

# Animal-level risk factors for *Trypanosoma evansi* infection in camels in eastern and central parts of Kenya

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#### **ABSTRACT**

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Point prevalences and animal-level risk factors for Trypanosoma evansi infection were investigated in a cross-sectional study that involved 2 227 camels from eastern and central parts of Kenya. The screening tests used were haematocrit centrifugation technique (HCT), mouse inoculation and latex agglutination (Suratex®). All camels were screened with HCT, while 396 and 961 of them were, in addition, screened with mouse inoculation and Suratex® tests, respectively. Parasitological and Suratex® test results were used in parallel to determine the number of camels exposed to T. evansi infections. Statistical analyses were conducted using Statistical Analysis Systems. Parasitological and Suratex® test results in parallel were dependent variables in multivariable logistic regression models that determined risk factors for T. evansi infection. Herd-level clustering was corrected with general estimation equations. The prevalences were 2.3 % and 19.6 %, using parasitological and Suratex® tests, respectively, and 21.7 % when both tests were used in parallel. There was a positive association between the screening tests (McNemar's test = 104.8, P = 0.001) although the strength of association was low (Kappa = 0.2; 95 % CI: 0.1-0.3). Before accounting for herd-level clustering, dry season (OR = 1.5; 95 % CI: 1.0, 2.1) and nomadic pastoralism (OR = 1.8; 95 % CI: 1.1, 3.2) were associated with increased odds of a camel being exposed to T. evansi infection compared to wet season and ranching, respectively. Following this correction, only nomadic pastoralism was significantly associated (OR = 3.1; 95 % CI = 1.0, 14.4) with T. evansi infection compared to ranching. It is concluded that camels managed under nomadic pastoralism had higher risk of being exposed to T. evansi infections than camels from ranching systems of management.

Keywords: Animal-level risk factors, camel, Kenya, pastoralism, Trypanosoma evansi

## INTRODUCTION

*Trypanosoma evansi*, the parasite that causes camel trypanosomosis is widespread throughout the world (Lesos 1980; Luckins 1988). In Kenya 95 % of camel trypanosomosis is attributed to *T. evansi* infections (Wilson, Dolan & Olaho-Mukani 1981).

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The disease manifests in different forms: acute, subacute, chronic and inapparent (Wilson, Schwartz, Dolan & Olaho-Mukani 1983). The chronic form is the most prevalent and is characterised by severe anaemia, general wasting, reduced production of milk, infertility, abortions, and in some animals, death (Olaho-Mukani, Munyua, Mutugi & Njogu 1993). The disease has also been identified as being of great economic importance in camel husbandry (Maina, Otieno, Okwara, Ngatia, Auma, Nyang'ao, Olaho-Mukani & Surtherland 1995).

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Effective control of camel trypanosomosis requires accurate epidemiological information regarding the occurrence of *T. evansi* infections in camels and other animal species. Factors associated with *T. evansi* infections in camels have been described in a number of publications. Dia, Dio, Aminetou, Jacquiet & Thiam (1997) reported a significant association between camel trypanosomosis and animals aged between 5–10 years, while Elamin, El Bashir & Saeed (1998) noted that nomadism and dry season were significantly associated with the disease.

Accurate diagnosis of T. evansi infection can be obtained by a combination of parasitological detection techniques and antigen detection. The latex agglutination test (Suratex®) for the detection of circulating trypanosome antigens has 100% specificity for trypanosome antigens (Nantulya 1994). Using this test in epidemiological studies in Kenya, Olaho-Mukani, Nyang'ao & Ouma (1996) showed good correlation between antigen detection and HCT. Test sensitivity was 88 % and seroprevalence 46.3 % compared to 23.1 % parasite prevalence. Chaudhary & Igbal (2000) used Suratex® to monitor seroprevalence in racing camels in United Arab Emirates and found good correlation between the positive results obtained by wet blood smear and Suratex®. On the basis of these reports, and because Suratex® is simple to use in field situations, the test was chosen to complement parasitological techniques, i.e. haematocrit centrifugation technique (HCT) and mouse inoculation test (MI) in the present study.

