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Efficacy of Cymelarsan® and Diminasan® against *Trypanosoma equiperdum* infections in mice and horses

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ABSTRACT

Trypanocidal sensitivity studies were conducted to assess the efficacy of Diminazene diaceturate (Diminasan®) and Bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) against *Trypanosoma equiperdum* (isolated from two mares with chronic cases of dourine) 713/943 and 834/940 Dodola strains in experimentally infected mice and horses. Diminasan® at doses from 3.5 mg/kg to 28 mg/kg and Cymelarsan® at doses of 0.25 mg/kg and 0.5 mg/kg body weight failed to cure any of the mice, indicating a clear dose dependent relationship in the mean time of relapse observed in mice. Indeed, mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. However, mice treated with Cymelarsan® at doses of 1.0 mg/kg and 2.0 mg/kg body weight were cured and no parasitemia was observed for 60 days. The efficacy of Cymelarsan® was also tested in horses. Two groups of horses containing two animals each were infected with *T. equiperdum* 834/940 Dodola strain and treated with Cymelarsan® at a dose rate of 0.25 mg/kg and 0.5 mg/kg, respectively. Cymelarsan® at 0.25 mg/kg and 0.5 mg/kg body weight cleared parasitemia within 24 h post treatment and none of the animals were found to show relapse throughout the 320 days of observation. The sensitivity of the particular trypanosome strain to Cymelarsan® was also supported by the relative improvement in the mean PCV levels of horses following treatment. A statistically significant difference ($p < 0.01$) in the mean PCV levels of horses treated with Cymelarsan® was observed between day 20 at peak parasitemia and days 40 as well as 60 of observation. The mean PCV levels of horses in the control group progressively decreased within the first 60 days of post infection. Two of the horses in the control group developed chronic form of dourine manifested by genital as well as nervous signs with progressive loss of body condition within 320 days post infection. The efficacy of Cymelarsan® against the chronic form of dourine was confirmed after treatment of one of the control horses with Cymelarsan® at a dose rate of 0.25 mg/kg body weight at day 282 post infection. It was noted that the treated horse improved overall body condition and clinical signs such as incoordination of hind legs, weakness and ventral oedema disappeared within 10 days of treatment. Thus, Cymelarsan® was found to be quite effective in curing horses in acute as well as chronic form of dourine. The results obtained from the present study will be important for designing effective control measures against dourine.

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1. Introduction

The causative agent of dourine *Trypanosoma equiperdum* differs from other mammalian trypanosomes due to the fact that it is primarily a tissue parasite and transmitted directly from one animal to another of the same species during coitus (Stephen, 1986). In practice, diagnosis is based on clinical evidence supported by serology (Alemu et al., 1997; Hagos, 2005). Since 1982 *T. equiperdum* has not been isolated in any country in the world. Moreover, most of the available *T. equiperdum* strains in different veterinary diagnostic laboratories are related to *T. evansi* rather than to *T. equiperdum* (Claes et al., 2003). Consequently, it appears difficult to identify which parasite is causing dourine. Diagnosis of the disease becomes more complicated in an area where the causative agents of surra or nagana occur. Moreover, isolation of *T. equiperdum*, the causative agent of dourine in horses, by standard parasitological techniques is usually difficult, due to low numbers of parasites in the blood or tissues fluids (Mulligan, 1970). Recently, the causative agent of dourine was isolated from two clinically sick horses in Dodola, Ethiopia. These horses were found to be positive in CATT/*T. evansi* (Bajyana Songa and Hamers, 1988) and RoTat 1.2 PCR (Claes et al., 2004) specifically developed for *T. evansi*. Yet, further analysis by RAPD (Claes et al., 2003) indicated that the Dodola strains have banding pattern similarity with *T. equiperdum* OVI strain, but not with the *T. evansi* strains tested. It can therefore be deduced that dourine in the Arsi–Bale highlands of Ethiopia is caused by *T. equiperdum* (unpublished data).

