Full Length Research Paper

Sensitivity of *Trypanosoma congolense* field isolates in experimentally infected calves in Konso district, Southern Ethiopia to isomethamidium and diminazene

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Study on sensitivity to diminazene aceturate 3.5 mg/kg and isometamidium chloride 0.5 mg/kg in tenzebu calves experimentally infected with two field isolates of *Trypanosoma congolense* (ET/07/Konso 59 and ET/07/Konso 114) was undertaken in Konso district, Southern Ethiopia. Calves were monitored for clinical and parasitological parameters during three months. At day 15 post-infection, corresponding to peak parasitaemia, they were treated with Diminasan® 3.5 mg/kg body weight via deep intramuscular route. Out of ten calves, only two remained parasitologically negative for 12 and 15 days after treatment with Diminasan®. Once relapse/breakthrough infection was detected in two calves, parasitemia persisted until they get second treatment with Veridium® 0.5 mg/kg body weight. However, linear regression analysis of the effects of persistent trypanosomal infections on mean packed cell volume (PCV) and loss of body condition in relation to the initial recordings in the relapsed calves was not statistically significant. Results of the trypanocidal drug sensitivity study revealed the presence of *T. congolense* populations exhibiting resistance to diminazene aceturate. It is strongly recommended that legislations be devised and implemented to ensure that only quality and effective trypanocidal drugs should get access to the market.

Key words: *Trypanosoma congolense*, trypanocidal drugs, sensitivity test, Southern Ethiopia, calves.

INTRODUCTION

Trypanosomosis is one of the major constraints on animal production in areas of Africa which have the greatest potential for significant increases in domestic livestock populations and livestock productivity. The disease affects animals and man, with direct and indirect losses estimated in billions of dollars annually. Control of animal trypanosomosis is one of the key components to improve the productive opportunities of rural communities in tsetse-infested areas. This has been addressed using trypanocidal drugs which are limited in number and have been under extensive use for over 40 years with little or no regular monitoring (Stein et al., 2011; Chitanga et al., 2011; Mungube et al., 2012; Sow et al., 2012).

Consequently, recent case surveys conducted in some African sub-Saharan countries, including Ethiopia, revealed that almost all of the commercially available trypanocidal drugs are gradually losing their efficacy due to the development of multiple drug resistance. For instance trypanocidal drug resistance was observed for isometamidium chloride and diminazene aceturate against *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma evansi* (Mungube et al., 2012; Sow et al., 2012; Kumar et al., 2012). The high infestation of low-lying areas by tsetse flies as well as *T. congolense*, *T. vivax* and *T. brucei* was also reported more than a decade back in Southern parts of Ethiopia (Abebe, 2005; Miruk et
al., 2008). *Glossina pallidipes* was found to be the only tsetse species prevalent in the study area and the greater section of the southern rift valley system of Ethiopia (Miruk et al., 2008; Moti et al., 2012).

Experimental studies conducted in different tsetse-infested zones of Ethiopia, using tests in ruminants, indicated the occurrence of varying degrees of resistance in trypanosomes to the commonly applied trypanocidal drugs (Yeshitila et al., 2006; Miruk et al., 2008; Moti et al., 2012). According to Kone (1999) there is no relation between curative and prophylactic doses for an individual trypanosome isolate in cattle and mice. Hence, it is necessary to ascertain whether or not treatment with a manufacturer’s recommended dosage is likely to be successful in cattle. As a prerequisite it was, therefore, essential to undertake study on sensitivity of selected trypanocidal drugs against field isolates of *T. congolense* in experimentally infected calves in Konso district, Southern Ethiopia.

**MATERIALS AND METHODS**

**Description of study area**

The present study was conducted in Konso district of Southern Ethiopia, located about 600 km away from Addis Ababa on the way to South Omo with an altitude of 550 to 2300 m above sea level. The population of the area is estimated to more than 250,000 where people secure subsistence livelihood through mixed agricultural farming practices. More than 150,000 heads of small east African zebu cattle and more than 500,000 heads of small ruminants (sheep and goats) maintained under traditional village management system with multiple ownership are raised mainly in the low lying areas (Neuromuscular Medicine Self-Assessment Examination (NMSAE), 2012). Tsetse transmitted animal trypanosomosis has been reported as the main impediment to the development of agriculture in the area.

