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Trypanosoma evansi IN A DROMEDARY CAMEL HERD IN THE UAE-PART II

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ABSTRACT

Trypanosoma evansi infection was diagnosed in 15 out of 17 adult dromedaries of a breeding herd in the Emirate of Dubai. The herd had a history of abortions in the previous years. Own observations on 16 adult females and one bull lasted 12 months and revealed trypanosomosis in 15 animals in monthly haematological and serological examinations. Despite three to four injections of the trypanocides melarsamine and/ or quinapyramine, only one camel was cured. One camel aborted in the 8th month of pregnancy. Four other dams delivered healthy calves.

Key words: Aantibody ELISA, buffy coat technique, melarsomine, quinapyramine, *Trypanosoma evansi*, United Arab Emirates

Surra, a protozoal disease caused by the salivarian flagellate *Trypanosoma evansi* is the most important parasitic disease in Old world camelids. While literature on surra in Bactrian camels known also under the name Su Auru[1] is scarce, many publications on *T. evansi* infections in dromedaries are available. Most of the papers dealt with prevalence of the disease but data are difficult to compare since different diagnostic methods were used. Among direct diagnostic methods, wet blood films and blood smears are less precise. The haematocrit centrifugation technique, the so called buffy coat technique (BCT), that can be carried out in a normally equipped laboratory gives more exact results. The mini-anion exchange centrifugation is more difficult to perform and the mouse inoculation test requires animal experimentation. Detection of *Trypansosoma* DNA can be performed with polymerase chain reaction (PCR). Amongst serological tests, the card agglutination test can be performed under field conditions while the indirect immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) require well equipped laboratories (OIE, 2018).

As summarised by Wernery *et al* (2014), clinical picture of surra is multifaceted. In places where the disease has occurred for a long time, it often runs a subclinical course without obvious signs and only abortions might give evidence that trypanosomes are involved. This was the case in a camel herd kept at the outskirts of an oasis next to Dubai. The herd consisting of 18 adult dromedaries had a history of abortions and seven animals had trypanosomes in their blood while 15 were serologically positive in ELISA at the start of our observation. Only two recently purchased females were negative for both trypanosomes in BCT and *T. evansi* antibodies.

Since in the past there were claims that available trypancides were not effective anymore, the aim of this paper was investigate blood samples of camels for the presence of trypanosomes and the serum for antibodies after multiple courses of treatment with melarsamine and/or quinapyramine. This paper is the resumption of an article by Wernery *et al* (2020).

Materials and Methods

Dromedary camels

A breeding herd of 17 dromedaries with a history of abortions consisted of 16 females and one bull. Of these, two adult females were recently introduced to the herd. The camel pens are situated on a farm 25 km east of the Dubai city centre in a desert area near a settlement (Al Aweer town) surrounded by irrigated farms and gardens with date palm plantations, cultivation of forage crops and vegetables. There are several small holder farms with cattle and small ruminants in a radius of one km around the camel pens. A 2.5 ha freshwater lake is

1. In the Russian literature surra is called Su Auru. Su Auru is Kazakh and means next to water since *T. evansi* also known in elder sources as *T. ninaekohlyakimovae* is transmitted by bloodsucking flies and these insects need water or at least a muddy or damp environment for their larval development.

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at a distance of 2.5 km. The farm on which also four Arabian horses were kept is protected from strong winds by a hedge of *Conocarpus lancifolius* trees and a 6 m high sand dune. Only the site to the west is open to a large irrigated alfalfa field. In November 2020, the whole herd was moved to a new territory in the desert, 1.5 km away from irrigated crop farms.

Observation started in June 2020 after the owner complained that pregnant camels of his herd again aborted. Abortions in the herd happened already in the previous year when a total of 15 abortions were observed. Some of the animals showed oedemas on ventral abdomen and distal legs (Wernery *et al,* 2020).

Test methods

From all 17 dromedaries blood was withdrawn from the jugular vein. The EDTA blood was analysed for haematological parameters using the automatic haematology analyzer Sysmex XN (Sysmex corporation, Japan). EDTA blood was then tested for the presence of trypanosomes with the BCT. A Giemsa stained blood smear was prepared when life trypanosomes were seen in BCT and the sample was 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 only,

+++: 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.

Sera 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 the 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. In May 2021 also the four camel calves that were borne during the observation period were tested for antibodies.

To exclude brucellosis as cause of abortion, all sera were also examined with the Rose Bengal Test at the beginning of our observations. All sera were negative for brucellosis.