Nowadays, dourine is dealt with international legislative measures imposed by the World Organization for Animal Health (OIE) aimed at isolation, castration or slaughtering of complement fixation test (CFT) positive horses (Zabotskij et al., 2003). There are no officially approved drugs to treat horses suffering from dourine although some older publications mentioned experimental treatment of horses with suramin and neoarsphenamine (Novarserobezol; Ciuca, 1933) or quinapyramine sulphate (Vaysse and Zottner, 1950). Evidence from *in vitro* drug sensitivity tests on *T. equiperdum* (Zhang et al., 1991; Brun and Lun, 1994) indicate that suramin, diminazene, quinapyramine and cymelarsan are effective, although no reports on clinical efficacy have been published. Hence, further *in vivo* studies should be conducted using experimental infections with parasites isolated from cases of dourine.

In Ethiopia, horses are treated against dourine on irregular basis when trypanocidal drugs are available and even such treated animals show frequent relapses. A survey conducted in the Arsi–Bale highlands of Ethiopia revealed that 53/60 (88.33%) of the interviewed animal owners and professionals claimed that horses treated against dourine show frequent relapses and generally treatment is not effective enough to cure clinical cases (Hagos, 2005). Thus, in order to prevent the phenomenon of frequent relapses in dourine cases and maintain the efficacy of the available trypanocidal drugs, it is important that chemotherapeutic regimens are rationalized on the basis of the drug sensitivity of trypanosome strains in a given locality.

Introduction and adaptation of *T. equiperdum* to laboratory animals is difficult (Brun et al., 1998). Since *T. equiperdum* is a tissue parasite naturally found in equines, its establishment in the blood of laboratory animals is extremely difficult. Hence, animal inoculation is of little use as a routine method of diagnosis because it is very difficult and often impossible to obtain a first passage. Mice, rats, rabbits and dogs are susceptible to infection with *T. equiperdum*, once it has been adapted in laboratory animals. Different routes of infection such as subcutaneous, intra peritoneal, intravenous, intraurethral and intravaginal transmission, were tested and all gave rise to clinical signs of dourine (Barrowman, 1976; Stephen, 1986). However, in a recent report, blood and genital washes from antigenaemic horses did not lead to infections when inoculated into mice and puppies (Alemu et al., 1997; Hagos, 2005). Due to these described difficulties one of the goals of this study was to adapt *T. equiperdum* in mice and assessing the therapeutic efficacy of trypanocidal drugs in experimentally infected mice and horses.

2. Materials and methods

2.1. Parasite strains

Trypanosoma parasites were isolated by the Woo technique (Woo, 1970) from two naturally infected mares designated 713 and 834 with chronic clinical signs of dourine in Dodola district of the Bale highlands Oromyia Regional State, Ethiopia in August 2008. Once mares 713 and 834 were identified as parasitologically positive in Woo test, fresh whole blood was collected from the jugular vein of the horses using heparinized vacutainer tubes and venoject needles. 250 μl of whole blood was mixed with an equal amount of cryomedium and cryostabilates were prepared and kept both at −70 °C as well as in liquid nitrogen according to Maina et al. (2007). Different characteristic signs of dourine were observed in the two mares including vaginal oedema and discharge, presence of depigmented scars over the external genitalia, partial dragging and stiffness of the hind legs, incoordination and loss of body condition. Subsequently, mares 713 and 834 were transported to Debre Zeit and housed in a fly proof stable. In order to maintain these trypanosome strains, two stallions, healthy and parasitologically (Woo test) and serologically (CATT/*T. evansi*) negative were purchased from the central highlands of Ethiopia (Ginchi district: 75 km s west of Addis Ababa and Cheffe Donsa district: 32 km s north of Debre Zeit) and infected by intravenous route with 100 ml fresh whole blood obtained from mares 834 and 713, respectively. The above strains were conveniently named as 834/940 and 713/943 Dodola strains.

2.2. Drug sensitivity studies in mice

2.2.1. Mice adaptation of strains

Swiss white mice, 8 weeks old, weighing 20–25 g, were obtained from the breeding colony of the National Veterinary Institute (NVI) at Debre Zeit and maintained on a commercial pelleted ration and water *ad libitum*. They were housed in a conducive environment at the laboratory.

