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Prevalence of camel trypanosomosis and its vectors in Fentale district, South East Shoa Zone, Ethiopia

Tadewos Kassa[1], Tadesse Eguale[2]*, and Hassen Chaka[1]

[1]*National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia*

[2]*Aklilu Lemma Institute of Pathobiology, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia*

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ABSTRACT

A cross-sectional study was conducted to determine the prevalence of camel trypanosomosis and assess the distribution and dynamics of the vectors responsible for transmission of the disease in five localities of Fentale district from September 2008 to January 2009. Parasitological examination was conducted using the microhaematocrit centrifugation technique (MHCT) and examination of Giemsa stained blood smears. The only species of trypanosome identified was *Trypanosoma evansi* with a prevalence of 4.7% by MHCT and 4.4% by blood smear. The prevalence was higher in male (6.8%) than female (4%) camels. With regard to age, calves (less than 2 years of age) were negative; the prevalence is high (7.7%) in young camels (between 3-4 years of age) and 4% in adult camels (older than 4 years of age). However, the difference in prevalence between sex and age groups was not statistically significant (P>0.05). The prevalence using blood smears was found to be different between different localities; the highest being 7.8% for Kobo and the lowest 2% for Haro kersa. The mean packed cell volume (PCV) of *Trypanosoma evansi* positive camels (22.43%) was significantly lower than that of negative camels (28.13%) (P<0.05). More than 99% of the biting flies captured from the study area were flies under the genus *Stomoxys,* while a few others such as *Tabanus, Chrysops* and *Lyperosia* were also captured. The highest fly count was recorded in September whilst the lowest was recorded in December. The current findings should not be generalized for all camel producing areas of the country or for all seasons in the same area. The prevalence of *Trypanosoma evansi* might be higher during the rainy season when the fly population (*Tabanus*) is expected to be high. Therefore, detailed studies should be carried out involving different seasons and the relative importance of different vectors in transmission of the disease in different ecologies.

Key words: camel, Ethiopia, Fentale, prevalence, *Trypanosoma evansi*

*Corresponding author:

Tadesse Eguale (DVM, MSc), Aklilu Lemma Institute of Pathobiology, College of Health Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia, Phone: +251 911 435 759; Fax- +251 112 755 296; E-mail: tadesseeguale@yahoo.com

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Introduction

Camels play a significant multi-purpose role in the dry lands of Ethiopia. The commonest uses of camels by the pastoralists are for transporting grain, water, salt and other goods as well as for milk and meat production. A study in Eastern Ethiopia indicated that camels work on average for 16 h per day, traveling 60 km (TEFERA and GEBREAB, 2004). They are very reliable milk producers even during the dry season and drought years when milk from cattle and goat is scarce (GEBRE and KAAYA, 2008). In spite of the valuable economic contribution to the pastoralist communities, as well as to national Gross Domestic Product, little effort is made to address the constraints of camel production. A few studies have been conducted however and they indicate that among other constraints, camel diseases are the major problems faced by camel producing communities throughout east Africa (TEKLE and ABEBE, 2001; DIRIE and ABDURAHMAN, 2003; GEBRE and KAAYA, 2008). Camels are comparatively hardy animals and are less susceptible to many of the devastating diseases that affect other livestock species, such as rinderpest, contagious pleuropneumonia and foot and mouth disease. However, they are affected by many other diseases (DIRIE and ABDURAHMAN, 2003).

Camel trypanosomosis, also called surra, caused by *Trypanosoma evansi*, is the main disease prevalent in most areas where camels are found (RICHARD, 1976). Although other species of trypanosomes like *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* have also been isolated from camels, their role in camel trypanosomosis is insignificant compared to *Trypanosoma evansi* (MAHMOUD and GRAY, 1980; ELAMIN et al., 1998). Camel trypanosomosis is the most important single cause of morbidity and mortality in camels. The disease is endemic in Africa, Asia and South America, and in addition to camels it is reported in other species of domesticated livestock (ENWEZOR and SACKEY, 2005)

Severe outbreaks of camel trypanosomosis causing the deaths of thousands of camels have been reported in different parts of the world (LUCKINS, 1998). Camel trypanosomosis causes anorexia, weakness and emaciation that lead to low milk and meat yield, poor traction power, increased abortion and death. The cost of treatment is also another economic loss to the camel breeders in particular and to the nation's economy in general (TEKLE and ABEBE, 2001). Recently, an outbreak of camel trypanosomosis characterized by extensive mortality and abortion was reported from Iran (DERAKHSHANFAR et al., 2010)

