J. Protozool. Res., 8, 120-125 (1998)

Prevalence of Surra in Dromedary Camels in Uganda

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Received 20 August 1998

Key words: Surra, dromedary camels, Uganda

ABSTRACT

Three herds, comprising 112 camels, were sampled and examined for *Trypanosoma evansi* infection (surra) in Moroto district, north-eastern Uganda. The Micro-Haematocrit Centrifuge Technique (MHCT) was used for parasitological diagnosis, while Suratex® and Enzyme-Linked immunosorbent assay (ELISA) were used for trypanosome antigen and anti-trypanosomal antibody detection, respectively. The haematocrit level of each camel was determined during examination with MHCT. Parasite prevalence ranged from 0% to 47%, while Suratex® showed positivity ranging from 35% to 65%. A high antibody prevalence ranging from 78-100% was recorded by ELISA. Low haematocrit values were associated with parasite or antigen-positive camels. Preliminary characterization of 8 trypanosome isolates from these camels by microscopic examination of Giemsa-stained bloodstream forms, transformation in SM-77 medium and isoenzyme characterization, shows characteristics similar to *Trypanosoma evansi*, as evidenced from their monomorphism, failure to transform in procyclic medium and zymodeme profiles. These findings show a high prevalence of *T. evansi* infection in Ugandan camels which is associated with anaemia and calls for the institution of interventional measures to save these useful animals. This is the first report of the existence of *T. evansi* in Uganda, which could be of considerable consequence in the epidemiology of animal trypanosomosis in the country.

INTRODUCTION

Trypanosomosis due to *Trypanosoma evansi*, also known as surra, is the most important protozoal disease affecting camels globally (Gatt-Ratter 1967). In Kenya for example, 95% of camel trypanosomosis is attributed to *T. evansi* infection (Wilson et al. 1981). *Trypanosoma evansi* is transmitted mechanically by biting flies such as tabanids and stomoxys. Epidemics of the disease often occur after peak fly activity, which usually occurs after the rains. Under field conditions the disease manifests different clinical forms, namely: acute, subacute, chronic and inapparent (Wilson et al. 1983). The chronic disease is the most common and is characterized by severe anaemia, general wasting, reduced milk production in lactating animals, infertility, abortions and death. The acute form of disease is more common in calves and in camels which are exposed to new strains of *T. evansi*. In chronic infections some animals may survive but remain infected for years and exhibit low-level fluctuating parasitaemia. These animals may serve as reservoirs of infection for the noninfected camels. There are no pathognomonic signs of the disease, so laboratory diagnosis has to be carried out to confirm infection. Traditionally, this involves parasitological and serological diagnosis. Parasitological diagnosis is mainly carried out by the direct microscopic examination of blood or buffy-coats and/or the subinoculation of camel blood into rodents such as mice or rats. These tests may fail to detect sub-patent infection when parasitaemia is scanty in peripheral blood (Killick-Kendrick 1968). On the other hand, antibody detection assays like, immunofluorescent antibody test (IFAT) or

enzyme linked immunsorbent assay (ELISA) (Luckins et al. 1979) and the card agglutination trypanosomosis test (CATT) (Bajyana-Songa and Hamers 1988) though sensitive, cannot distinguish current from cured infections (Luckins 1988). Recent tests based on trypanosome-antigen detection in blood or serum are more reliable and have shown a high correlation with patent or sub-patent disease in camels (Nantulya et al. 1989, 1994; Olaho-Mukani et al. 1993a, 1996).

In Africa, surra was at first believed to be confined to the area north of the line drawn from northern Nigeria through, Lake Chad, Barhel-el-Ghazal and northern Kenya , (Gatt-Rutter 1967). However, recent studies have shown that surra occurs in camels kept as far south as Masailand in Kajiado district of Kenya and probably northern Tanzania (Olaho-Mukani et al. 1990). The present study was undertaken to establish the status of surra in Ugandan camels.

MATERIALS AND METHODS

Camel Herds: Three camel herds comprising 112 camels were sampled in Moroto district of Karomoja. Two of the herds were in Rupa sub-county of Matheniko county and one in Kangole-Lotome sub-county of Bokora county. In total, there were 44 males and 68 females, of which 20 were calves. These camels are of Turkana breed and Somali-Turkana or Rendille-Turkana crosses found in neighbouring Kenya.

Parasitological diagnosis: Camel blood for parasitological and serological diagnosis was obtained by jugular venipuncture. Ten mls of which was allowed to clot in vacutainer tubes for serum preparation, while 1ml was heparinised and used for buffy-coat examination and haematocrit determination by the Micro-Haematocrit Centrifugation Technique (MHCT) (Woo 1969). Blood from parasitaemic camels was subinoculated into mice (Molyneux 1973) or stabilated directly in liquid nitrogen for laboratory characterization of trypanosome isolates.