In this study, we investigated the factors associated with camel trypanosomosis in three districts of Kenya. Parasitological techniques and Suratex® test were used in parallel to identify risk factors for exposure of camels to *T. evansi* infection. We also assessed the agreement between the parasitological techniques and Suratex® test in the diagnosis of *T. evansi*.

## **MATERIALS AND METHODS**

# Study areas and camel population

The study was carried out between October 1996 and July 1997. Three areas selected for the survey were Athi in Machakos district, Mugwoni in Laikipia district and Isiolo in Isiolo district.

Athi falls in the Upper Midland Ranching agro-ecological zone (UM 6). The annual rainfall averages 557 mm and is distributed in two rainy seasons: March to May (143 mm) and November to Decem-

ber (171 mm). The dominant black soil supports treeless short grass savannah and a stocking rate of more than 4 ha per livestock unit (LU) (Jaetzold & Schmidt 1983a). Mugwoni, similarly, falls in the Upper Midland Ranching zone (UM 6). It receives, on average, an annual rainfall of 600-850 mm distributed in three seasons: April to May (100 mm), June to July (120 mm) and October to November (> 60 mm). The predominant vegetation is short grass and thorn bush savannah that supports a stocking rate of more than 4.8 ha/LU (Jaetzold & Schmidt 1983b). Isiolo is in the Lower Midland Ranching zone (LM 6). Mean minimum and maximum temperatures range between 15.5 and 32.3 °C. The short grass savannah supports a stocking rate of more than 4 ha/LU, but reduces to 12 ha/LU in eroded areas (Jaetzold & Schmidt 1983a).

It is estimated that there are about 810 000 camels in Kenya currently (Köhler-Rollefson, Mundy & Mathias 2001). The largest camel numbers are found in North Eastern Province and Eastern Province. Grill (1985) indicated that there were 96 680 and 733 camels in Isiolo and Laikipia districts, respectively. Less than 1 % was found in Machakos district.

# Study design and sample selection

A cross-sectional study design was adopted in this survey. The selection of the areas was based on the reported claims that camel trypanosomosis was prevalent in Isiolo District and Mugwoni in Laikipia District. Athi area was known to be free from the disease. However, because of the rough terrain in the study sites the prepared sampling frame could not be strictly adhered to. Camel owners were requested to participate in the study through the local leaders. All the camels in the herds presented for screening were sampled and screened with HCT. A total of 396 blood samples that had packed cell volumes (PCVs) less than 22 were inoculated in mice. Herds were randomised and every other herd was selected for screening. Out of a total of 86 herds, 40 (with a total of 961 camels) were selected for screening with Suratex®.

## Trypanosomosis diagnosis

Camels were examined clinically, and a general history of each herd and each individual camel was recorded. Blood was drawn from the jugular vein using a 20 m $\ell$  sterile syringe and transferred to 5 m $\ell$  vacutainer tubes and to 15 m $\ell$  non-heparinised (plain) vacutainer tubes. Heparinised blood

was centrifuged in microhaematocrit tubes and the PCV determined using a microhaematocrit reader. HCT (Woo 1970) and MI were conducted for the detection of infection. Briefly, in the HCT, heparinised blood was centrifuged in microhaematocrit tubes that were then examined microscopically for the presence of live trypanosomes near the buffy layer. In the MI technique, pairs of laboratory-bred mice were inoculated intraperitoneally with 0.5 ml of blood per mouse. Parasitaemia was monitored by daily examination of tail blood for 60 days. The blood in the plain vacutainer tubes was allowed to clot, serum harvested after 24 h then stored at -20 °C. Trypanosoma evansi circulating antigens were detected using the latex agglutination test, Suratex® as described by Nantulya (1994).

# Data management and statistical analysis

Data generated from the study were entered in a database designed using Microsoft Excel. It was then exported to Statistical Analysis Systems (SAS 1990) software for statistical analysis. The level of significance for all the analyses was set at P = 0.05 (2-sided).

