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Short communication

Chemotherapy of surra in horses and mules with diminazene aceturate

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Abstract

During June–July 2000, an outbreak of surra occurred on an equine breeding farm in Khonkaen Province, Thailand. Forty-two percent of pregnant mares aborted or gave stillbirth and 40% (19/47) of horses and 10% (1/10) of mules died from surra. In August 2000 *Trypanosoma evansi* were detected in the remaining animals (28 horses and nine mules) on the farm by blood smear and/or the haematocrit centrifuge technique. All animals were treated with diminazene aceturate at 3.5 mg/kg body weight by intramuscular injection on days 0 and 41 of the study. Blood samples of eight randomly selected horses and mules were collected on days 0, 1 and once a week until day 56 and examined for *T. evansi* by various parasitological techniques. The sera were tested for antibodies against *T. evansi* using an indirect enzyme linked immunosorbent assay (ELISA).

The results revealed that diminazene aceturate at 3.5 mg/kg appeared to be effective in the first treatment of horses and mules infected with *T. evansi*. Parasites were cleared from the peripheral blood of horses on days 1 and 7 and mules on days 1 and 14. Thereafter the number of positive animals increased. After the second treatment, 50% of horses and 25% of mules were still positive to surra 24 h after treatment demonstrating that diminazene had no protective effect. Mild to severe toxicity of diminazene was seen in the horses and mules after injection.

Keywords: Diminazene aceturate; *Trypanosoma evansi*; Surra; Horse; Mule; Toxicity; ELISA

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1. Introduction

Surra, caused by *Trypanosoma evansi*, was first detected in mules in Rachaburi Province, Thailand in 1949 (Sananraksat, 1949). Following this first case, many other outbreaks were reported by Kashemsant et al. (1989) reporting more than 92 outbreaks of surra in nine provinces in Northeast Thailand from 1984 to 1989. The infection rates with *T. evansi* in cattle, buffalo, horses and dogs using parasitological tests were 13, 20, 57 and 100%, respectively, while Nishikawa et al. (1990), using the indirect fluorescent antibody test (IFAT) found that the prevalence of surra in buffalo and cattle was 38.6 and 50%, respectively. Subsequently, surra has been reported throughout Thailand in horses, cattle, buffalo, pigs, dogs, cats, deer, hog deer (Tuntasuvan and Luckins, 1998) and elephants with varying clinical manifestations.

Treatment of surra relies on the use of diminazene aceturate which is effective for the treatment of surra in cattle, buffalo, sheep, pigs and camels (Peregrine and Mamman, 1993; Sirivan et al., 1994). Since 1998 diminazene aceturate (Berenil®, Hoechst, Germany) is the only trypanocide sold in Thailand and the objective of the study was to determine the activity of diminazene aceturate against *T. evansi* infection in horses and mules on an endemic farm in Thailand.

2. Materials and methods

2.1. Experimental design

During June–July 2000, an outbreak of surra occurred on a breeding equine farm in Khonkaen Province, Thailand where 42% of pregnant mares aborted or gave stillbirth. About 40% (19/47) of horses and 10% (1/10) of mules were suspected to have died from surra. In August 2000, blood samples of the remaining animals (28 horses and nine mules) on the farm were collected and examined for blood parasites at the National Institute of Animal Health (NIAH), Bangkok. *T. evansi* was detected by blood smear test and/or the haematocrit centrifuge technique (OIE, 1996). Animal body weights were determined using the Lincoln Equimeasure tape® (Hayward and Bower Ltd., Lincoln) and all animals treated with diminazene aceturate at 3.5 mg/kg body weight by intramuscular injection on days 0 and 41 of the study. Eight horses and mules at varying degree of parasitaemia were randomly selected for the study.

Blood samples of the selected animals were collected on day 0 (before treatment), day 1 and once a week from day 7 to 56. EDTA blood samples were examined for *T. evansi* using parasitological tests. Haematocrit values (%PCV) were also measured. Sera were separated from blood and assayed for antibodies to *T. evansi* using an indirect enzyme linked immunosorbent assay (ELISA). In addition, creatinine values were determined to assess the kidney function of the animals.

2.2. Parasitological examination

Blood samples were examined for *T. evansi* by thin blood smear test, haematocrit centrifuge test (HCT) and mouse inoculation test (MIT) (OIE, 1996).

2.3. Indirect ELISA

The *T. evansi* antigen preparation and indirect ELISA were conducted (Tuntasuvan et al., 1996). The antigen was a sonicated *T. evansi* NIAH41 strain collected from cattle in Thailand and the antigen protein concentration was 4.45 mg/ml.