Trypanosoma evansi cannot undergo growth and differentiation in the insect vector because it lacks the genes necessary for mitochondrial development (SONGA et al., 1990). It is transmitted mechanically by the bites of haematophagous flies, such as T*abanus* and *Stomoxys* (ENWEZOR and SACKEY, 2005). Several species of *Tabanus* have been experimentally shown to transmit *Trypanosoma evansi* (LUCKINS, 1998) and a definite

correlation between increase in number of *Tabanus* during the rainy season and the seasonal outbreaks of *Trypanosoma evansi* infections have been reported in various tropical areas (MAHMOUD and GRAY, 1980; NJIRU et al., 2002). The efficiency of the different flies to transmit *Trypanosoma evansi* varies in different geographic conditions and is also dependent on the interval between two successive feeds and intensity of the fly challenge (LUCKINS, 1998). An essential factor in mechanical transmission of trypanosome is interrupted feeding; the flies move quickly from one host to the other to transmit the parasite within a short period before the parasite dies. Trypanosomes do not survive for more than 10-15 minutes in the proboscis of the fly (SOULSBY, 1986). The tsetse fly (Glossina species), like other blood sucking flies, can act as a mechanical vector for *Trypanosoma evansi* in the areas where they co-exist. However, since camels are usually kept in non-tsetse endemic areas, the role of the tsetse fly in transmission of surra is insignificant (BRUN et al., 1998; RICHARD, 1976; SCHWARTZ and DIOLI, 1992).

In Ethiopia, the prevalence of camel trypanosomosis and its vectors have not yet been fully documented in most parts of the country. A study conducted in southern Ethiopia indicates that trypanosomosis is one of the leading health problems (TEFERA and GEBREAB, 2004) and a prevalence of 21% has been reported in eastern Ethiopia (ZELEKE and BEKELE, 2001). Baseline information on the prevalence of the disease and the distribution of its vectors is essential to establish effective control strategy. Therefore, the objective of the current study is to determine the prevalence of camel trypanosomosis in Fentale district and to identify the possible vectors involved in the transmission of the disease.

Materials and methods

Study area. The study was conducted in Fentale district, which is located in the eastern dry lowlands of the Rift Valley, situated 200 km east of Addis Ababa. The altitude of the study area ranges from 943 to 1135 meters above sea level (Fig. 1).

The study area has a semi-arid and arid climate with mean annual rainfall of 520 mm, mean annual minimum and maximum temperature of 28 °C and 40 °C, respectively. The vegetation type in the area is mostly dominated by Acacia species, small bushes and thorny shrubs. Some of the common tree species in the area are *Acacia senegal, Grewia flavescens, Balanites aegyptiaca* and *Acalypha fruticosa*. Laboratory examination was conducted at the National Animal Health Diagnostic and Investigation Center (NAHDIC) Sebeta, Ethiopia.

Study animals. The study population consisted of camels of all age groups residing in Fentale district and managed under pastoral production systems. Specific study sites were selected purposively where camels congregate for watering and browsing purposes. A total of 383 camels of different ages and both sexes were used in this study. All study animals were randomly selected from the population at grazing and watering points. The

age of the animals was recorded based on information from the owners. Camels below 2 years of age were considered as calves, those between 2-4 years as young animals, while those above 4 years of age were considered as adults.