Fly trapping

A Malaise trap and two sticky traps were set for one week next to the camel pens and were checked daily in July 2020. The trap was also set in November 2020 when temperatures had dropped considerably and lots of houseflies appeared on the farm.

Treatment

Twelve non-pregnant camels including the bull were treated with quinapyrimidine (Triquine®) at a dose of 2.5 ml/ 100 kg b.wt. s.c. while three pregnant camels received an i.m. injection of melarsomine (Cymelarsan®) at a dose of 0.25 mg/ kg b.wt. in June 2020. In a second treatment, all positive animals were treated with melarsomine at the same dosage in July 2020. Since examination of EDTA blood after the second treatment still revealed trypanosomes, all non-pregnant camels and the bull received a further treatment with quinapyrimidine and four pregnant were treated with melarsomine in August 2020 and melarsomine treatment of pregnant females was repeated the next day (Table 1).

Reproduction

During the 12 months lasting observation period camel No 9 aborted in the 8th month of pregnancy despite four treatments with melarsomine. Four other females delivered healthy calves despite showing active infection with *T. evansi* (Table 1).

Results

All camels on the farm were in fair to good condition, food and water uptake was normal. Camels Nos. 1 and 7 had oedemas that disappeared after treatment but reoccurred later on.

Parasitological examination of EDTA blood with BCT during the twelve-month observation period revealed that 15 of the 17 adult dromedaries were at least one time positive for trypanosomes despite several applications of trypanocide drugs. Fourteen animals remained all the time serologically positive. One camel (No 15) was initially diagnosed positive with trypanosomes in the blood but was cured since further testing did not reveal positive blood samples and antibodies measured as optical density faded. Two dromedaries (Nos 5 and 16) remained negative for trypanosomes over the whole observation period and remained serologically negative (Table 2, 3). Four calves that were borne in the examination period were included in the last testing in May 2021. No trypanosomes were found in BCT but two calves in an age of 8 and 11 weeks showed antibodies (optical density of 0.5).

Haematological parameters of the two negative (Nos. 5 and 16) and the cured camel (no. 15) were in

Table 1. Treatment of a camel herd against *T. evansi* and reproduction success (quina = quinapyramin, melars = melarsomine).

No.	Sex	Age (years)	Pregnant since	Treatment				Reproduction success
				8.06. 20	09.07.20	4.08. 20	5.08. 20	
1	Fem	8	no	quina	melars	quina	—	
2	Fem	10	Oct-2019	melars	melars	melars	melars	delivered 19 Novmber 2020
3	Fem	6	Jan-2020	melars	melars	melars	melars	delivered 25 March 2021
4	Fem	9	no	melars	melars	quina	—	
5	Fem	>10	no	quina	melars	quina	—	
6	Fem	10	no	quina	melars	quina	—	
7	Fem	10	Jan-2020	melars	melars	melars	melars	delivered 18 February 2021
8	Fem	9	Jan-2020	melars	melars	melars	melars	delivered 2 March 2021
9	Fem	7	Jan-2020	melars	melars	melars	melars	aborted 20 Oct 2020
10	Fem	>10	no	quina	melars	quina	—	
11	Fem	4	no	quina	melars	quina	—	
12	Fem	4	no	quina	melars	quina	—	
13	Fem	4	no	quina	melars	quina	—	
14	Fem	4	no	quina	melars	quina	—	
15	Fem	2	no	quina	melars	quina	—	
16	Fem	2	no	quina	melars	quina	—	
17	Male	4	—	quina	melars	quina	—	

Table 2. Detection of *T. evansi* in blood samples of a camel herd during a 12 month lasting observation period. (June 2020-May 2021).

No	8 Jun	7 Jul	4 Aug	26 Aug	15 Sept	15 Oct	15 Nov	15 Dec	31 Jan	24 Feb.	25 Mar	27 Apr	26 May
1	-	-	-	-	-	-	-	+	-	-	-	-	-
2	++	+	-	+	++++	-	-	++++	+++	++	-	-	-
3	-		-	-	-	+	-	-	-	-	-	-	-
4	+	+	-	-	-	-	++++	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	+	-	-	-	-	-	-	-	-	-	-	-
7	-	-	+++	-	-	-	-	-	-	-	-	-	no blood
8	-	-	-	-	-	-	+	-	-	+++	-	-	no blood
9	++	+	-	-	+	-	+	-	++	-	-	-	no blood
10	+	-	++++	++	++	++++	++	++	-	-	-	-	no blood
11	-	-	+++	++	-	-	+	+	+	+	-	+	-
12	+	+	-	-	-	-	-	+	-	-	-	-	-
13	-	+	-	-	-	-	-	-	-	-	-	-	no blood
14	+	-	-	-	-	-	++++	-	-	-	-	-	no blood
15	+	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	-	+	+	-	-	-	-	-	+	+	-	-

(- : no parasites in BCT,+: single parasites in BCT but not in the blood smear, ++: few parasites in BCT but occasionally in the blood smear only, +++: 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; no blood: animals were not available)

Table 3. Optical density of an indirect ELISA for the detection of *T. evansi* antibodies in serum samples of a camel herd during a 12 month lasting observation period (June 2020-May 2021).