The antigen was diluted in coating buffer (carbonate–bicarbonate buffer, Sigma C-3041) at 1:100 and filled 100 μl/well of microplate (MaxiSorp®, Nunc). Thereafter it was incubated at 4 °C overnight then washed with washing buffer (PBS-Tween20) three times. Serum was diluted in serum buffer (phosphate buffered saline, Sigma P-4711) at 1:50 and 100 μl/well was added in duplicate and incubated at 37 °C for 1 h. The microplate was washed with washing buffer three times. Protein G peroxidase conjugate (Zymed®) was diluted in conjugate buffer (phosphate buffered saline, Sigma P-4711) at 1:5000 and 100 μl/well added to the plate and incubated at 37 °C for 1 h. Tetramethyl benzidine hydrochloride (TMB) (Sigma T-3405) was used as substrate which was diluted in phosphate–citrate buffer with sodium perborate (Sigma P-4922) and added at 100 μl/well. After incubation at 37 °C for 15 min, 50 μl/well of 1 N sulfuric acid was added to stop the reaction with the OD read at 450 nm using ELISA reader (Biorad®).

In each plate there was a positive control serum, negative control serum and PBS. The positive control serum was from a horse infected with *T. evansi* in Aytthaya Province in 1994. The negative control serum was pooled sera of horses from Central Europe that attended the Asian Games held in Thailand in 1998.

2.4. Blood creatinine

Blood creatinine values were determined using the picric acid method (BMLab, 1987). All data were analyzed by Student's *t*-test using Epistat program.

3. Results

3.1. T. evansi detection

The results of various parasitological tests showed that *T. evansi* was cleared from the peripheral blood of all animals 24 h after the first treatment. *T. evansi* was detected again in horses and mules on days 14 and 21, respectively. Thereafter the number of positive horses and mules increased. On day 35, 87.5% (7/8) of horses and 25% (2/8) of mules were positive by parasitological tests. After the second treatment the number of positive horses decreased but *T. evansi* could be still detected in 50% of horses and 25% of mules on day 42. At the end of the study (day 56) 25% of horses and 12.5% of mules were *T. evansi* positive (Fig. 1). The detection of *T. evansi* in horses was found to be greatest with the MIT, followed by HCT and blood smear tests (Table 1).

3.2. Detection of T. evansi antibodies

By indirect ELISA on day 0 the mean OD values of the infected horses and mules were 0.447 and 0.579, respectively. Whilst the mean OD values of positive control serum and

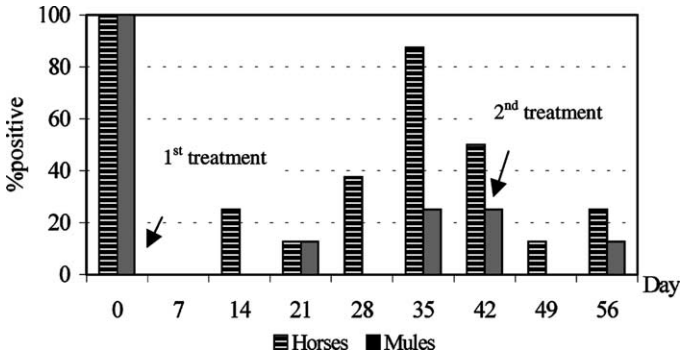

Fig. 1. Percentage of horses and mules in which *T. evansi* was detected by parasitological tests from day 0 to 56 before treatment.

negative control serum were 0.999 and 0.249, respectively. By the end of the study the mean OD values in horses was significantly higher than on day 0 ($P < 0.05$) and the highest mean OD value was 0.743 on day 42 (Table 2).

3.3. Clinical signs

Before treatment, the clinical signs of the infected horses and mules were fever, weakness, emaciation, a staring coat, anemia or icterus, edema of brisket and belly, lameness, but appetite. Some showed nervous signs; including restlessness and a circling gait. After the first injection of diminazene, five horses and one mule showed mild allergic signs. These were salivation, restlessness, recumbency and slight dyspnea. After 30 min the mule was normal, but not horses and these animals were given an injection of antihistamine (chlorphenilamine hydrochloride) to relieve allergic signs.

Table 1
Number of horses testing positive by detection of *T. evansi* in blood stream using various parasitological techniques

Collection time	Blood smear	HCT	MIT	Total positive samples
Day 0	6	7	8	8
Day 1	0	0	0	0
Week 1	0	0	0	0
Week 2	2	1	2	2
Week 3	1	1	1	1
Week 4	1	2	3	3
Week 5	3	5	7	7
Week 6	3	3	2	4
Week 7	0	1	1	1
Week 8	1	1	0	2
Total	17	21	24	28

Table 2
OD values of *T. evansi* antibodies in the horses and mules using an indirect ELISA