The association and strength of association between parasitological (HCT and mouse inoculation tests used in parallel) and Suratex® tests were determined using McNemar's Chi-square test and Kappa statistic, respectively. The differences in mean PCV (± SE) between HCT-positive/negative, Suratex®positive/negative and Suratex® positive/ HCT negative camels were analysed using Student's T-test. Chi-square tests were used to determine if univariable associations existed between the independent variables and the occurrence of trypanosomosis. Exposure to T. evansi infection was determined using parasitological tests, Suratex® test and both parasitological and Suratex® tests in parallel. Independent variables included age (calf or adult), sex, breed (Somali, Turkana, Pakistan, Somali-Pakistan or Somali-Turkana crosses), management (ranching or pastoralism), season (wet or dry) and area (Isiolo, Athi or Mugwoni).

Two multivariable logistic regression models were fitted to the data with parasitological/Suratex® tests used in parallel as dependent variable. The first model was an ordinary logistic-regression model built without accounting for internal correlation at herd level. Clustering at herd level was accounted for using generalised estimation equations (GEE) in the second model.

Backward elimination was used to determine the variable(s) that could be dropped from the models based on Wald's tests. A variable was dropped from the list of those initially offered to the model if it was highly collinear with others already fitted. Main effects models were fitted first and two-way interaction terms were later included. The difference in deviance of models with and without the interaction term compared with chi-square distribution was used to test the significance of the interaction. Adjusted odds ratios and their 95 % confidence intervals were computed.

## **RESULTS**

# Trypanosomosis diagnosis

Diagnostic tests

A total of 2 227 camels were screened for trypanosomosis with HCT. Twenty-seven were found parasitaemic. Of the 396 pairs of mice inoculated intraperitoneally with 0.5 m $\ell$  of blood from anaemic camels, 47 pairs developed patent parasitaemia within 60 days. When HCT and MI were used in parallel for trypanosomosis diagnosis, a total of 51 camels were found infected, indicating a parasitological prevalence of 2.3 % (95 % CI: 1.6, 2.9). All the parasite populations were slender and monomorphic, a characteristic suggestive of T. evansi. Parasitaemia varied, and was scored between 1-3 trypanosomes per field. Out of 961 sera analysed for T. evansi antigens with Suratex®, 188 tested positive. The prevalence of T. evansi antigens, therefore, was 19.6 % (95 % CI: 17.1, 22.1). Parasitological and SuratexÒ tests used in parallel gave a prevalence of 21.7% (95% CI: 19.1, 24.4).

There was a significant association between parasitological and Suratex® tests (McNemar's test = 104.8, P = 0.001); though the strength of association was low (Kappa = 0.2, 95% CI: 0.1-0.3) (Table 1).

#### Packed cell volume

The mean PCV of parasitologically positive camels (22.7  $\pm$  0.1) was significantly (P < 0.05) lower than the mean PCV of HCT-negative camels (25.3  $\pm$  0.6). Similarly, the mean PCV of Suratex®-positive camels (24.0  $\pm$  0.2) was significantly (P < 0.05) lower than the mean PCV of Suratex®-negative ones (25.2  $\pm$  0.1). The mean PCV of Suratex® positive camels (24.0  $\pm$  0.2) was also significantly (P < 0.05) lower than the mean PCV of parasitologically negative camels (25.4  $\pm$  0.17).

## Univariable analyses

There was a positive association (P = 0.003) between wet season and a high parasitological prevalence (Table 2). *T. evansi* antigens were significantly more prevalent amongst camels managed under nomadic pastoralism (P = 0.05) (Table 3). Higher *T. evansi* antigenaemia was also identified in camels from Isiolo and Mugwoni than those from Athi area (P = 0.001).

Significantly higher parasitological prevalence was recorded during the wet than the dry season, although higher *T. evansi* antigen prevalence was realised during the dry than the wet season.

When parasitological and Suratex® tests were used in parallel (Table 4), management and area were identified as the two variables that significantly influenced the variation of T. evansi prevalence. Nomadic pastoralism was associated with significantly (P = 0.01) higher T. evansi prevalence than ranching, whereas Isiolo and Mugwoni areas had significantly (P = 0.001) higher prevalences than Athi area.