Ten mice were allotted to two groups and infected with blood containing 834/940 or 713/943 Dodola strains, respectively. The mice were daily injected intraperitoneal (i.p.) with 0.2 ml blood for seven consecutive days. In order to adapt the strains to mice the immune system was suppressed by daily administration of 0.33 ml of dexamethasone sodium phosphate (Dexamethason®, Tal: Sanand, Dist: Ahmadabad 382 210, Indiana) i.p. for seven consecutive days immediately following infection of the mice.

2.2.2. Experimental design

Drug sensitivity studies were conducted on mice infected with 713/943 or 834/940 Dodola strains using Bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®, Lot B 09108A, MERIAL-17, rue Bourgelat 69002 Lyon–France) and Diminazene diaceturate (Diminasan®, Batch DG/20337 Kuipersweg 9, 3449 JA Woerden, Holland). For each drug, sensitivity studies were performed on 20 mice randomly divided into four experimental groups of five animals each. Four groups (I–IV) formed the infected groups treated with different doses of trypanocidal drug. Another group (V) with five mice served as untreated infected control for both drugs. Cymelarsan® was administered i.p. at doses of 0.25 mg/kg (standard dose in camels), 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg body weight. Diminasan® was given i.p. at doses of 3.5 mg/kg (standard dose in cattle), 7.0 mg/kg, 14.0 mg/kg and 28.0 mg/kg body weight. Treatment was administered once parasitemia peaked at 1–3 days post infection. Mice were weighed on a digital balance for calculation of drug dosages. The mice were monitored every other day for up to 60 days for the presence of trypanosomes on wet blood smears obtained by tail bleeding. The control groups received the same placebo volume of sterile distilled water by i.p. route.

2.3. Drug sensitivity studies in horses

2.3.1. Experimental design

Six adult horses (four mares and two stallions) parasitologically (Woo test) and serologically (CATT/*T. evansi*) negative were selected from the central highlands of Ethiopia. The animals were transported and kept under closed confinement in a fly proof stable.

The six horses were divided into three treatment groups of two horses each designated as groups I and II and control group III, respectively. Horses were infected with the 834/940 Dodola strain. About 50,000 trypanosomes per ml were inoculated directly into the jugular vein. After peak parasitemia, Cymelarsan® was administered by deep intramuscular route in the middle third of the neck. Horses in groups I and II were treated once at a dose of 0.25 mg/kg as a 0.5% solution, and 0.5 mg/kg as a 1% solution, respectively.

Horses in the control group remained untreated and allowed to develop chronic form of dourine. These horses were also challenged with 1 ml of dexamethasone sodium phosphate (intra muscular) for five consecutive days at three different occasions, at days 148–152, 193–197 and 232–236 to induce immunosuppression.

2.3.2. Measured parameters

Experimental animals were examined regularly for parasitemia, packed cell volume (PCV) and serological status using CATT/*T. evansi* up to 320 days post infection. Blood samples were collected by bleeding animals from marginal ear veins into paired heparinized microhaematocrit capillary tubes sealed at one end with creastaseal (Hawaksly, England) and centrifuged at 12000 rpm for 5 min (Woo, 1970). Each microhaematocrit tube was examined microscopically for trypanosomes by the buffy zone by placing the tubes in a viewing chamber (Woo, 1970). Moreover, the buffy coat was spread on clean slide and examined under a 40× objective microscope by buffy coat method (Murray et al., 1977) and intensity of infection was graded from 0 to 6 as per the standard scores described by Paris et al. (1982).

Serological analysis was performed using CATT/*T. evansi*. This is a direct card agglutination test, which uses formaldehyde fixed, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2 stained with Coomassie blue (Bajyana Songa and Hamers, 1988). The test was executed on the horse sera, diluted 1:4 in Phosphate Buffered Saline (PBS).

2.3.3. Data analysis

Experimental animals were considered cured when no trypanosomes were observed for 320 days post treatment. The mean PCV levels of the horses and the relapse interval in mice were calculated over the course of the experiment. The mean PCV levels on days 20, 40 and 60 post infection were compared in each of the treatment and control horse groups using student's *t*-test with a 95% confidence limit.