	Day								
	0	7	14	21	28	35	42	49	56
Horse (eight)									
Minimum	0.26	0.23	0.31	0.32	0.30	0.32	0.51	0.45	0.32
Maximum	0.64	0.65	0.67	0.74	0.62	0.69	1.07	0.91	1.01
Mean	0.45	0.46	0.52	0.51	0.47	0.57	0.74	0.66	0.68
S.D.	0.14	0.14	0.12	0.13	0.10	0.14	0.22	0.19	0.24
Mule (eight)									
Minimum	0.29	0.37	0.4	0.41	0.39	0.51	0.39	0.45	0.46
Maximum	0.90	0.89	0.87	0.69	0.75	0.72	0.79	0.63	0.77
Mean	0.58	0.67	0.67	0.59	0.62	0.62	0.54	0.54	0.59
S.D.	0.26	0.16	0.16	0.10	0.14	0.07	0.12	0.07	0.10

After the second treatment about 50% of horses and mules showed moderate to severe allergic signs. These were edema of the lips, salivation, recumbency, restlessness, dyspnea and some of the animals could not eat or drink. This lasted for 3 days. The sick animals were given injections of antihistamine. One horse on the farm died on the next day. At the end of the study the mules were in good condition but the horses were in poor condition. However no animals on the farm died from surra during the study.

3.4. %PCV

Before treatment the experimental animals were anemic. The mean %PCV of the horses and mules were 20.1 and 23.3, respectively. By day 7 the mean %PCV of the mules increased significantly to 30.1 ($P < 0.05$) whereas the mean %PCV of the horses on day 0 was not different from the other days ($P > 0.05$) (Table 3).

Table 3
%PCV of horses and mules infected with *T. evansi* and treated with diminazene

	Day								
	0	7	14	21	28	35	42	49	56
Mules (eight)									
Maximum	34	38	39	42	45	44	43	45	41
Minimum	17	24	25	25	26	25	20	23	22
Mean	23.3	30.1	31.4	34	36.4	35.6	32.6	32.8	31.6
S.D.	6.3	4.8	5.2	6.6	6.9	6.7	7.1	6.9	6.1
Horses (eight)									
Maximum	32	35	35	29	28	30	30	34	38
Minimum	11	13	15	18	12	15	14	14	11
Mean	20.1	22	24.5	22	21.5	22.8	22.4	22.9	24.3
S.D.	7.5	6.5	7.2	3.8	5.6	5.6	6.2	7	8.8

Table 4
Mean and S.D. of creatinine values (mg%) of the horses and mules[a]

	Day				
	1	7	14	21	28
Horses (eight)					
Mean	1.37	1.12	1.35	1.21	1.67
S.D.	0.19	0.35	0.39	0.22	0.24
Mules (eight)					
Mean	1.33	1.19	1.17	1.4	1.67
S.D.	0.30	0.20	0.22	0.46	0.21

[a] Values in parenthesis indicate the number of examined animals.

3.5. *Creatinine levels*

There were no significant differences in creatinine levels between serum collection dates for the treated horses and mules ($P > 0.05$) (Table 4).

4. Discussion

It was found that among various parasitological tests, MIT detected the greatest number of infections of *T. evansi* which corresponded to the study of Paris et al. (1982) and Tuntasuvan et al. (2000). However two negative samples by MIT at weeks 6 and 8 were positive by blood smear and/or MHCT (Table 1). Diaminazene aceturate at 3.5 mg/kg appeared to be initially effective in treatment of horses and mules infected with *T. evansi* because it cleared *T. evansi* from the peripheral blood of all animals on days 1–7 of the study. This differed from the second treatment when 50% of horses and 25% of mules were still positive to surra using parasitological tests 24 h after treatment.

Besides parasitological tests, it was shown that the indirect ELISA was a useful technique for the surveillance of surra in horses and mules. However, the sensitivity of the test varied from 37.5 to 100% due to the duration of *T. evansi* infection compared with the specificity of ELISA of surra in pigs which is 94.7%, respectively, (Tuntasuvan et al., 1996).

The dose rate for diminazene aceturate for the treatment of surra depends on the host species. Pholpark et al. (1984) treated experimentally infected buffalo with diminazene at 5 mg/kg but the parasites could be detected after treatment. In contrast, injection of diminazene at 3.5 mg/kg could clear sows infected with *T. evansi* on day 1 post-treatment (Sirivan et al., 1994). In China, suramin and diminazene have been the most important drugs used for treatment of surra. The dosage of diminazene was 3.5 mg/kg in horses (Lun et al., 1993). However, significant levels of resistance to diminazene have been reported for populations of *T. evansi* in horses in China (Zhang et al., 1992). In the present study diminazene had no protection efficacy in either horses or mules.

Leach (1961) reported that a single dose of 7 mg/kg could be highly toxic in camels and dogs. Whereas cattle can tolerate diminazene as high as 21 mg/kg without signs of toxicity (Fairclough, 1963). From the study toxicity of diminazene was found in the experimental

animals but the horses showed more severe systemic toxicity than mules. However diminazene had minor affection to the mean values of creatinine in the treated horses and mules, these values were in the normal range (creatinine = 1.2–1.9 mg%) (Kaneko, 1989).

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