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Treatment Failure in Camel Trypanosomosis in Uaso Region of Kenya

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SUMMARY

A study was conducted in Uaso area on the border of Isiolo and Laikipia districts in Kenya to determine the cause(s) of treatment failure in camel trypanosomosis. Ten trypanosome isolates from infected camels were characterized by morphology and procyclic transformation test. Their sensitivity to quinapyramine sulphate and melarsomine were then determined using an in vitro assay system. Six isolates were *Trypanosoma evansi*, three *T. congolense* and one had both *T. congolense* and *T. evansi*. All the *T. congolense* isolates were resistant to melarsomine at 1.2 mg/kg bwt whereas one was resistant to the quinapyramine sulphate at 7.4mg/kg bwt. On the other hand one of the six *T. evansi* isolates, one was resistant to melarsomine at 1.2 mg/kg bwt and three to quinapyramine sulphate. Camel owners got little veterinary advice leading to wrong models administration of trypanocides, underdosing and use of unrecommended drugs. The efficacy of trypanocides in the area appears to be hampered by drug resistance and inappropriate drug use. Uaso falls within a tsetse belt and as such camels are susceptible to infection with tsetse transmitted trypanosomes. A drug effective against *T. evansi*, *T. congolense* and *T. brucei brucei* should therefore be used in the treatment of trypanosomosis in camels in this area. They should be educated on the proper use of trypanocidal drugs.

INTRODUCTION

Camel trypanosomosis (surra) caused by *T. evansi* is the most important disease affecting camels in Kenya (Wilson et al. 1981). The tryanocides currently used in the management of this disease are quinapyramine sulphate and quinapyramine chloride (Triquin^R), Wockhardt (Europe) Ltd. and melarsomine (Cymelarsan, Roving Merieux presently Merial). *Trypanosoma evansi* isolates resistant to quinapyramine sulphate were reported in Kenya as early as 1984 (Schillinger et al. 1984). Recently, resistance to quinapyramine sulphate was found to be high and widespread in Kenya (Maina et al. 1996). In their study, Maina and colleagues found all eight *T. evansi* isolates from Isiolo district resistant to quinapyramine sulphate at 500ng/ml whereas three isolates had reduced sensitivity (IC_{80} 50-150ng/ml) to melarsomine. Camel owners in the district, and particularly in Uaso region, reported high incidences of trypanosomosis, treatment failures, still births and high mortality rate. In a study to determine the causes of treatment failure the various types and methods of application of trypanocides in the area, and the trypanosome species infecting camel were determined. Sensitivities of the isolates to melarsomine and quinapyramine sulphate using an in vitro system were also determined.

MATERIALS and METHODS

Appraisal interviews : Five camel owners in Uaso area at the border of Isiolo and Laikipia districts (Fig. 1), were interviewed on various aspects of camel trypanosomosis,

especially disease detection and methods of intervention.

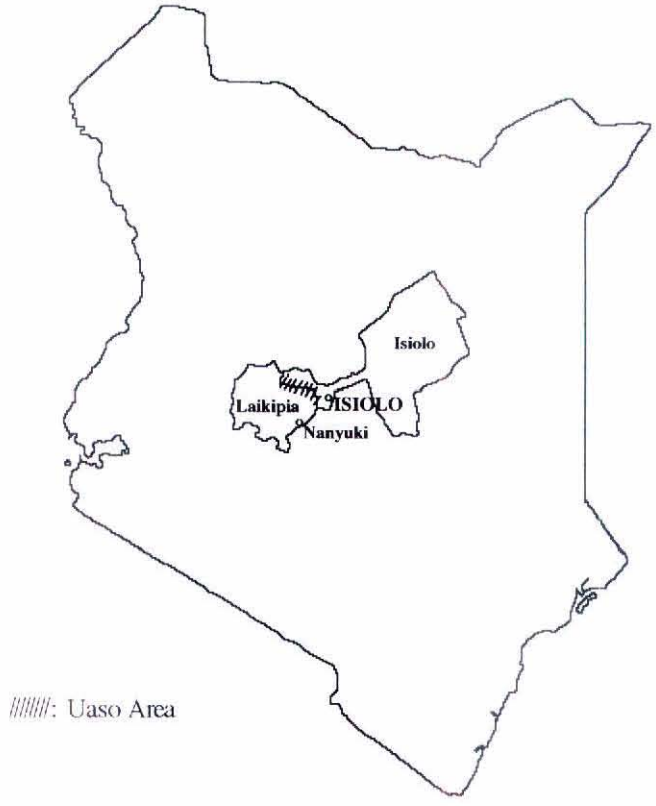

Figure 1. Map of Kenya showing the districts from which *T. evansi* isolates were collected.