## Multivariable analyses

Because of the collinearity between management and area ( $\chi^2$  = 1772.6, P = 0.001), the parameter estimates for the area were not generated, and

TABLE 1 The relationship between parasitological and SuratexÒ tests used in the screening for T. evansi infections in camels in selected areas of Kenya between 1996 and 1997

Curatav@ taat	Parsitological test	Parsitological tests			
Suratex® test	Positive	Negative	Total		
Positive Negative	30 21	158 752	188 773		
Total	51	910	961		

McNemar's test = 104.8, P = 0.001Kappa = 0.2, 95 % CI: 0.1-0.3

TABLE 2 Descriptive statistics of T. evansi parasitological prevalences in camels screened for trypanosomosis using both the HCT and mouse inoculation test in selected areas of Kenya between 1996 and 1997

	Levels	No. of camels		Prevalence		
Variable		n	Parasitological tests (positive)	% positive	95 % CI	P > Chi
Age	Adult (> 5 years) Calf (< 5 years)	1303 341	38 4	2.9 1.2	2.0, 3.8 0.0, 2.3	0.07
Sex	Male Female	576 1083	14 28	2.4 1.4	1.2, 3.7 0.7, 2.0	0.85
Breed	Somali Turkana Pakistan Somali-Pakistan Somali-Turkana	1913 301 5 7	51 0 0 0 0	2.7 0 0 0 0	1.9, 3.4 - - - -	0.07
Management	Pastoralism Ranching	2104 123	51 0	2.4	1.8, 3.1 –	0.81
Season	Dry Wet	1671 556	31 20	1.9 3.6	1.2, 2.5 2.0, 5.14	0.003
Area	Isiolo Athi River Mugwoni	2008 103 86	51 0 0	2.5 0.0 0.0	1.8, 3.2 - -	0.13

TABLE 3 Descriptive statistics of *T.evansi* sero-prevalences in camels screened with Suratex® in selected areas of Kenya between 1996 and 1997

Variable	Levels	No. of camels		Prevalence		- P > Chi
		n	Suratex® positive	% positive	95 % CI	P > GIII
Age	Adult (> 5 years) Calf (< 5 years)	612 121	106 22	17.3 18.1	14.3, 20.3 11.3, 25.1	0.81
Sex	Male Female	271 446	45 80	16.7 17.9	12.2, 21.0 14.4, 21.5	0.66
Breed	Somali Turkana Pakistan Somali-Pakistan Somali-Turkana	880 68 5 7	176 9 1 2	20.0 13.2 20.0 28.6 0	17.4, 22.6 5.2, 21.3 - -	0.65
Management	Ranching Pastoralism	123 838	16 172	13.0 20.5	7.1, 18.9 17.8, 23.3	0.05
Season	Dry Wet	884 77	179 9	20.3 11.7	17.6, 22.9 4.5, 18.9	0.07
Area	Athi River Isiolo Mugwoni	103 749 86	3 169 16	2.9 22.6 18.6	0, 6.2 19.6, 25.6 10.4, 26.8	0.001

TABLE 4 Prevalence of *T. evans*i in camels from selected areas of Kenya screened with parasitological and Suratex® tests in parallel between 1996 and 1997

Variable	Levels	No. of camels		Prevalence		P > Chi
		п	Positive (parallel)	% positive	95 % CI	PSGIII
Age	Adult (> 5 years) Calf (< 5 years)	613 121	124 22	20.2 18.2	17.0, 23.4 11.3, 25.1	0.77
Sex	Male Female	270 446	52 91	19.3 20.4	14.6, 24.0 16.7, 24.1	0.71
Breed	Somali Turkana Pakistan Somali-Pakistan Somali-Turkana	880 68 5 7	197 9 1 2	22.4 13.2 20.0 28.6	19.6, 25.1 5.2, 21.3 - - -	0.47
Management	Ranching Pastoralism	123 838	16 193	13.0 23.0	7.1, 18.9 20.2, 25.9	0.01
Season	Dry Wet	884 77	179 9	20.3 11.7	17.6, 22.9 4.5, 18.9	0.07
Area	Athi River Isiolo Mugwoni	103 749 86	0 190 16	2.9 25.4 18.6	0, 6.2 22.3, 28.5 10.4, 26.8	0.001