Latex Agglutination (Suratex©): The test was performed on serum following the guidelines provided by the manufacturer (Brentex, Narobi) (Nantulya 1994). Briefly, a drop of serum (approx. 50μl) was transferred onto the circle of the test-card and an equal volume of Suratex® reagent placed next to it. The two drops were mixed with a stirring rod and spread out to cover the entire surface area of the circle. The test-card was then tilted and rotated manually for 2 minutes and viewed for evidence of agglutination, then rotated for additional 3 minutes, to allow weak reactions to develop before viewing again for agglutination.

Antibody Micro-ELISA: A micro-enzyme linked immunosorbent assay (ELISA) was performed as described by Olaho-Mukani et al. (1993a) using lysate antigen prepared from *T. evansi* isolate 0198A, and goat anticamel IgG-peroxidase conjugate.

In vitro Procyclic Transformation: In vitro transformation of bloodstream forms of trypanosomes isolated from camels was carried out as described by Zweygarth and Kaminsky (1989) and Olaho-Mukani et al. (1993b). Briefly, trypanosomes were aseptically isolated from parasitaemic mouse blood by anion exchange chromatography (Lanham and Godfrey 1970) and seeded into SM-77 transformation medium (Cunningham 1977) supplemented with 20% foetal calf serum. The cultures were incubated at 27℃ to initiate procyclic transformation and observed for 7 days.

Morphological Identification: Morphological characterization was carried out by microscopic examination of Giemsa-stained thin blood smears using morphological features described by Hoare (1956).

Isoenzyme Characterization: Isoenzyme analysis was performed on enzyme extracts of trypanosome stocks as described by Stevens et al. (1989). The enzymes analysed were alanine amino transferase (ALAT), aspartate amino transferase (ASAT), isocitrate dehydrogenase (ICD), phosphoglucomutase (PMG), malate dehydrogenase (MDH), nucleoside hydrolases (NHI and NHD), threonine dehydrogenase (TDH) and superoxidase dismutase (SOD).

RESULTS AND DISCUSSION

Prevalence of surra in the three herds is shown in Table 1. The highest prevalence of infection was observed at Matheniko-Rupa, which recorded 100% anti-trypanosomal antibody prevalence, 65% Suratex® positivity and 47% parasite prevalence. The camel herd at Rupa-Acholi Inn also recorded fairly light prevalence rates: 85% antibody prevalence, 62% Suratex® positivity and 26% Parasite prevalence. The disease in these two herds had reached epidemic proportions and there was indication that most of the camels had been exposed to infection. Though no parasites were detected in Kangole-Latome camels, an antibody prevalence of 78% is indicative of a high exposure state. Furthermore, a Suratex® positivity of 35% could be indicative of sub-patent disease undetectable by MHCT, as observed by Killick-Kendrick (1968) and Olaho-Mukani et al. (1996).

Generally, the haematocrit status of the three herds showed evidence of anaernia as shown by the mean PCV levels. This was more so in the Matheniko-Rupa herd which recorded mean PCV values below the normal range (25-43% dromendary camels (Mutug, et al. 1993). The mean PCV values for Rupa-Acholi Inn and Kangole-Latome camels were at the lower limit for the normal range for dromendary camels. Under field conditions several factors including surra and helminthosis may affect the haematocrit status of camels (Wilson et al. 1981).

Table 1. Prevalence of surra by MHCT, Suratex® and antibody-ELISA and the haematocrit status of three herds of camels from Moroto district

Camel herd	n	Mean PCV (±SE)	Prevalence (%)		
			Suratex®	HCT	Ab -ELISA
Matheniko-Rupa	55	22.1±1.2	65	47	100
Rupa-Acholi Iun	34	25.1±0.7	62	26	85
Kangole-Lotome	23	24.7±0.6	35	0	78

Table 2 : Haemotocrit status of parasite-positive and Suratex®-positive camels and those that were negative

Group	n	Mean PCV (±SE)
Parasite-positive	35	21.5 ± 0.6
Parasite-negative	77	24.3 ± 0.9
Suratex®-positive	69	22.3 ± 0.7
Suratex®-negative	43	25.5 ± 0.3

That notwithstanding, surra appeared to play a direct influence on the haematocrit status of these camels. As shown in Table 2, camels with patent or sub-patent (MHCT and/or Suratex®-positive), recorded lower PCV values than those that were without. This would be expected because *T. evansi* infection in camels is known to cause anaemia (Gatt-Ruther 1967).