**Study design**

Ten local East African zebu calves (*Bos Indicus*) 5 to 6 months old were obtained from Durro site (2,268 m above sea level) from a place where tsetse and trypanosomosis is not present and then randomly included in one of the two groups. One month prior to the challenge, calves were moved to a fly proof facility and treated with anthelmintics (ivermectin 0.2 mg/kg subcutaneously) and diminazene aceturate (3.5 mg/kg body weight intramuscularly). After two weeks, the animals were examined for *Trypanosoma* infection by thin blood smears stained with rapid differential Diff quick staining solution. The first group received intravenously 0.2 ml solution containing approximately 5 × 10^6 *T. congolense* diluted in phosphate saline glucose (PSG) solution.

Calves were monitored regularly three times per week for a 90 days period to monitor packed cell volume (PCV) and parasitemia level using the phase contrast buffy coat method (Murray et al., 1977). The calves from treated group received diminazene aceturate at 3.5 mg/kg body weight with deep intramuscular route of injection (*Diminisan®*, Batch DG/20337 Kuipersweg 9, 3449 JA Woerden, Holland). Relapsed cases were treated with Isometamidium chloride (*Veridium®*, Lot No. 113A2; Libourne, France) at 0.5 mg/kg body weight intramuscularly. In order to detect any clinical relapse and to confirm the appearance of trypanosomes in blood, all the calves were bled and examined three times per week for 90 days post treatment please put the sentence in the good position.

**Parasite isolation and infection of calves**

Two isolates of *T. congolense* (ET/07/Konso-59 and ET/07/Konso-114) were obtained from two trypanosomosis infected calves with high parasitemia score 5 × 10^6 in Konso, Southern Ethiopia. Thin blood smears were prepared and air dried, then stained with rapid differential Diff quick staining solution. Following, *T. congolense* was identified morphologically based on absence of free flagellum, marginal and medium size kinetoplast (OIE, 2004; Urquhart et al., 1996). Eight week aged Swiss white mice, weighing 25 to 30 g, were used to amplify the two field isolates of *T. congolense*, they were maintained on a commercial pelleted ration and water *ad libitum* in a fly-proof room at Konso district veterinary clinic. Mice were injected with 0.2 ml of fresh blood containing trypanosomes through intra-peritoneal route; the strain was passaged twice on mice. One milliliter of blood was aseptically collected from ether anaesthetized donor mouse by cardiac puncture, corresponding to 5 × 10^6 trypanosomes (Paris et al., 1982). Each calf received intravenously 0.2 ml solution containing approximately 5 × 10^6 trypanosomes diluted in phosphate saline glucose (PSG) solution (Eisler et al., 2001).

The present experimental study involving infection of calves with two isolates of *T. congolense* was authorized by the Ethical Clearance Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University.

**Data analysis**

The rate of relapse/breakthrough infections was calculated as the number of animals with parasitemia on the day of monitoring divided by the total number of animals. Interpretation of the results was made according to the descriptions given by Eister et al. (2001). Data analysis was performed with Statistical Package for Social Sciences (SPSS 11.5) software. The 95% confidence level was estimated and a P<0.05 was considered as statistically significant difference.

**RESULTS**

Peak parasitemia was reached in the experimentally infected calves between 13 and 15 days and treated with *Diminisan®* exactly at day 15 post-infection. Infected calves manifested typical clinical signs of trypanosomosis (between days 9 and 15 post-infection), such as depression, fever, inappetence, swelling of pre-scapular and pre-femoral lymph nodes, rough hair coat, and overall reduction in mean PCV below 20%.

Calves remained parasitologically negative only for 9 days after treatment with *Diminisan®*. However, after 12 and 15 days treatment with *Diminisan®*, relapse/breakthrough infection was detected in two calves. Parasitemia in these two calves persisted until they get second treatment with *Veridium®* on day 15.
On the contrary, no relapse/breakthrough trypanosomal infections were detected in any of the remaining eight calves which received Diminasan® and two calves treated with Veridium® until the end of the experiment.

The overall relapse/breakthrough infection rate and mean relapse duration was found to be 2/10 and 13.5 days, respectively. Patterns of infections of *T. congolense* isolates in experimentally infected ten calves after treatment with Diminasan® revealed that only two animals showed relapse/breakthrough infection between days 12 and 15 post-infection. Calves with relapse/breakthrough infections revealed reduced PCV and loss of body condition until they were treated with Veridium®. However, linear regression analysis of the effects of persistent trypanosomal infections on mean PCV and loss of body weight in relation to the initial recordings in the relapsed calves was not statistically significant (P>0.05).