TABLE 5 Multivariable ordinary logistic-regression model of *T. evans*i prevalence in camels in selected areas of Kenya between 1996 and 1997

Variables	Levels	Ь	S.E. (b)	P	OR	95 % CI
Intercept		-1.9	0.27	0.0001		
Season	Dry Wet	0.37 0.00	0.18 0.00	0.045 0.000	1.5	1.0, 2.1
Management	Pastoralism Ranching	0.60 0.00	0.29 0.00	0.035 0.000	1.8	1.1, 3.2

Log likelihood = -497.82

Scale = 1

TABLE 6 Multivariable logistic-regression model in which clustering at herd-level has been accounted for using GEE in the analysis of *T. evansi* prevalence in camels in selected areas of Kenya between 1996 and 1997

Variables	Levels	b	S.E. (b)	P	OR	95 % CI
Intercept		-2.24	0.76	0.003		
Management	Pastoralism Ranching	1.13 0.00	0.78 0.00	0.056 0.000	3.1	1.0, 14.4

Scale = 1

were therefore dropped from the logistic regression model. The ordinary regression model indicated that dry season (OR = 1.5; 95 % CI: 1.0, 2.1) and nomadic pastoralism (OR = 1.8; 95 % CI: 1.1, 3.2) were associated with increased odds of a camel being exposed to T. evansi infection (Table 5). None of the interaction terms tested significantly explained the variation in trypanosomosis prevalence.

Accounting for clustering at herd level increased parameter standard error estimates (Table 6). The parameter estimates also increased. Exchangeable covariance structure fitted the data well. The other covariance structures that were tried but gave poor model diagnostic results include auto regressive, independent and unstructured. Nomadic pastoralism remained the only factor associated with increased odds of a camel being exposed to *T. evansi* infection. A camel managed under pastoralist production system was three times more likely to be exposed to *T. evansi* than a camel managed under a ranching system (OR = 3.1; 95 % CI: 1.0, 14.4).

#### DISCUSSION

The parasitological prevalence of 2.3% reported in this study is lower than the 20% and 5.4% obtained

by Olaho-Mukhani *et al.* (1993) and Elamin *et al.* (1998) in Kenya and Mid Eastern Sudan, respectively. Suratex® test gave a higher prevalence than HCT because of its superior sensitivity (Nantulya 1994). The demonstration of trypanosomes in blood is quite unreliable since large proportions of the infections (50–80 %) in the field do not develop detectable level of parasitaemia (Killick-Kendrick 1968). Parasitological and Suratex® tests were therefore used in parallel in this study to increase the overall sensitivity in the identification of camels exposed *to T. evansi* infection. A prevalence of 21.7% was observed.

There was a low level of agreement between parasitological and Suratex® tests in the detection of *T. evansi* infections although they were positively associated. The discordant pairs could have resulted from the following likely scenarios. Firstly, it is probable that *T. evansi* antigens persisted for varying lengths of time after possible trypanocidal therapy or there was a continued release of these antigens from tissue foci where the trypanosomes had localised (Waitumbi & Nantulya 1993). In the majority of cases, 30 days is the maximum period that antigens remain in the blood of animals following successful trypanocide therapy (Waitumbi & Nantulya 1993). Considering that antibodies can persist for up to 3 months in circulation (Luckins *et al.* 

1978, 1979 cited by Waitumbi & Nantulya 1993), antigen detection is more accurate than antibody assays in the diagnosis of current infections. It follows that accurate interpretation of serological results relies heavily on the knowledge of the health history of the individual camel and of the herds. In the present study, information from the camelherders and veterinary personnel in Isiolo indicated that the large majority of camels had not been treated with trypanocidal drugs. Also, it was evident that the treatment administered by camel herders was often ineffective due to improper use of drugs (e.g. sub curative dosage) or use of expired or fake drugs. Such practices increase the chances of trypanosomes clearing from circulation and localising in tissue foci. Moreover, the majority of Suratex® positive camels exhibited signs and symptoms of surra even when parasites could not be detected. Thus Suratex® was of significant importance in revealing latent infections of T. evansi.

