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SURRA IN THE UAE: DO WE HAVE DRUG RESISTANT *Trypanosoma evansi*?–PART 1

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ABSTRACT

An experimentally *Trypanosoma evansi* infected dromedary (intravenous injection of 3 million parasites) was under investigation for 200 days. Already after 24 h, living trypanosomes were detected in the blood. On day 6, parasites reached a high density and the host showed first clinical signs. Several treatments with melarsomin on day 6 and in combination with quinapyramin on day 23, 68, 102 and 198 decreased the number of trypanosomes in the blood but did not eliminate the parasite indicating a drug resistant *T. evansi* strain. While throughout the observation period there was no signs of anaemia, the number of leucocytes increased shortly after infection and remained high later on. Antibodies indicating a positive surra infection appeared on day 20 and remained on a high level.

Key words: Dromedary, melarsomin, quinapyramin, Surra, *Trypanosoma evansi*

Of eight world-wide used veterinary trypanocides for animals (suramin, homidium bromide, homidium chloride, isometamidium chloride, quinapyramine sulfate, quinapyramine sulfate:chloride diminazene aceturate, melarsomine dihydrochloride) (Giordani *et al,* 2016), six products are registered for the use in camels in the UAE. These are Trypanosoma^R (isometamidium), CymelarsanR (melarsomine dihydrochloride = melaminylthioarsenate) and the quinapyramines Triquin^R, Triquin inj. 2.5 g^R, Interquin^R and Asypur^R. A further product PiroplasminR that contains diminazen aceturate is registered only for horses and dogs and has a claim also for Babesia and Trypanosoma infections in these hosts.

Quinapyramine was developed in 1950 and has long metabolic half-life also thus bears a prophylactic effect (Curd and Davey, 1950). The large scale use of quinapyramine has been interrupted because of serious drug resistance problems in cattle trypanosomosis in Africa in the mid-1970s. Quinapyramine is now produced mainly for the treatment of surra in camels and horses, in particular where there is resistance of *T. evansi* to suramin (Uilenberg, 1998).

The phenanthridinium, isometamidium chloride (Samorin^R), a conjugate of the homidium (ethidium) and part of the diminacene molecule launched in the 1960s, is used exclusively as a veterinary trypanocide, and it is used both prophylactically and therapeutically.

Melarsomine dihydrochloride, a member of the triazines family is the latest drug that was produced by combining melarsenoxide with two cysteamine equivalents. Melaminophenylarsenicals were already used in the late 1940s to treat Rhodesian sleeping sickness and melarsopol (Mel B^R) was the drug of choice in human medicine. It was less toxic than melarsenoxide (De Koning, 2020). Melarsomine was developed in the 1980s and was commercially available in 1992 for the treatment of surra in camels.

Complaints of camel owners in the UAE led to a survey where a small camel herd was monitored over a period of 12 months including repeated treatments with melarsomine and quinapyramine (Wernery *et al,* 2020; Schuster *et al,* 2021). As a result, only one camel was successfully treated and all other positive animals remained serologically positive and trypanosomes were detected in their blood at least once after the last treatment.

In order to find out if this was a result of drug failure or reinfection, an infection trial was carried out and melarsomine and quinapyramine were administered to the experimentally infected camels kept at Central Veterinary Research Laboratory in Dubai.

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[1]It is noteworthy that in our treatment trials we used Triquin^R that is a combination of quinapyramine sulfate and quinapyramine chloride.

Materials and Methods

The Central Veterinary Research Laboratory in Dubai keeps 24 adult dromedaries for experimental and teaching purposes. Three to four dromedaries are accommodated in 225 m^2 (15x15 m) partially shaded pens on sandy grounds. The animals received hay and crushed cereals twice a day and drinking water *ad libitum* through automatic drinkers.

The Trypanosoma strain that was used in the trial originated from a camel of the farm of a previous investigation (Schuster *et al,* 2021). Trypanosomes were injected into the jugular vein in a dose of 3 million parasites. Prior to the start of our investigations, all dromedaries at CVRL were haematologically examined with negative results for trypanosomes. Blood sampling of the experimentally infected animal was done daily for the first 31 days and later in weekly intervals.