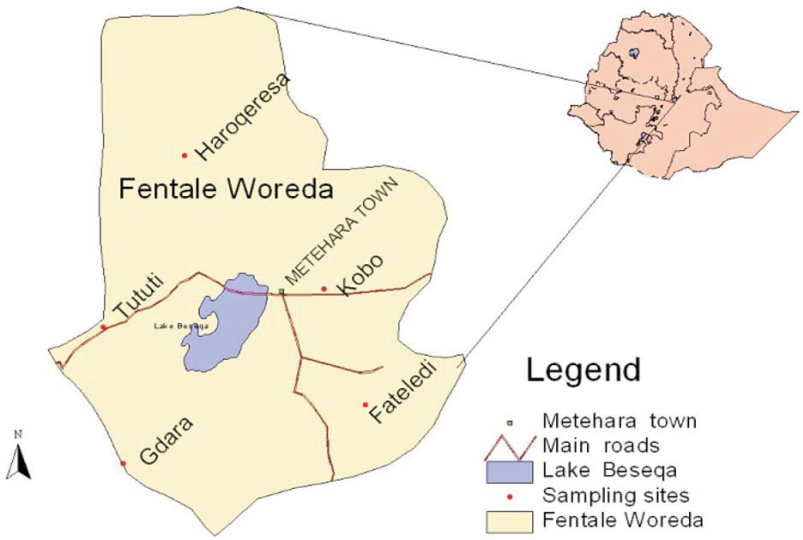

Fig. 1. Map of Fentale district showing study area in Ethiopia

Collection of blood samples and preparation. Blood samples were collected by pricking the ear vein of each animal with the help of a sterile lancet after disinfecting the site with 70% alcohol. MHCT was employed by drawing up blood directly into 75 x 1.5 mm heparinized capillary tubes in duplicate up to 3/4th of its length and one end was sealed with crystaseal. Thin blood smears were prepared from all 383 camels, while MHCT was employed for only 150 camels according to MURRAY et al. (1977). Prepared smears were fixed for 5 minutes in absolute methanol on the day of preparation and transported to NAHDIC for laboratory investigation.

Collection of entomological samples and identification. In addition to parasitological studies, an entomological survey was also conducted around Metehara town, which is located at the center of the study area, to identify potential vectors for transmission of *Trypanosoma evansi* and to monitor the monthly dynamics of the flies in the area, which was believed to be representative of other localities. The potential mechanical vectors/ biting flies were captured using three modified Vavoua traps baited with acetone and cattle urine according to CHALLIER and LAVEISSIÈRE (1973). Three traps were deployed for two consecutive days per month for five successive months continually at the same

trapping sites 500 meters apart. The collecting cages of the traps were emptied daily; biting flies and other flies caught were killed by 70% ethanol spray, and preserved in screw capped sample bottles filled with 70% ethanol. The space left above the flies was filled with cotton wool to avoid damage during transportation. In NAHDIC, identification of the flies to genus level was conducted using identification keys (MURRAY et al., 1977).

Examination of blood samples. Parasitological examination of blood samples was conducted using MHCT and stained blood smears according to MURRAY et al. (1977). The MHCT was conducted immediately after collection to estimate the level of parasitemia and anemia at the site of collection. The sealed blood filled capillary tubes were centrifuged at 12,000 rpm for 5 minutes with a microhaematocrit centrifuge. Packed cell volume (PCV) was determined by using a microhaematocrit reader. The capillary tube was then cut at 1 mm below the buffy coat with a diamond tipped pencil. Contents of the capillary tube was then poured onto a clean slide, and mixed and covered with a 22 × 22 mm cover slip. The preparation was then examined using a bright field microscope with the condenser top out and the diaphragm closed.

The fixed blood smears were immersed in upright position in Giemsa stain solution for 30 minutes. The stain was then poured off, the slide washed thoroughly in running tap water and allowed to drip-dry in an upright position before microscopic examination. The slides were examined with a microscope using oil immersion at 1000X magnification. Species identification was based on the morphological characteristics of trypanosomes depending on the size of the trypanosome, the shape of the posterior end, the size and position of kinetoplast and absence or presence of flagellum according to MURRAY et al. (1977). The proportions were compared by chi-square test.

Results

Out of 383 blood smears examined, 17 (4.4%) were positive for *Trypanosoma evansi*. No other haemoparasites were detected (Table 1). On the other hand out of 150 camels examined using MHCT, *Trypanosoma evansi* was detected in 7 (4.7%) of them.

Table 1. Prevalence of *Trypanosoma evansi* using microscopic examination of blood smears and MHCT techniques

Technique	No. examined	No. positive (%)
MHCT	150	7 (4.7)
Blood smear	383	17 (4.4)

The prevalence was found to be different among camels from different localities, the highest being 7.8% in Kobo, 4.2% in Fateledi, 2.6% in Gidara, 3.7% in Tututi, and 2% in Haroqarsa (Fig. 2)

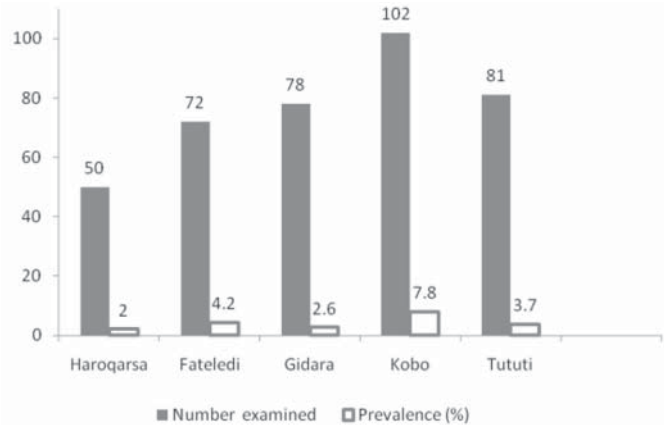

Fig. 2. Prevalence of *Trypanosoma evansi* at different localities of the study area determined by microscopic examination of blood smears. The mean PCV of *Trypanosoma evansi* positive animals (22.43%) was significantly lower than the mean PCV of negative animals (28.13%) ($P<0.05$).