Trypanosome stocks : Blood samples were collected from 130 camels to determine the presence of trypanosomes infection. Twenty one them were found positive for trypanosomes by mouse inoculation. Ten out of the 21 isolates, whose details are shown in Table 1, were stabilated as described by Olaho-Mukani et al. (1996).

Experimental mice : Swiss White mice weighing between 20 and 25g were obtained from the Small Animal Breeding Unit of the Kenya Trypanosomiasis Research Institute (KAURI). They were maintained on a commercial ration and water *ad libitum*.

In vitro culture media : Semi-defined medium (SD, Gibco, Scotland) supplemented with10% heat inactivated fetal bovine serum, 4mM L-glutamine, 25mM Hepes and 3mM cisaconitase was used.

Characterization of trypanosome isolates : The characterization of trypanosomes was based on the morphological features from Giemsa stained smears of the bloodstream forms as described by Hoare (1972). *Trypanosoma b. brucei* and *T. evansi* were differentiated using the procyclic transformation test. Initiation of the trypanosome cultures was done as described by Zweygarth et al. (1989a). Briefly, the flask was incubated at 28℃and 5% $C0_2$ and examined daily to record the appearance of trypanosomes in relation to the life-cycle stage present as described by Olaho-Mukani et al. (1993) and Zwerygarth et al. (1989b).

Trypanocidal drugs : Quinapyramine sulphate (Trypacide^R, May and Baker, Germany) and melarsomine (Mel-CY, Rhino Merieux, France) were purchased. Stock

solution of 100mg/ml of drug were prepared and diluted to appropriate concentrations in double distilled deionised water.

In vitro trypanocidal activity : Trypanosomes were harvested from donor mice and diluted to $1x10^5$ trypanosomes/ml. 0.2 ml of this suspension was inoculated intraperitoneally (i.p.) into each of 35 male mice. The mice were divided into six treatment groups and one control group of five mice each. Treatment with either quinapyramine (1.85, 3.7 and 7.4mg/kg bwt) or melarsomine (0.2, 0.6 and 1.2mg/kg bwt) was done two hours after experimental infection. Parasitaemia was monitored daily by examination of wet smear preparations of tail blood for a period of 60 days and the minimum effective dose that cured 50% (ED_{50}) of the mice determined.

RESULTS

Interviews : The camel 'breeds in Uaso are Small, Turban and their crosses. The drugs that have been used for the treatment camel trypanosomosis are quinapyramine sulphate, melarsomine, diminazene aceturate and homidium. The route of administration and/or dosage of trypanocides used were usually incorrect. There were reports of quinapyramine sulphate having been administered intravenously, as opposed to the recommended intravascular route. Relapses after treatment with these drugs were reported to be high. The farmers also reported that abortions and still births were high in the camel herds, and associated the conditions with the administration of melarsomine. The farmers said they received very little advice from veterinary officers, which led to self-diagnosis and treatment of the sick camels. Farmers also reported the presence of biting flies and tsetse flies along the rivers.

Characterization of trypanosome species : Six of the isolates were *T. evansi*, three *T. congolense* and one isolate had both *T. congolense* and *T. evansi*. The details are shown in Table 1.

Table 1.