Secondly, it is possible that Suratex® is unsuitable for detecting trypanosome infections in the multiplicative growth phase of the organism, as there is insufficient parasite destruction to produce detectable levels of antigens in circulation (Nantulva 1994). Similar findings have been reported in the detection of trypanosome antigens by enzymelinked immunosorbent assays (ELISA) (Nantulya 1989; Pathak, Singh, Meirvenne, Kapoor, 1997; Waitumbi & Nantulya 1993). Thirdly, antigens could also exist in immune complexes and hence remain undetected since complexing antibodies would mask epitopes (Nantulya 1989). Although Suratex® was sensitive in identifying 158 latent/aparasitaemic infections, the test was unable to detect 21 of 51 (41.2%) patent infections (Table 1). This confirmed that antigen detection is complementary and not an alternative to parasitological methods (Nantulya 1994). The explanations given for false negative results of Suratex® may assist in future studies to improve test accuracy.

There was an association between low PCV levels and the presence of trypanosomes in blood. This is in agreement with the reports of Raisinghani, Lodha, Bhatia, & Dwarakanath (1981) and Diall, Bajyana Songa, Magnus, Kouyate, Diallo, Meirvenne & Hamers (1994). We therefore suggest that a low PCV of < 23 associated with a Suratex® positive status is indicative of *T. evansi* infection.

Camels managed under nomadic pastoral system were three times more likely to be infected by *T. evansi* compared to those managed under ranch system. This supports the reports of Elamin *et al.* 

(1998) and Kalu *et al.* (2001). The association between pastoralism and *T. evansi* infection could, however, be an indirect one. One possible linkage is the inefficiency of the systems for the delivery of veterinary services to pastoral communities. It has been reported that the long distances involved in the provision of these services increases transaction costs of service delivery (McDermott, Randolph & Staal 1999). In intensive livestock production systems, such as ranching, infrastructures for the delivery of veterinary services exist. This allows for the consumption of promotive and preventive services such as the use of prophylactic drugs that have an overall effect of reducing the incidence of endemic diseases.

With the adjustment of error and parameter variance estimates in the correction for herd-level correlation, the association between season and *T. evansi* antigenaemia became insignificant. This indicates that the variation in prevalence attributed to season may actually be due to management factors such as grazing patterns. However, dry season has been reported severally as a risk factor for trypanosomosis infection (Dia *et al.* 1997; Elamin *et al.* 1998 and Kalu, Oboegbulem & Uzoukwu 2001). This is because camels congregate at scarce water points during this period which allows for an efficient transmission of the parasites between hosts to take place (Elamin *et al.* 1998).

No association between camel trypanosomosis and the host factors of age, sex and breed was found. Host factors are often of secondary interest in epidemiological studies and are only considered when they are known to exert a significant effect on the occurrence of disease (Martin & Meek 1987). Age is probably more important than other host factors because the risk of disease usually is more closely related to it. In the present study, the prevalence of the *T. evansi* infections was higher in adults than calves, although this was not significant. Wilson *et al.* (1983) also reported a very low incidence of trypanosome infections in calves as compared to older age groups.

A large proportion (84 %) of Suratex® positive camels were undetectable by parasitological methods (Table 1). This is because endemic situations are characterised by a high proportion of animals in sub-acute and chronic stages of infection in which parasitaemia is below the detection limit of parasitological detection techniques. The results of the present study have demonstrated the potential usefulness of Suratex® as an epidemiological tool, confirming findings by (Nantulya) 1994, Olaho-Mukani

et al. (1996) and Chaudhary & Iqbad (2000). Further detailed assessment of risk factors of *T.evansi* infections requires that test validity parameters be applied including diagnostic sensitivity and specificity and predictive values. Therefore extensive field evaluation of Suratex® would be beneficial in providing more insight into the usefulness of the test.

## CONCLUSION

We conclude that camels managed under nomadic pastoralism are at a greater risk of acquiring trypanosomosis caused by *T.evansi* than are camels maintained under ranch system of management. The former population could therefore be targeted in the control of camel trypanosomosis in the regions surveyed.

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