A comparison of the prevalence of *Trypanosoma evansi* between different age groups and sex is shown in Table 2. Although the number of male camels examined was lower, due to the low number of breeding male animals kept by pastoralists in the study area, the prevalence was relatively higher (6.8%) as compared to the females (4%) but not statistically significant ($P>0.05$). Young age groups were found to be more affected (7.7%) than adult camels (4%) relatively, while all examined calves were found negative. However, the difference in prevalence between the age groups was not statistically significant ($P>0.05$).

Table 2. Effects of sex and age on the prevalence of *Trypanosoma evansi* using microscopic examination of blood smears

Group	No. examined	No. positive	% prevalence	P-value
Sex				
Male	59	4	6.8	0.312
Female	324	13	4	
Total	383	17	4.4	
Age				
Calf	16	0	0	0.494
Young	39	3	7.7	
Adult	328	14	4	
Total	383	17	4.4	

The monthly collection of biting flies from September 2008 to January 2009 is shown in Table 3. The most abundant genus of biting flies during the whole collection period was *Stomoxys* (99.8%). Of the sampling months, they were highly abundant during September and least abundant during December. Other genera like *Tabanus, Chrysops* and *Lyperosia* were also collected although their proportion was very minimal compared to Stomoxys.

Table 3. Relative abundance of biting flies collected from Fentale district at genus level during different months.

Month	Type of biting flies at genus level			
	Stomoxys	*Tabanus*	*Chrysops*	*Lyperosia*
September	2575	0	1	2
October	1178	0	1	2
November	1363	1	0	1
December	392	0	2	0
January	511	2	0	1
Total	6019	3	4	6

Discussion

The overall prevalence of 4.4% recorded in the current study is in agreement with the prevalences of 3.3% and 4% reported from parasitological and serological examinations respectively, in the Punjab region of Pakistan in camels (MURTAZ et al., 2006). However, the most recent report from Pakistan indicated a prevalence rate of 11.5% (BHUTTO et al., 2010). A study conducted in Somalia also showed a prevalence of 5.3% for *Trypanosoma evansi,* while only 0.06% were infected by *Trypanosoma congolense* and *Trypanosoma vivax* (DIRIE et al., 1989). However, the prevalence in the current study is lower than the findings by previous workers who reported a prevalence of 21% in eastern Ethiopia (ZELEKE and BEKELE, 2001), 28% in Kenya (NJIRU et al., 2001) and 33% in Sudan (ELAMIN et al., 1998). This type of discrepancy might be attributed to variations in the ecology of the study areas and seasons of the year when the studies were conducted which have a direct effect on the distribution of biting flies responsible for the mechanical transmission of *Trypanosoma evansi.* The presence of rainfall, moisture-retaining clay soil and surface water pools where *Acacia senegal* shrubs grow in abundance are suitable for the survival and propagation of the vector (ENWEZOR and SACKEY, 2005). The current study was conducted during the dry season. The possible reason for the difference in prevalence among camel herds in different localities in the district could also be due to differences in the microclimates of the areas.

The most important biting flies for transmission of *Trypanosoma evansi* are species of the genus *Tabanus* (ENWEZOR and SACKEY, 2005). Surveys in various tropical areas

have shown a definite correlation between seasonal outbreaks of *Trypanosoma evansi* infections and the increase in number of *Tabanus* during the rainy season (MAHMOUD and GRAY, 1980; NJIRU et al., 2002). In the current study, very few Tabanus were collected, which may probably be attributed to the low prevalence of *Trypanosoma evansi* in the study area during the study period. More than 20 different species of *Tabanus* have been shown experimentally to transmit *Trypanosoma evansi* (LUCKINS, 1998). The presence of *Tabanus* species all year round ensures that the transmission of the parasite occurs wherever there is co-existence of reservoir hosts and susceptible hosts. Sporadic occurrence of the disease during the dry season and outbreaks during the rainy season has been reported to be associated with the abundance of *Tabanus* species (NJIRU et al., 2002).