Haematological examination

The EDTA blood was analysed for haematological parameters using the automatic haematology analyser Sysmex XN (Sysmex Corporation, Japan) and was examined for the presence of trypanosomes with the buffy coat test (BCT). Giemsa stained blood smears were prepared when life trypanosomes were seen in BCT and the sample was semi-quantitatively assessed as follows:

negative : no parasites in BCT

\+ : single parasites in BCT but not in the blood smear

++ : few parasites in BCT but occasionally in the blood smear

+++ : many parasites in BCT and single parasites in each field in the blood smear

++++ : many parasites in BCT and more than one parasite in each field in the blood smear.

Serological examination

Serum samples were examined for *T. evansi* antibodies with an indirect ELISA. The antigen for coating the ELISA plates was prepared by filtration of EDTA blood of experimentally with *T. evansi* infected rats by anion exchange chromatography followed by hypotonic shock lysis. For evaluation of the test results, an optical density determined by the ELISA reader <0.3 was considered negative, 0.3-0.5 was dubious and >0.5 was positive. The commercially available CATT test was also employed.

The experimentally infected female dromedary No 26 was treated several times with melarsomin and quinapyramine (Table 1) and blood samples were taken prior and 3 days after treatment of the subsequent treatment.

Results

The buffy coat test

The infection dose of 3 million parasite cells was relatively high and there was no surprise, that trypanosomes were already seen 24 h after infection. Already on day 5, the number of trypanosomes reached ++++ in our semi quantitative assessment. On day 6, the camel showed clinical signs (depression, inappetence). An effect of the treatment was seen on day 9 when the concentration of trypanosomes dropped to +. On day 15, the number of trypanosomes reached again ++++.

Knowing that melarsomin is quickly excreted, for the second treatment on day 23, quinapyramin and melarsomin was given. The effect was seen between day 25 and day 27 when only single trypanosomes remained in the buffy coat. However, on day 39, again many trypanosomes were observed in the Giemsa stained blood smears. It seemed that the concentration of trypanosomes got less when the examination of blood on day 67 revealed

Table 1. Treatment of an experimentally with *T. evansi* infected dromedary. The dose route of application of the trypanocides was according to recommendations of the producer.

Treatment		**Haematology result (presence of *T. evansi*)**			
Trypanocide drug	**At day**	**Day prior treatment**	**Days after treatment**		
			1	**2**	**3**
melarsomin	6	++++	+++	++	+
melarsomin + quinapyramin	23	++++	++++	+	+
melarsomin + quinapyramin	68	++	+	+	+
quinapyramin	198	+++			
melarsomin	199		-	+	+

only occasional findings of trypanosomes in microscopic fields in Giemsa stained blood smears (++). A combined treatment of quinapyramin and melarsomin on day 68 decreased the number of trypanosomes again to (+) but did not eliminate the infection, although, no trypanosomes were seen at some occasions, later on. When the concentration of *T. evansi* reached high values (++++) again, quinapyramine was given on day 198, followed by melarsomin the next day. The haematological examination on the day after treatment gave a negative result but later, *T. evansi* occurred again (Table 1) and stayed until the end of observation.

Antibodies

The first measurable ELISA antibodies against *T. evansi* were detected on day 20, and by day 27, they reached a value of 1.0. Further testing revealed rising concentrations of antibodies to 1.3 on day 67, 1.8 on day 90 and 2.3 on day 196.

Haematology

Contrary to the number of erythrocytes and the concentration of haemoglobin that remained in the normal range, there were changes in the number of leucocytes that rose quickly after infection and remained on a high level between 20 and 25 x 10^9/ L and reached values of 30 x 10^9/ L at the end of the observation period. A severe neutrophilia and lymphopenia was observed in the initial phase of the infection but at the end of the observation period neutrophils and lymphocytes reached normal values. The concentration of monocytes was in the normal; range at the beginning of the trial but doubled starting from day 8 (two days after the first treatment) and remained at a high level and reached a maximum of 25% on day 25. Later, this value declined but still with values slightly above the norm. Eosinophile and basophile leucocytes were in normal range throughout the whole observation period.

Discussion

Following the introduction of a latently Trypanosoma infected dromedary to the small camel herd of CVRL in winter 2009/2010, 4 of the resident dromedaries got infected. It required 2 treatments with melarsomine to eliminate the infection. While trypanosomes and trypanosoma DNA disappeared after the 2nd treatment, antibodies remained up to 10 months. This small trial showed that melarsomine has been an effective drug.

According to Holmes *et al* (2004), trypanocide resistance occur under large-scale drug use, by using inadequate dosing and by using correct dosing with drugs that are slowly eliminated from the body.

