (1983) observed at 25 DAI locomotor disturbs associated to the presence of trypanosomes in the cerebrospinal fluid. Also in experimentally infected horses MONZON *et alli* (1984) isolated *T. evansi* from the cerebrospinal fluid by the 7th DAI when no apparent nervous signs could be detected.

The alterations in the haematological indices observed during the course of the infection are consistent with the findings of previous workers using horses infected with *T. evansi* in which falls in hematocrit, erythrocyte counts and hemoglobin concentration were described. Anemia was a consistent finding in all infected horses throughout the experimental period. Despite being a significant feature of the disease, the origin of the anemia in trypanosomiais is not completely elucidated. Evidences suggest that its etiology is multifactorial and hemolysis, hemodilution and disorder and/or noncompensatory erythropoiesis are some of the mechanisms proposed (JENKINS & FACER, 1985). No significant change in total leukocyte count was detected in infected horses, eventhough individual analysis revealed that some of them presented leukocytosis in variable days. Moreover, leukocyte changes were more frequently observed in infected horses than in control ones. MANOHAR *et alli* (1984a) reported leukopenia with monocytosis in experimentally infected horses. MONZON *et alli* (1991) observed leukocytosis with lymphocytosis until 40 day and relative neutrophilia until 105 day of experimental disease of a horse. These findings reveal that there is not a defined trend for leukocytes changes in horses infected with *T. evansi.*

Increases in icterus index can be observed in cases of hemolysis, biliary obstructions and destruction of hepatocytes while decreases in this parameter occur in cases of bone marrow depression. In the present study, increases in icterus index occurs coincidently with high parasitaemia periods, suggesting that jaundice is consequent to hemolytic action of the parasites.

No statistically significant changes were detected in total seric protein levels, corroborating with the observations previously made by WINKLER (1982), BREM *et alli* (1984), MANOHAR *et alli* (1984b) and MONZON *et alli* (1990) and differing from those observations made by BOERO (1974) who states that a significant decrease in plasma protein occurs in chronic stage of the disease, leading to the outcoming of hypoproteinemic edema.

We conclude that the clinical signs and the haematological features of the horses infected by *T. evansi* are quite variable and depend largely on the stage of the disease process.

SUMÁRIO

Objetivando estudar a evolução clínica e as alterações hematológicas, seis eqüinos foram inoculados individualmente, via intravenosa, com 10 x 10^6 tripomastigota sangüícolas de *Trypanosoma evansi*, provenientes de uma cepa criopreservada, originária de um cão naturalmente infectado. Três outros animais clinicamente sadios foram mantidos como testemunhos. Exames clínicos foram realizados duas vezes ao dia durante 11 semanas consecutivas. Ainda foram estudadas as alterações hematológicas, bioquímicas e parasitológicas. Hipertermia foi notada a partir do 2º dia após as inoculações, caracterizando febre do tipo intermitente. Membranas mucosas amareladas, alternadas com palidez, hiporexia, edemas, aumento de volume de linfonodos externos, emaciação, fraqueza progressiva, tosse, sufusões hemorrágicas nas conjuntivas e incoordenação motora foram outros sinais clínicos evidenciados. A presença de tripomastigotas sangüícolas foi observada a partir do 4º DAI, sendo os maiores picos parasitêmicos evidenciados durante as sete primeiras semanas de evolução. As contagens de hemácias, as determinações dos volumes globulares e dos teores de hemoglobina diminuíram até a 3ª semana após as inoculações, e subseqüentemente sofreram, em média, apenas pequenas variações. Os leucogramas revelaram não existir um padrão de resposta leucocitária bem definido em eqüinos portadores de infecção por *T. evansi*. Entretanto, as análises individuais revelaram, em alguns animais, em tempos variáveis, leucocitose com neutrofilia e linfocitopenia relativa. Os índices ictéricos mantiveram-se elevados entre a 1ª e 7ª semanas de evolução e diminuíram após a 9ª semana. Não se evidenciaram variações significativas nos níveis séricos de proteínas.

PALAVRAS-CHAVE: *Trypanosoma evansi*; tripanossomiases; eqüinos; sinais clínicos.

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