**DISCUSSION**

*T. congolense* the identified species in the present study area, is in accordance with most of the previously conducted studies in the southern rift valley of Ethiopia (Abebe, 2005; Miruk et al., 2008) and in the Ghibe valley (Rowlands et al., 2001; Moti et al., 2012) where it is the dominant prevailing species. In general, it has been proved that *T. congolense* is the most prevalent and virulent trypanosome species in Eastern Africa, although certain hemorrhagic *T. vivax* strains still prevail in Eastern African countries (Taylor and Authie, 2004). An in-vivo experimental study was carried out to assess the trypanocidal activities of Diminasan® and Veridium® most frequently used drugs in the study area. This approach had the advantage that it could generate direct information about the success of treatment with the recommended drug dosage in cattle infected with trypanosomes. And this is in accordance with the standardized test protocols described for drug sensitivity in tsetse-transmitted trypanosomes of African domestic cattle (Eisler et al., 2001).

The fact that the present test was conducted in a fly-proof accommodation and in tsetse free area has reliably avoided the confounding effects attributed to the risk of vector-borne infection during the study period. It was essential to include a relatively large number of experimental calves, since previous studies show that results obtained on reduced animal groups are not always reliable (Eisler et al., 2001).

In this trial, the detection of relapse infections in some of the experimental calves as revealed by parasitemia in direct smear following treatment with Diminasan® is clearly indicative of the presence of a drug resistant sub-population. This conclusion stems from the fact that Diminasan® could maintain therapeutic blood levels until 22 days following treatment, unless resistance is present (Gilbert and Newton, 1982). The relapse delay in resistant strains is comparable with inferences drawn from a recent study at the Ghibe valley, where trypanosomes resistant to Diminasan® have relapsed more than 14 days following treatment (Moti et al., 2012). As there is an increasing number of case reports from other trypanosomosis endemic areas of Ethiopia, disclosing range of prevalence of *T. congolense* resistant to Diminasan® and Veridium® (Codjia et al., 1993; Mulugeta et al., 1997; Rowlands et al., 2001; Afework et al., 2004; Tewelde et al., 2004), the demonstration of resistance to Diminasan® (about 33% from the present study) manifested by the current *T. congolense* isolates at Jarso, was unsurprisingly an expected outcome. Furthermore, it has been observed under longitudinal studies that there was an association between the initial trypanosome prevalence and the occurrence, and thus the degree, of drug resistance (Mungube et al., 2012).

Despite a considerable initial deterioration in mean PCV of the relapsed calves, this study revealed a significant improvement in mean PCV following administration of Diminasan® and Veridium® (P<0.01). Firstly, the trypanosome isolates under investigation might have entailed a heterogeneous population of trypanosomes and treatment with Diminasan® could have eliminated the sensitive sub-population through its therapeutic effects, so that the parasite burden was limited to the resistant population. Furthermore, re-treatment of the calves with Veridium® could have resulted in a complete elimination of the sub-population that revealed resistance to Diminasan®. Initial treatment with Diminasan® followed by Veridium® could trigger better improvements in the relapse conditions. Even the sub-population resistant to Diminasan® may be less pathogenic, with insignificant impacts on productive performances of the calves. Only a limited number of studies have demonstrated a loss of virulence and/or loss of fitness in drug resistant trypanosomes (Mulugeta et al., 1997). A useful study that assessed this important issue was conducted in the Ghibe valley, where *T. congolense* strains demonstrated multiple resistance to all available drugs. In that study, it was deduced that, despite the occurrence of high degrees of drug resistance, local zebu cattle could yield profitable productivity, so that attractive economic returns were generated for herd owners (International Livestock Research Institute (ILRI), 2002; Stein et al., 2011; Moti et al., 2012).

In the present trial, the relapsed calves were treated with Veridium®, 45 days later, and no calves relapsed post-treatment. This observation is in accordance with the currently available information indicating the trypanocidal effects of treatment with Veridium® in cattle (Stevenson et al., 2000). Delaying of Veridium® treatment by 45 days after the administration of Diminasan® was a reasonable process in light with the possible side effects that could be associated with the injection of the second
drug in a short period following the administration of the first (Eisler et al., 2001; Stein et al., 2011; Mungube et al., 2012). The sensitivity of the isolates to Veridium® is conclusive. The possible effects of selection biases are of crucial importance, against which the trypanosome population against which the second drug was administered might not be a complete representative of the original field population. This can be further explained by the fact that the initial treatment with Diminasan® might have eliminated the sub-population resistant to Veridium® (Eisler et al., 2001). *T. congolense* isolated from Konso district Southern Ethiopia exhibited resistance to Diminasan®, which could also be Veridium®. However, this need to be confirmed by deriving clones from the trypanosome population in cattle or mice (Eisler et al., 2001; Stein et al., 2011). Nevertheless, the chemotherapy is still the most convenient way of fighting against the disease. In that way, trypanocidal drug sensitivity tests should be conducted before application in field.

**REFERENCES**


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