Although *Stomoxys* are also incriminated as vectors of *Trypanosoma evansi*, experimental trials regarding transmission between horses, guinea pigs and dogs, did not prove these flies to be important vectors (LOSOS, 1980). This might be the reason for the low prevalence observed in the current study despite large number of *Stomoxys* counted throughout the study period. However, the efficiency of the different flies in transmitting *Trypanosoma evansi* is reported to vary in different geographic conditions and is also dependent on the interval between two successive feeds and the intensity of the fly challenge (LUCKINS, 1998).

The possible reason why calves were less infected than other age groups could be due to the fact that pastoralists keep them in the residence area and they do not go to distant areas where the fly burden is high. A previous report from Mauritania is also in agreement with the current finding that young calves below one year of age were free of *Trypanosoma evansi* infection (JACQUIET et al., 1994). Unlike a previous report from Pakistan in which the prevalence of *Trypanosoma evansi* was higher in female than male camels (BHUTTO et al., 2010), in the current study the prevalence in male camels was higher than in females although this was not statistically significant. This could be due to the fact that male camels travel from one place to another place to provide transportation service more than female camels, so that they have a higher probability of acquiring an infection. Frequent travel could also compromise their immune response to infection due to the stress of fatigue. The previous workers attributed the higher infection in females than males to stress during pregnancy and lactation, which could decrease resistance in female camels and render them more susceptible to infection (BHUTTO et al., 2010).

In conclusion, the study revealed that camel trypanosomosis is prevalent in Fentale district at relatively low levels during the dry season of the year, using parasitological techniques. The findings in this study might not reflect the real situation because the sensitivity of parasitological techniques in the diagnosis of *Trypanosoma evansi* has been reported to be low and most of the time it is under-diagnosed (PATHAK et al., 1997; YADVENDRA et al., 1998). The prevalence was also reported to be higher in populations

of camels with a large herd size (BHUTTO et al., 2010). The real infection status could be higher than reported here. Moreover, the findings of this study should not be generalized for all camel producing areas of the country or for all seasons in the same area. The prevalence might be higher during the rainy season when the fly population (*Tabanus*) is expected to be high. Therefore, detailed studies should be carried out involving both dry and rainy seasons and the relative importance of various biting flies in transmission of the disease in different ecologies. More sensitive diagnostic methods such as PCR could give a higher prevalence.

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SAŽETAK

Provedeno je istraživanje u svrhu određivanja prevalencija tripanosomoze u deva te procjene rasprostranjenosti i dinamike prijenosnika bolesti na pet mjesta na području Fentale u razdoblju od rujna 2008. do siječnja 2009. Parazitološke pretrage provedene su na osnovi mikrohematokrita te bojenja krvnog razmaska po Giemsi. Na osnovi mikrohematokritskog nalaza dokazana je samo *Trypanosoma evansi* s prevalencijom od 4,7%. Pretragom krvnih razmazaka ustanovljena je nešto niža prevalencija (4,4%). Prevalencija je bila viša u mužjaka (6,8%) nego u ženki (4%). Invazija tripanosomama nije bila dokazana u deva mlađih od dvije godine. Prevalencija u deva u dobi od tri do četiri godine iznosila je 7,7%, a u deva starijih od četiri godine bila je 4%. Razlike u odnosu na spol i dob nisu bile statistički značajne (P>0,05). Prevalencija na temelju pretrage krvnih razmazaka bila je različita u životinja iz različitih područja. Najveća prevalencija dokazana je u životinja na području Kobo (7,8%), dok je najmanja (2%) bila na području Haro Kersa. Srednja vrijednost hematokrita u invadiranih deva bila je statistički značajno manja (22,43%) u usporedbi s neinvadiranim devama (28,13%) (P<0,05). Više od 99% ulovljenih kukaca pripadalo je muhama roda *Stomoxys*. Preostali su kukci pripadali rodovima *Tabanus*, *Chrysops* i *Lyperosia.* Najveći broj kukaca bio je ulovljen tijekom rujna, a najmanji tijekom prosinca. Razumljivo je da je prevalencija nametnika *Trypanosoma evansi* bila viša tijekom kišnih razdoblja. Autori smatraju da se dobiveni nalazi ne mogu odnositi na sva područja kao i sva godišnja doba pa zato predlažu da se nastave istraživanja, koja će obuhvatiti različita razdoblja te ulogu različitih prijenosnika bolesti.

Ključne riječi: deva, Etiopija, Fentale, prevalencija, *Trypanosoma evansi*