Characterization of 8 trypanosome stocks by morphological examination of Giemsa-stained thin blood smrears revealed slender monomorphic forms characteristic of *T. evansi* as observed by Hoare (1956). This was further supported by the failure of these isolates to transform into procyclic forms unlike *T. b. rhodesiense*, as shown in Table 3.

Table 3. Procyclic transformation in SM-77 medium by selected trypanosome stocks isolated from camels in Moroto district.

Stock	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Camel 1197A	+	+	+	+/-	-	-	-
Carnel 0198A	+	+	+/-	-	-	-	-
Camel 1197B	+	+	+/-	-	-	-	-
Camel 0398A	+	+	+/-	-	-	-	-
Man Utat 4. 1	+	+	+	+	+	+	+

+ Trypanosomes present and alive; +/- Many trypanosomes dead, few alive
- All trypanosomes dead.

All the stocks from camels had the same isoenzyme profile 346 which differed from those of *T. b. brucei, T. b. rhodesiense* and *T. b. gambiense* included in the study. The SOD 1.3 pattern was observed in the camel isolates only and has not been observed in the Ugandan 230 *T. brucei* ssp. isolates so far analysed (Enyaru 1993; Enyaru et al. 1997). However, Lun et al. (1992) reported the same SOD 1.3 pattern in *T. evansi* from China. Similarly, in a study involving stocks from Africa and South America, Stevens et al. (1989) mentions SOD$_B$ 3 as being a characteristic of *T. evansi*. *Trypanosoma evansi* has never been reported in Uganda.

Table 4. Isoenzyme profiles of selected trypanosome stocks isolated from camels in Moroto district

Stock	Profile	ALAT	ASAT	PGM	ICD	MDH	NHI	NHD	IDH	SOD A.B
Man NWS[1]	378	2	3	2	2	1	1	1	1	3.5
Camel 0198A	346	10	1	2	2	1	1	1	1	1.3
Camel 1197B	346	10	1	2	2	1	1	1	1	1.3
Camel 11974	346	10	1	2	2	1	1	1	1	1.3
Dog 250594[2]	444	10	1	3	1	1	1	3	1	1.9
Dog 290494[2]	639	10	1	2	2	1	1	5	1	1.4
Man 120394[3]	444	10	1	3	1	1	1	3	1	1.9

[1]*T. b. gambiense;* [2]*T. b. brucei* ; [3]*T. b. rhodesiense* ; ALAT = Alamine amino transferase; ASAT=Aspartate amino transferase; ICD = Isocitrate dehydrogenase; PGM = Phosphoglucomutase; MDH = Malate dehydrogenase; NH1/NHD = Nucleoside dehydrolases; TDH = Threonine dehydrogenase; SOD = Superoxidase dismutase

This is the first report of its accurrence in that country. The high prevalence of *T. evansi* infection in Uganda camels calls for the institution interventional measures to save these useful animals. This should employ diagnosis using Suratex® and MHCT and prompt treatment with Cymelarsan® or Triquin®. The existence of *T. evansi* in Uganda could bear significant consequences on the epidemiology of animal trypanosomosis in the country, given the fact that the parasite is pathogenic to other domestic animals. The present study shows further proof that *T. evansi* in Africa, has spread southward beyond the line defined by Gatt-Rutter (1967).

ACKNOWLEDGEMENT

This study was supported by World Bank and Uganda Government. The authors would like to thank the Director General NARO for permission to publish this work and the DVO Moroto for his assistance.

REFERENCES

Bajyana-Songa, E. & Hamers, R. 1988. A card agglutination test (CATT) for veterinary use based on early VAT RoT. at. 1/2 of *Trypanosoma evansi*. *Ann. Soc. Belg. Med. Trop. 67:51-57.*

Cunningharn, I. 1977. New culture medium for the maintenance of tsetse tissues and growth of trypanosomatids. *J. Protozool. 24: 325-329.*

Enyaru, J.C.K. 1993. Biochemical characterisation of *Trypanosoma (Trypanozoon) brucei* species in Uganda. *Ph.D. Thesis, Makerere University.*

Enyaru, J.C.K., Matovu, E., Okedi, L.A., Rwendeire, A.J.J. & Stevens, J.R. 1997. Genetic diversity in *Trypanosoma (Trypanozoon) brucei* isolates from mainland and L.Victoria island populations in South-Eastem Uganda: Epidemiological and control implications. *Ann. Trop. Med. Parasit. 91 : 107-113.*

Gatt-Rutter, T.E. 1967. Diseases of camels. II. Protozoal diseases. *Vet. Bull. 37: 611-618.*