3. Results

3.1. Mice adaptation

Mice were challenged daily for seven consecutive days with infected horse blood and Dexamethason®. First parasitemia was evident at day 3 and peaked at day 5 after the last challenge with horse blood. Subsequently, it was possible to serially pass *T. equiperdum* every 4–5 days by i.p. transfer of 0.2 ml blood into the immunocompetent mice. In the first three passages parasitemia peaked at 5 days of post infection with a score of +5. From the fourth serial passages onwards parasitemia peaked at 3 days of post infection with a score of +6. Twenty-three serial passages were conducted and cryostabilates prepared at passages 2, 7, 15, 19 and 23 were subsequently kept in liquid nitrogen.

3.2. Drug sensitivity studies in mice

The results of the drug sensitivity studies in mice are summarized in Tables 1 and 2. Diminasan® at doses ranging from 3.5 mg/kg to 28 mg/kg body weight failed to cure any of the mice infected with 713/943 or 834/940 Dodola strains. Cymelarsan® treatment of mice infected with 713/943 or 834/940 Dodola strains at doses of 0.25 mg/kg and 0.5 mg/kg also failed to cure the infection. There was a clear relationship between the time of relapse and the dose of the drug used. Mice treated with lower doses relapsed after a shorter time than mice treated with higher doses. In contrast, Cymelarsan® at doses of 1 mg/kg and 2 mg/kg

Table 1
Sensitivity of 713/943 and 834/940 Dodola strains to Diminasan® and Cymelarsan® in mice.

Drugs	Doses (mg/kg)	Number of mice treated/relapsed	Mean relapse interval in days ± SD	
			(713/943 Dodola strain)	(834/940 Dodola strain)
Diminasan®	3.5	5/5	1.25 ± 0.043	1.5 ± 0.54
	7.0	5/5	1.5 ± 0.5	2 ± 0.65
	14.0	5/5	4.5 ± 1.22	3.5 ± 1.32
	28.0	5/5	6 ± 1.94	4 ± 1.89
Cymelarsan®	0.25	5/5	6.5 ± 1.83	6 ± 1.73
	0.50	5/5	7.5 ± 1.72	6.5 ± 1.22
	1.0	5/0	Cured	Cured
	2.0	5/0	Cured	Cured
Control	Distilled water	5/5	Died 3–5 days post infection	

Table 2
Change in the mean PCV levels of horses experimentally infected with 834/940 Dodola strain and treated with 0.25 mg/kg (group I) and 0.5 mg/kg (group II) Cymelarsan® and untreated control (group III).

Mean PCV levels				
Groups	Day of infection	Peak parasitemia (day of treatment)	Day post infection	
	Day 0	Day 20	Day 40	Day 60
I	32^{a*}	24^{b}	30^{a}	34^{a}
II	34^{a}	20^{b}	28^{a}	32^{a}
III	33^{a}	24^{b}	20^{b}	16^{b}

* Mean PCV values with different superscripts across a row represent statistically significant difference ($p < 0.05$).

body weight effectively cured all mice regardless of the strain used for 60 days of observation.

3.3. Drug sensitivity studies in horses

Six horses were infected and became parasitologically positive in Woo test (+2 score, 1–10 trypanosomes per preparation) 13–15 days post infection with 10^3–10^4 trypanosomes per ml. Parasitemia peaked (+6 score, swarming trypanosomes in every field) in horses 17–20 days post infection.

Based on the results of drug sensitivity testing in mice, only Cymelarsan® was selected to be used in the horse experiment since Dimanasan® already showed relapses in mice. Single shot treatment with Cymelarsan® 0.25 mg/kg and 0.5 mg/kg body weight was administered to horses of groups I and II, respectively, 20 days post infection after peak parasitemia was evident. Treated horses in groups I and II had no detectable parasitemia within 24 h of treatment. The mean PCV levels of horses in groups I and II treated with Cymelarsan® showed improvement starting from day 20 of observation (Fig. 1). At day 40 and day 60 post infection the mean PCV was significantly ($p < 0.05$) higher than on day 20 (Table 3). In contrast, there was a progressive depression in the mean PCV levels of horses in the control group III (Fig. 1 and Table 2).

