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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.