Hoare, C. A. 1956. Morphological and taxonomic studies on mammalian Trypanosomes. VII. Revision on *Trypanosoma evansi. Parasitology 46: 130-172.*

Killick-Kendrick, R. 1968. The diagnosis of Trypanosomiasis in livestock; a review of current techniques. *Vet. Bull. 38: 191-197.*

Lanham, S.M. & Godfrey, D.G. 1970. Isolation of Salivarian trypanosomes from man other mammals using DEAE-cellulose. *Exp. Parasit. 28: 521-534.*

Luckins, A.G., Gray, A.R. & Rae, P.F. 1978. Comparison of diagnostic value of serum immunoglobulin levels, an enzyme-linked immunosorbent assay and fluorescent antibody test in experimental infections with *Trypanosoma evansi* in rabbits. *Ann. Trop. Med. Parasitol. 72: 429-441.*

Luckins, A.G. 1988. *Trypanosoma evansi* in Asia. *Parasitol. Today. 4: 137-142.*

Lun, Z.R., Alligham, R., Brun, R. & Lanham, S.M. 1992. The isoenzyme characteristics of *Trypanosoma evansi* and *Tyrpanosoma equiperdum* isolated from domestic livestock in China. *Ann. Trop. Med. Parasit. 86: 333-340.*

Molynenx, D.K. 1973. Isolation of *Trypanosoma (Trypanozoon) brucei gambiense* in rabbits by the intra-testicular inoculation technique. Ann. *Trop. Med. Parasitol. 67:391-397.*

Mutugi, M.W., Olaho-Mukani, W., Kuto, B., Alushula, H. & Njogu, A.R. 1993. Haematological characteristics of the one humped camel, *Camelus dromedarius* in Kenya. *Bull. Anim. Prod. Afr. 41: 181-184.*

Nantulya, V.M., Lindqvist, K.J., Diall, O. & Olaho-Mukani, W. 1989. Two simple antigen-detection enzyme immunoassays for the diagnosis of *Trypanosoma evansi* infection in the dromedary camels (*Camelus dromendarius*). *Trop. Med. Parasitol. 40: 415-418.*

Nantulya, V.M. 1994. Suratex®. A simple Latex agglutination antigen test for the diagnosis of *Trypanosoma evansi* infection (surra). *Trop. Med. Parasitol. 45: 9-12.*

Olaho-Mukani, W., Omuse, J.K., Nyangao, J.M.N, Mutugi, M.W., Ngaira J. M., Luckins, A. G. & Jeffreis, P. 1990. Use of ELISA in the diagnosis of infection and evaluation of treatment with *Trypanosoma evansi* in Kenya. *IAEA-TECDOC-707: 121-130.*

Olaho-Mukani, W. Munyua, W.K., Mutugi M.W. & Njogu, A. R. 1993a. Comparison of antibody and antigen-detection enzymes immunoassays for the diagnosis of *Trypanosoma evansi* infection in camels. *Vet. Parasitol. 45:23 1-240.*

Olaho-Mukani,W., Mkunza, F., Kimani, J. K., Njoka, P. K. & Walubengo. J. 1993b. Evaluation of the in vitro transformation technique to distinguish *Trypanosome evansi* from cyclically transmitted *Trypanozoon* stocks. *Trop. Med. Parasitol. 44: 108-110.*

Olaho-Mukani, W. Nyangao, J.M.N. & Ouma, J.O. 1996. Comparison of Suratex® , parasite detection and Antigen-ELISA for the evaluation of treatment efficacy and diagnosis of surra in dromedary camels. *J. Cam. Pr. Res. 2: 1-5.*

Stevens, J.R., Nunes, V.L.B., Lanham, S.M. & Oshiero, E.T. 1989. Isoenzyme characterization of *Trypanosoma evansi* isolated from capybaras and dogs in Brazil. *Acta Tropica. 46: 213-222.*

Wilson, A.J., Dolan, R. & Olaho-Mukani, W. 1981. Important camel diseases in selected areas of Kenya. *IPAC/UNESCO REP. No. E6.*

Wilson, A.J., Schwartz, H.J., Dolan, R. & Olaho-Mukani, W. 1983. A simple classification of different types of trypanosomosis occurring in four camel herds in selected areas of Kenya. *Tropenmed. Parasitol. 34: 220-224.*

Woo, P.K. 1969. The haematocrit centrifuge for the detection of trypanosomes in blood. *Canadian J. Protocol. 47: 921-923.*

Zweygarth, E. & Kaminsky, R. 1989. In vitro differentiation between *Trypanosoma brucei* and *T. b. evansi. Trop. Med. Parasitol. 40: 115-116.*