The two untreated control stallions developed chronic forms of dourine with clinical signs such as urethral discharge, oedema of the scrotum and prepuce, incoordination of the hind legs, weakness, emaciation, and ventral oedema. Following treatment of one of the horses with Cymelarsan® at day 282 post infection at a dose of 0.25 mg/kg body weight improvement in body condition was observed. No relapses were observed up to date, i.e. 367 days post infection and 85 days after Cymelarsan® treatment, whereas the non-treated control did relapse (data not shown). Moreover, clinical signs of incoordination of the hind legs, weakness and ventral edema disappeared within 10 days and the PCV increased in this treated horse (Fig. 2). These findings demonstrated the efficacy of Cymelarsan® in a chronic dourine case as well.

Interestingly, the two control stallions became aparasitemic 80 days post infection in the Woo test, even though the general body condition was progressively deteriorating. When the two horses in the control group were challenged with 1 ml of dexamethasone sodium phosphate for five consecutive days at three different occasions, reoccurrence of parasitemia and decline in PCV were always evident within 7 days of immunosuppressive treatment (Fig. 2).

The horses in the control group were found to be positive for CATT/*T. evansi* test throughout the course of the experiment. However, in horses treated with Cymelarsan® at a doses of 0.25 mg/kg and 0.5 mg/kg body weight seroreversion was observed starting from 150 and 170 days post treatment, respectively (Table 3).

Table 3
CATT/*T. evansi* seroconversion or seroreversion status of the experimental horses.

Experimental groups	Seroconversion (days post infection)	Seroreversion (days post treatment)
I	32	150
II	32	170
III treated at day 282	32	n/a
III non-treated	32	n/a