Isolate NO.	Date of isolation	PTT	morphology	ED_{50} Mel.	ED_{50} quina.
1	15.08.1995	NT	*T. evansi*	0.6mg	3.7mg/kg
173	23.07.1995	NT	*T. evansi*	0.6mg/kg	R
194	23.07.1995	NT	*T. evansi*	0.6mg/kg	3.7mg/kg
108	22.08.1995	NT	T. evansi	R	R
109	22.08.1995	T	*T. congolense* and *T. evansi*	R	3.7mg/kg
115	22.08.1995	T	*T. congolense*	R	R
114	22.08.1995	NT	*T. evansi*	0.6mg/kg	R
89	20.08.1995	T	*T. congolense*	R	3.7mg/kg
116	22.08.1995	T	*T. congolense*	R	7.4mg/kg
106	23.07.1995	T	*T.evansi*	0.6mg/kg	3.7mg/kg

KEY
T. Transformed
NT. Not transformed
R. resistant at 1.2mg/kg bwt for melarsomine and 7.4mg/kg bwt for quinapyramine sulphate chloride

In vitro sensitivities : The sensitivities of trypanosome isolates to various trypanocides

are shown in Table 1.

***Trypanosoma evansi* isolates** : Five out of six isolates, were sensitive (ED_{50} 0.6mg/kg bwt) to melarsomine whereas one was resistant to melarsomine at 1.2mg/kg bwt. Three of isolates were sensitive (ED_{50} 3.7mg/kg bwt) while the rest were resistant to quinapyramine sulphate at 7.4mg/kg bwt. One *T. evansi* isolate was resistant to both quinapyramine sulphate and melarsomine.

***Trypanosoma congolense* isolates** : All the three *T. congolense* isolates were however refractory to melarsomine. Two of them were sensitive to quinapyramine sulphate (ED_{50} of 3.7 and 7.4mg/kg bwt) and one resistant at 7.4mg/kg bwt.

Trypanosoma evansi* and *T. congolense : The isolate that was a mixture of *T. evansi* and *T. congolense* was resistant to melarsomine at 1.2mg/kg bwt but sensitive to quinapyramine sulphate at an ED_{50} of 3.7mg/kg.

DISCUSSION

The trypanocides currently used in the management of camel trypanosomosis are the quainapyramine salt, Triquin® and melarsomine (Cymelarsan®). Melarsomine, an arsenical introduced in 1993, is effective against *T. brucei* subgroup trypanosomes especially to *T. evansi* (Zweygarth et al. 1990). Camel owners in the study had questioned the efficacy of this drug and from this study one out of the six *T. evansi* isolates was found to be resistant to melarsomine. In an earlier study, three *T. evansi* isolates had reduced sensitivity to melarsomine (Maina et al. 1996). These resistance could be due to cross resistance with diminazene aceturate (Zweygarth et al. 1990). Camel owners in the study area and from other North Eastern parts of Kenya (Mahanga et al. 1982) use diminazene aceturate in the treatment of camel trypanosomosis although it is not recommended for camel trypanosomosis (Homeida et al. 1981).

The presence of tsetse flies and hence tsetse transmitted trypanosomosis in the region, indicates that the appropriate drugs for the treatment of camel trypanosomosis should be selected on the basis of infecting trypanosome species since trypanosomes respond differently to trypanocides. Reports from the manufactures of melarsomine indicate that melarsomine is not effective against *T. congolense* (Rhino merieux, personal communication). The present study has also confirmed that melarsomine is not effective against *T. congolense*.

Triquin® is effective against *T. b. brucei*, *T. evansi* and *T. congolense* species and therefore should be the drug of choice in the study area. Resistance to this drug is however reported to be high and widespread in Kenya (Maina et al. 1996). In the present study, resistance to quinapyramine sulphate was found to be higher in *T. evansi* than in *T. congolense* isolates. This may be because quinapyramine salts have been used on *T. evansi* infections in camels since 1940. The improper drug usage noted in the study area could also play an important role in the development of drug resistant trypanosomes. The study therefore recommends that the camel farmers be educated on proper dosage and the appropriate route of drug administration of trypanocides.

The efficacy of trypanocides in Uaso region appears to be hampered by both drug resistance and improper diagnosis of the infecting species of trypanosomes. Since the development of new drugs is expensive and time consuming, the study recommends combination therapy for additive effects. Quinapyramine salts could be combined with melarsomine either during manufacture or use. In this way the problem of resistance of *T. evansi* to quinapyramine salts and ineffectiveness of melarsomine on *T. congolense* may be

eliminated.

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