No	8 Jun	7 Jul	4 Aug	26 Aug	15 Sept	15 Oct	15 Nov	15Dec	31 Jan	24 Feb	25 Mar	27.Apr	26 May
1	**2**	**1.9**	**2.4**	**1.9**	**2.2**	**2.1**	**2.2**	**2**	**2.1**	**2**	**2.2**	**2.3**	**2.3**
2	**1.1**	**0.7**	**2.2**	**1**	**1.1**	**1.1**	**1.3**	**1.1**	**0.94**	**1**	**1.3**	**0.92**	**0.8**
3	**2.3**	**2.2**	**2.5**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.5**	**2.5**	**2.2**
4	**2.2**	**2**	**2.4**	**2.1**	**2.1**	**2.1**	**2.2**	**2.1**	**2**	**1.9**	**2**	**2.3**	no blood
5	0.08	0.08	0.07	0.08	0.08	0.08	0.1	0.08	0.09	0.09	0.09	0.08	0.06
6	**2.1**	**2.2**	**2.5**	**2.4**	**2.4**	**2.4**	**2.4**	**2.3**	**2.3**	**2.2**	**2.4**	**2.3**	**1.8**
7	**2**	**1.9**	**1.2**	**2**	**2.2**	**1.9**	**2.3**	**2.3**	**2.3**	**2.2**	**2.3**	**2.4**	no blood
8	**1.7**	**1.7**	**2.3**	**1.9**	**2.1**	**1.9**	**2.3**	**2.3**	**2.3**	**2.1**	**2.3**	**2.2**	no blood
9	**2.3**	**2.3**	**2.6**	**2.4**	**2.5**	**1.9**	**2.5**	**2.4**	**2.4**	**2.3**	**2.4**	**2.5**	no blood
10	**2.2**	**2.2**	**2.4**	**2.4**	**2.4**	**1.8**	**2.4**	**2.4**	**2.4**	**2.3**	**2.5**	**2.4**	no blood
11	**2**	**2.1**	**2.3**	**2.2**	**2.2**	**2**	**2.1**	**2.1**	**2.1**	**2.1**	**2.3**	**2.3**	**2.0**
12	**1.5**	**1.5**	**2.2**	**2.2**	**2**	**1.9**	**2.2**	**2.1**	**2.2**	**2.2**	**2.4**	**2.5**	**2.2**
13	**2.2**	**2.2**	**2.5**	**2.2**	**2.3**	**2.2**	**2.4**	**2.3**	**2.3**	**2.3**	**2.4**	**2.4**	no blood
14	**1.6**	**1.2**	**1.7**	**1.2**	**1.1**	**0.7**	**0.98**	**1**	**0.9**	**0.86**	**0.96**	**0.98**	no blood
15	**0.4**	**0.4**	0.3	0.09	0.07	0.1	0.1	0.1	0.1	0.1	0.08	0.11	0.01
16	0.07	0.08	0.07	0.07	0.08	0.07	0.1	0.1	0.1	0.1	0.055	0.06	0.5
17	**0.8**	**0.7**	**1.1**	**0.6**	**1.1**	**0.6**	**0.9**	**0.7**	**0.74**	**0.71**	**0.63**	**1.1**	**0.9**

(<0.3 was considered negative, 0.3-0.5 was dubious and >0.5 was positive; no blood: animals were not available).

the normal range, except for platelets that were low at few sampling dates and leucocytes for No. 16 that showed slightly enlarged numbers 15.1-18.2x10^9/l but without lymphocytosis.

For all the other camels, erythrocyte and haemoglobin values were in the normal range but platelets were below the reference value of 270 x 10^9/l at most of the sampling dates. Twelve of the trypanosome positive camels showed enlarged leucocyte counts at all collecting dates. In 10 animals, this value exceeded 20x10^9/l and reached up to 56.8x10^9/l in camel No. 3. Lymphocytosis and neutrophilopenia was observed in most of the *Trypanosoma* positive camels.

Fig 1. *Tabanus sufis*, female.