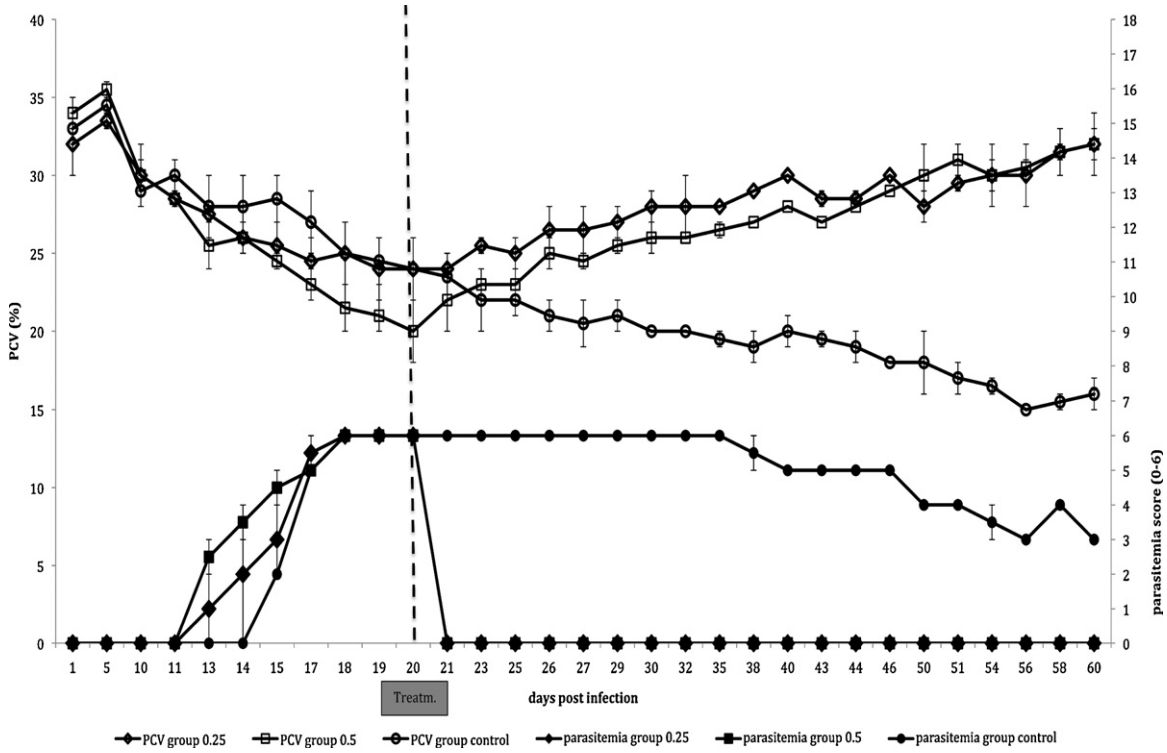

Fig. 1. Follow-up of acute infection by *T. equiperdum* Dodola 834/940 in horses. The graph shows the profiles of mean packed cell volume (PCV) and parasitemia levels of experimentally infected horses with *T. equiperdum* and treated with Cymelarsan® at 0.25 mg/kg (◊) and 0.5 mg/kg (□) body weight and the untreated control (○) during the acute phase of the infection (day 0–60). Open symbols indicate the PCV levels, closed symbols the parasitemia levels, respectively. The dotted line at day 20 indicates the time of treatment in groups I and II.