Our treatment trials showed that the tested *T. evansi* strain could not be eliminated neither with melarsomine nor in combination with quinapyramine. While melarsomine levels quickly reach a maximum level in the blood stream, this drug is also quickly eliminated from the body (Kasozi *et al,* 2022). Contrary to this, quinapyramine unfurls a prolonged action. TriquinR, used in the trial is a combination of quinapyramine dichloride and quinapyramine sulfate. While dichloride salt is slowly adsorbed and protects the host of up to 2 months from susceptible trypanosomes, the sulfate is easily dissolved in water and subsequently resorbed but the protection lasts only for 2 weeks (Steuber and Kroker, 2002).

The mode of action of the melamino-phenylarsenical substance, melarsomine, is the neutralisation of enzymes and the interruption of the ATP generation of the trypanosomes. As other chemicals of this group, it irreversibly binds to sulfhydryl groups on the enzyme pyruvatekinase and leads to disrupting energy production. In addition, the trypanothion reductase is inhibited, and this causes death of the trypanosomes (Steuber and Kroker 2002). Resistances are subsequently related to the adenine-adenosine transporter, P2, due to point mutations within this transporter. Adenosine and adenine and the transport inhibitor dipyridamole are able to block the trypanocidal activity of melarsen oxide that has a similar mode of action as melarsopol and melarsomine. The P 2 encoding gene, TbAT1, and an allele bearing multiple polymorphisms were found to be responsible for the resistance. In case of resistance, use of isometamidium is recommended (Kasozi *et al,* 2022).

Quinapyramin interferes with DNA synthesis and suppression of cytoplasmatic ribosomal activity in the mitochondria of the trypanosomes. Resistance to quinapyramin is due to variations in the potential of the parasite's mitochondrial membrane (Kasozi *et al,* 2022). Quinapyramin chloride is slowly adsorbed and protects infection for up to 2 months and therefore, has a prophylactic effect too. The disadvantage is that resistance occurs when infection takes place at a time when the drug becomes sub therapeutic concentration. Also, for the management of resistance, isometamidium is recommended.

Another trypanocide is isometamidium. It freely crosses the plasma membrane of the parasite by facilitating diffusion and is subsequently

actively accumulated into the mitochondria using the mitochondrial potential as a driving force. In resistant parasites with a low mitochondrial potential, isometamidium rapidly diffuses from the cell when placed in isometamidium-free medium. In sensitive *T. congolense*, a similar amount of isometamidium diffuses from the cell under these conditions but a large proportion of the drug, sequestered in the mitochondrion, is retained (De Konig, 2001).

Diminazene aceturate has a claim for treatment of trypanosomosis, the effective dose for the treatment of *T. evansi* is 5 – 10 mg/kg body weight, however, this dose is toxic for camels (Homeida *et al,* 1981; Peregrine and Mamman,1993).

Resistance mechanisms of trypanosomes to trypanocides are well investigated but there are only speculations about the genesis of *T. evansi* resistance in Dubai camels. It is a fact that trypanocides can be purchased without prescription and it is believed among camel owners that melarsomin enhances racing performance of camels. Since the active substance is quickly eliminated from the body, it cannot be detected in forensic examination after the race. It is also believed by camel owners that a subtherapeutic dose of melarsomin "cleans" the body of the camel. Thus, frequent applications and underdosing might be the reasons for the loss of efficacy.

Except for the 1st week after infection when the camel was depressed and lost appetite, it did not show obvious clinical signs. This agrees with our experience in a naturally infected camel herd (Schuster *et al,* 2021) and confirms that the used *T. evansi* strain causes mild clinical signs. With regards to haematological values, anaemia is often described in Trypanosoma infected dromedaries but was not observed in present study. However, observations in this study lasted only 200 days.

The antibody titre measured by the CVRL in house ELISA became positive at day 20. This is in agreement with Luckins and Dwinger (2004) who stated that antibodies against *T. evansi* occur 14 to 21 days after infection.

Contrary to the number of erythrocytes and the concentration of haemoglobin that remained in the normal range, there were changes in the number of leucocytes that rose quickly after infection and remained on a high level. Platelets were already low prior to infection and stayed low over a longer period but reached normal values at the end of observation period. A severe neutrophilia and lymphopenia was observed in the initial phase of the infection but at the end of the observation period neutrophils and lymphocytes reached normal values.

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