Fly trapping with a Malaise trap in July revealed seven horseflies of the species *Tabanus sufis* (Fig 1) as the only bloodsucking insect. In November four horseflies of the same species were trapped. Due to sand and dust in the air, sticky traps were not effective.

Discussion

Reoccurring of trypanosomes in the bloodstream and/ or persisting antibodies against trypanosomes in the blood serum after numerous applications of trypanocidal drugs suggest a resistance of the parasites to melarsomine and quinapyrimidine in the investigated camel herd. While quinapyramine was developed in the late 1940th and is on the market since the 1950th (Hawking and Sen, 1960) first data on the efficacy of melarsomine against *T. evansi* in naturally infected dromedaries were obtained by Zelleke *et al* (1989). A single dose of 0.3 or 0.6 mg kg b.wt. eliminated blood trypanosomes and treated animals remained negative till the end of the observation periode of 14 weeks.

It is believed by camel owners and trainers of racing camels, that an injection of a subtherapeutic

dose of melarsomine cleans the body and enhances performance of racing camels. This underdosing is the reason for resistance of *T. evansi* to melarsomine. Mass treatments using a single syringe and blood doping in racing camels can also be *Trypanosoma* infection sources.

Of the 17 dromedaries, only two were negative for trypanosomes and for antibodies throughout the whole observation period. A further camel (No. 15) had trypanosomes at the beginning of the observation and became negative after the first treatment. Serologically it became negative in August and remained negative at all further sampling dates. All other adult camels of the herd remained serologically positive and 10 out of them even showed trypanosomes in the bloodstream after treatments.

In a classical surra case in camels, the disease, if not treated, develops into a chronical course with weight loss, weakness, loss of condition, rough coat and oedema at different locations but mainly under the belly. This clinical sign develops also during the acute stage and was seen in two of the infected camels. According to textbooks, animals develop anaemia, mucous membranes are pale and changes in the haematological parameters are often significant with low red blood cell count (RBC), low haemoglobin, low packed cell volume and decreased platelets. Anaemia is often believed to be a reliable indicator of a chronic trypanosome infection, but it is not pathognomonic as mild subclinical and acute infections often show no evidence of anaemia.

Although there was a difference in haematological values of the 15 infected dromedaries in comparison to the 3 non-infected including the successfully treated camel, most of the parameters indicating anaemia were still in the normal reference range for erythrocytes and haemoglobin (7.0-10.5x10^{12}/l and 10.5-14.5 g/dl, respectively). In some of the *Trypanosoma* positive camels these parameters even were slightly above these values. This shows that it is difficult to suggest anaemia as a reliable sign of *T. evansi* infection. However, the total leucocyte counts and the share of lymphocytes were significantly increased and lay outside the reference values in serologically positive animals while platelets were decreased. It is worthwhile to mention that although *T. evansi* is a parasite, the eosinophil count was elevated only at few sampling dates in four camels.

The transmission of *T. evansi* under natural conditions is mechanically by interrupted feeding of bloodsucking flies. Horse flies (Tabanidae) with their complex mouthparts are the main vectors (Luckins, 1998). During our investigations we detected so far only one (*T. sufis*) out of 11 described species in the UAE (Ježek *et al,* 2017). A whole list of tabanids that are relevant for surra in camels is given by Wernery *et al* (2014). Both sexes of tabanids feed on plant pollen and sugars, but females need blood meals prior to oviposition. The larval development of tabanids takes place in wet or damp soil. Such conditions were present in the surroundings of the farm since forage crops in surrounding areas in this desert environment need permanent irrigation. The movement of the camels away from tabanid breeding sites reduces the chance of a natural transmission of the parasite.

The role of stable flies (*Stomoxys* spp.) as vectors for *T. evansi* is disputed and was reviewed by Baldacchino *et al* (2013) and Desquesnes *et al* (2013). During our entomological survey no stable flies were trapped but they might have been present in previous months. It is our experience that *Stomoxys calcitrans* occurs in Dubai after rainfalls in winter and reaches high population densities in March and April. With rising temperatures stable flies fade (Schuster and Sivakumar 2013a,b).

Several parasitic infections may cause abortions. According to Shaapan (2015) these are toxoplasmosis, neosporosis, sarcocystosis, trypanosomosis, tritrichomonosis and babesiosis. With regards to dromedaries, only trypanosomosis is proven to be a cause of abortion and this was the cause in the camel herd under investigation. Right from the beginning, brucellosis as another disease that has to be considered was excluded by negative results of the Rose Bengal Test. Noteworthy, four *Trypanosoma* positive dams delivered healthy calves. Low titer antibodies that were found in two of the calves most probably originated from colostrum of the mothers.

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