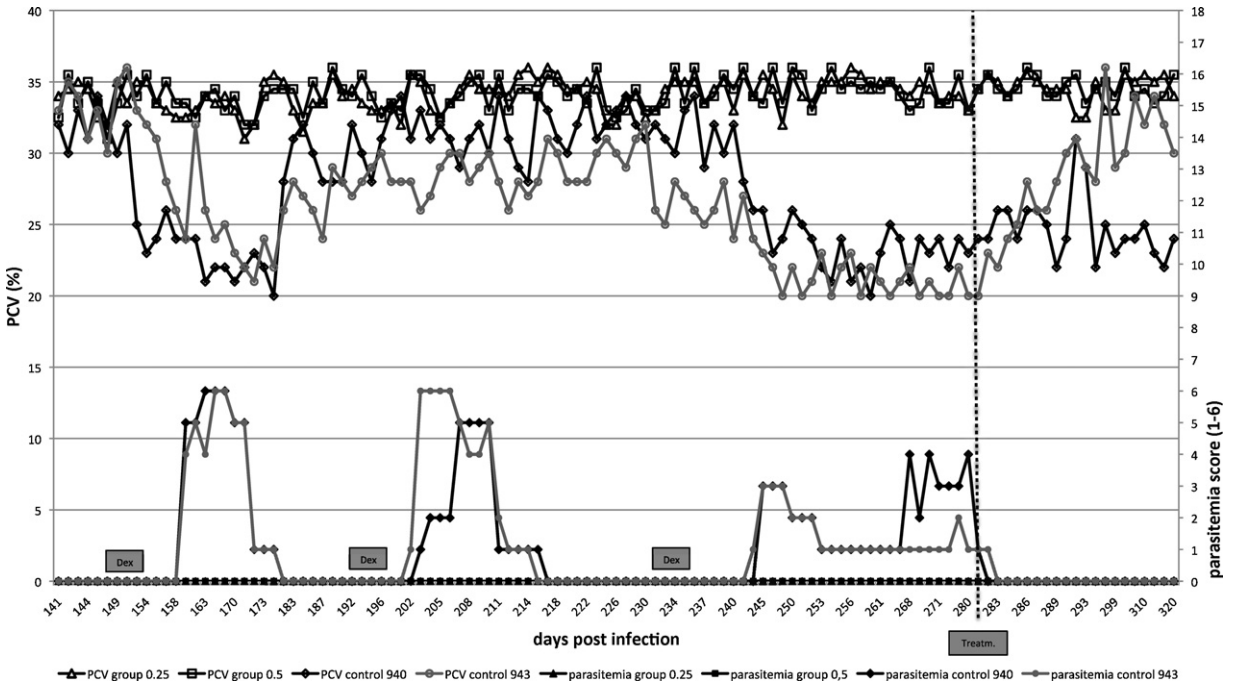

Fig. 2. Follow-up of chronic infection by *T. equiperdum* Dodola 834/940 in horses. The graph shows the profiles of mean packed cell volume (PCV) and parasitemia levels of experimentally infected horses with *T. equiperdum* and treated with Cymelarsan® at 0.25 mg/kg (△) and 0.5 mg/kg (□) body weight, and control horses 940 (◊) and 943 (○) during the chronic phase of infection (day 141–320), respectively. Open symbols indicate the PCV levels, closed symbols the parasitemia levels. Grey boxes (DEX) indicate the period of dexamethasone treatment in the control horses. The vertical striped line at day 282 indicates the time of treatment of control horse 943 with Cymelarsan® at a dose of 0.25 mg/kg.

4. Discussion

One of the characteristics of *T. equiperdum* is that it is often impossible to obtain a first passage in mice or any other rodent when infected with blood (Stephen, 1986; Claes et al., 2003) and genital washes from antigenaemic horses (Alemu et al., 1997; Hagos, 2005). In order to facilitate the adaptation of trypanosomes in mice, the immune system of the mice was suppressed with Dexamethason®. Dexamethasone® is one of the most frequently used glucocorticoides for immunosuppression. In high doses glucocorticoides have a profound anti-inflammatory and immunosuppressive effect, whereas low physiological concentrations rather have an immunostimulatory function (Wiegers et al., 1993; Wiegers and Reul, 1998). In this study, immunosuppressive treatment with Dexamethason® improved the growth and adaptation of parasites in mice and the reoccurrence of peak parasitemia in chronically infected horses. It can be concluded that Dexamethason® shows the potential to increase success rates of parasite isolation of *T. equiperdum* from chronic dourine cases or to confirm parasite presence in seropositive yet apparently aparasitemic cases of dourine.

Diminasan® is effective against *T. congolense* and *T. vivax* but less effective against other trypanosomes (Mulligan, 1970). Yet, a relative efficacy of Diminazene aceturate on *T. equiperdum* isolates was observed following *in vitro* drug sensitivity tests (Zhang et al., 1991; Brun and Lun, 1994). In contrast, it was shown by Tuntasuvan et al. (2003) that Diminazene aceturate was ineffective in curing and preventing relapses of *T. evansi* infections in horses and mules. Despite this knowledge, local veterinarians and veterinary assistants in the highlands of Ethiopia still use diminazene to treat suspected trypanosome infections. In our study Diminasan® at doses ranging from 3.5 mg/kg to 28.0 mg/kg body weight failed to eliminate 713/943 or 834/940 Dodola strains in mice. Hence, the dose necessary to cure mice might be >28.0 mg/kg body weight. However, higher doses of Diminasan® can cause severe toxicity rather than cure infection in mice (Gilbert and Newton, 1982). Drug failure could be attributed to the relatively rapid excretion of the drug (Mulligan, 1970) or its pharmacokinetics. Diminasan® cannot cross the blood brain barrier and enter somatic tissues as a result it cannot be the curative drug for trypanosomes with tissue and central nervous system affinity. Indeed, Diminasan®, was found to be ineffective against infections involving central nervous system as parasitemia returned rapidly after a few days of post treatment and the central nervous system was demonstrated to be the source of relapsing parasitemia (Jennings et al., 1979, 1980).

Cymelarsan® was found to be very effective against *T. brucei brucei*, *T. equiperdum* and *T. evansi* in camels, buffalo, goats and pigs (Zweygarth and Kaminsky, 1990; Lun et al., 1991; Otsyula et al., 1992; Zhang et al., 1991; Zweygarth et al., 1992). Currently, under field conditions, Cymelarsan® is only used to cure *T. evansi* infections in camels at a recommended dose of 0.25 mg/kg body weight (Musa et al., 1994). This arsenical compound contains the trivalent arsenic element with a markedly reactive arsenoxide group. The presence of arsenoxide confers the physiochemical ability of lipid solubility that allows passage across the blood brain barrier (BBB) (Pepin and Milord, 1994). The arsenical compound melarsoprol possesses the remarkable ability to cross the BBB and kill *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* parasites residing in the central nervous system (CSF). In cerebrospinal fluid as well *T. equiperdum* infections, the occurrence of nervous symptoms and lesions is associated with the presence of parasites in cerebrospinal fluid (Barrowman, 1976). Central nervous system involvement was also demonstrated in horses suffering from *T. evansi* infection (surra). This infection was characterized by severe neurological abnormalities such as progressive ataxia, head tilt, nystagmus and cranial nerve deficits. *T. evansi* trypomastigote were also detected in the cerebrospinal fluid using cytology (Berlin et al., 2009). Since Cymelarsan® can penetrate the BBB and dourine and surra can be associated with parasite occurrence in CSF evaluation, an *in vivo* evaluation of this drug in mice and equines was necessary.

In mice, Cymelarsan® failed to cure infection with *T. equiperdum* Dodola strains at 0.25 mg/kg and 0.5 mg/kg doses. However, at higher doses of 1.0 mg/kg and 2.0 mg/kg body weight the drug effectively cleared the mice from parasites with no relapse for 60 days. The inability of Cymelarsan® to clear parasitemia in mice as well as in other domestic animals at the standard dose of 0.25 mg/kg used against *T. evansi* in camels has previously been reported. For example, Cymelarsan® was ineffective in buffaloes treated at doses ranging from 0.25 mg/kg to 3 mg/kg (Lun et al., 1991), in goats treated at a dose of 0.3 mg/kg (Zweygarth et al., 1992), in mice treated at doses of 0.25 mg and 0.5 mg/kg (Syakalima et al., 1995) and in cattle treated at a dose of 0.5 mg/kg (Payne et al., 1994). Zweygarth et al. (1992) suggested that this dosage should be used only in camels and that higher doses are needed to treat *T. evansi* in smaller animals. Indeed, in our study mice treated with Cymelarsan® at doses of 0.25 mg/kg and 0.5 mg/kg body weight could have been under dosed since the differences in the metabolic rates between camels and mice were not taken into consideration.

In the present study horses, Cymelarsan® seemed to cure acute infections without relapses for 320 days post infection at doses of 0.25 mg/kg and 0.5 mg/kg. No parasites were observed after treatment and the observed seroreversion of the horses indicates the efficacy of the drug in clearing the infection. Further, the relative improvement in PCV levels of animals also appeared to reflect the efficacy of the trypanocidal drug used in our experiment. The horses in the non-treated control group developed the chronic form of dourine with genital as well as nervous forms of the disease. One of the two stallions in this group seemed to be completely cured after treatment at 282 days post infection with Cymelarsan® at a dose of 0.25 mg/kg body weight. This stallion demonstrated marked improvement in the overall body condition with disappearance of clinical signs such incoordination, weakness and ventral oedema within 10 days of treatment. Moreover, eight chronic clinical field cases of dourine in the highlands of Arsi–Bale of Ethiopia were treated on site with Cymelarsan® at a dose of 0.25 mg/kg body weight. So far no relapses and parasites were found after revisiting and testing of the animals six and twelve months following initial treatment (personal

observations). Thus, this study indicates that Cymelarsan® seems effective in horses at both 0.25 mg/kg and 0.5 mg/kg in acute as well as in chronic *T. equiperdum* infections. This is a first promising result in the possible cure of dourine-infected horses, yet a thorough evaluation of Cymelarsan® in horses should be performed on a larger scale to validate these findings.

Currently an eradication strategy is imposed by the World Organization for Animal Health (OIE) with slaughtering of seropositive horses while treatment is prohibited (Zabotskij et al., 2003). However, it is not economically feasible to apply strict test and slaughter policy to control dourine in developing countries. Equines in developing countries have a significant role in the transport and agriculture. Moreover, more than 60% of all horses on Earth are found in developing countries (Pritchard et al., 2005). It might be worth treating clinical cases to alleviate the disease, enable animals to perform well and thereby reduce mortality. In light of the results of this study, a revised strategy of the appropriate treatment with Cymelarsan® instead of eradication could be recommended to the OIE.

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