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Parasitological and serological study of camel trypanosomosis (surra) and associated risk factors in Gabi Rasu Zone, Afar, Ethiopia

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Camel trypanosomosis (surra), caused by *Trypanosoma evansi*, is the most important single cause of morbidity and mortality in camels. Thus, a cross-sectional study was conducted from February to June, 2012 to investigate the parasitological and serological prevalence and associated risk factors of camel trypanosomosis in two camel rearing districts of Gabi Rasu zone, Afar region, Ethiopia. A total of 408 randomly selected camels reared under extensive husbandry management system were sampled for this study. Parasitological and serological examination was carried out by using haematocrit centrifugation technique (HCT) also known as Woo's technique and card agglutination test for trypanosomes (CATT/*T. evansi*), respectively. The overall parasitological and serological prevalence of camel trypanosomosis was found to be 5.15 and 23.77%, respectively. Nine out of twenty one camels that scored positive by the haematocrit centrifugation technique (HCT) test were negative by card agglutination test for trypanosomes (CATT/*T. evansi*), and the relative sensitivity of CATT/*T. evansi* test was found to be 57.14% (12/21). The mean packed cell volume (PCV) of parasitologically negative camels (24.27 ± 0.18) was significantly higher ($p < 0.05$) than that of parasitologically positive camels (20.71 ± 0.58). Serologically negative camels had a mean PCV of (24.27%) which was not significantly different from that of positive camels (23.48%). Risk factors associated with parasitological and serological prevalence were found to be "study district" and "age". Accordingly, camels in Awash Fentale district had significantly higher ($p < 0.05$) parasitological and serological prevalence of camel trypanosomosis than in Amibara district. Generally, surra was found to be prevalent in Awash Fentale district during the study period. Therefore detailed studies should be carried out on the seasonality of the disease and its vectors in order to establish the clear epidemiology of the disease.

Key words: Camel, Gabi Rasu, haematocrit centrifugation technique (HCT), prevalence, trypanosomosis.

INTRODUCTION

According to the Unite Nations (UN) Food and Agriculture Organization, the total world camel population is approxi-

mately 23 million animals (http://faostat.fao.org). In Ethiopia, around 1.7 million camels are estimated, which are mainly distributed in arid and semi arid lowlands of Borena, Ogaden and Afar regions, which cover 50% of pastoral areas of the country (CSA, 2007).

In Ethiopia, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists. The commonest uses of camels by the pastoralists are for milk and meat production, transporting grain, water, salt and other goods as well as for the determination of wealth and social status of pastoralists. They are very reliable milk producers even during the dry season and drought periods, when milk from cattle and goat becomes scarce (Gebre and Kayaa, 2008). In addition, camels play a central role in providing draught power and determining the wealth and social status of pastoralists. A study in Eastern Ethiopia indicated that camels work on average for 16 h per day, traveling 60 km (Tefera and Gebreab, 2004).

Inspite of the valuable economic contribution to the pastoral communities, as well as to the National Gross Domestic Product (NGDP), little effort has been made so far to address the constraints of camel production. A few studies have been conducted however and these studies indicated 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, Megersa, 2010). Among the diseases, camel trypanosomosis also called surra, caused by *Trypanosoma evansi*, is the most important cause of morbidity and mortality in camels (Enwezor and Sackey, 2005). It is the most important single cause of economic losses of camel production, causing morbidity of up to 30% and mortality of around 3% in different camel rearing areas of the world (Enwezor and Sackey, 2005). 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 and 10.5% were reported from Eastern and Southern parts of the country, respectively (Zeleke and Bekele, 2001; Megersa, 2010). Despite many studies from Southern and Easten parts of the country, to the best of our knowledge, there is no comprehensive information or valid literature on the prevalence of camel trypanosomosis in afar regions and specifically in the current study area. However, effective control of camel trypanosomosis requires accurate baseline information on the prevalence and epidemiology of the disease and its vector. Therefore, the objective of this study was to investigate the prevalence of camel trypanososmosis and associated risk factors parasitologically and serologically.

MATERIALS AND METHODS

Description of the study area

The present study was conducted in two selected districts of Gabi Rasu zone, of Afar National Regional State, which is situated in the North Eastern part of the country. These two districts, namely Amibara and Awash Fentale, are located in the dry lowlands of the rift valley, at about 230 and 280 km, respectively from the capital Addis Ababa. The zone consists of six districts predominantly occupied by pastoral and agro-pastoral communities and it is characterized by arid and semi arid agro-climatic condition with ranging annual rainfall of 550 to 580 mm. Specifically, a long term average annual rainfall of 550 mm was reported for Awash Fentale by Abule et al. (2007), while 560 and 578 mm were reported for Amibara by Kidane (2005) and Kidanie (2010), respectively. The mean annual minimum and maximum temperature at Awash Fentale is 17.4 and 32.7°C (Abule et al., 2007), respectively, while the temperature is 19.5 and 34.4°C, respectively at Amibara (Kidanie, 2010). The area has two (a bimodal) rainy seasons with the main rainy season occurring from July to September and a short rainy season occurring from February to April (Abule et al., 2007; Kidane 2005). Land is generally flat and fertile with altitude ranges from 500 to 1500 metres above sea level (Abule et al., 2007; Kidane, 2005; Kidanie 2010). The predominant vegetation includes acacia species, mesquite (Prosopis juliflora), different bushes and other thorny shrubs (Kidane, 2005; Kidanie 2010). Some of the common important tree species in the area are *Acacia senegal*, *Acacia nilotica*, *Acacia melifera*, *Acacia nubica* and *Balenitus* spp.

Study design, sampling strategies and animals

A cross-sectional study was conducted from February to June, 2012, based on parasitological and serological examination, in a total of 408 randomly selected camels from Amibara and Awash Fentale districts. The two study districts were purposively selected to represent major camel rearing districts of the zone, based on their camel population and accessibility to vehicles. The sampling method for camel herds was also purposive (based on willingness of the owners) and simple random selection for the respective study animals. The total numbers of camels were proportionally sampled from both districts. Accordingly, 208 (51%) camel were sampled from Amibara district, and 200 (49%) were sampled from Awash Fentale district. The study animals included camels of different ages (young and adult) and of both sexes reared under extensive husbandry management system. The age of camels was determined based on the information obtained from the owners and were grouped as young (< 4 years old) and adult (> 4 years old).

Sample collection

After physical restraining of each selected camel, two parallel blood samples were collected through the jugular vein. Whole blood samples collected by jugular venipuncture into 5 ml ethylene diaminetetra acetate (EDTA) coated vacutainer tubes were subjected to parasitological examination using haematocrit centrifugation technique (HCT) also known as the Woo's technique (OIE territorial manual, 2010). On the other hand, blood samples collected using 10 ml plain vacutainer tubes were allowed to clot

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Table 1. Parasitological and Serological prevalence of camel trypanososmosis in Amibara and Awash Fentale districts of Gabi Rasu zone, Afar Region, Ethiopia.

District	No. of camels examined	Parasitological (HCT/Woo's)		Serological (CATT/T.evansi)	
		No. of positive	Prevalence (%)	No. of positive	Prevalence (%)
Amibara	208	6	2.88	37	17.8
Awash Fentale	200	15	7.5	60	30
Overall	408	21	5.15	97	23.77

and then serum was harvested after 24 h. These serum samples were preserved at -20°C until they were used for detection of trypanosome antibodies using CATT/*T. evansi* test.

Laboratory examination procedures

Packed cell volume (PCV) and parasitology

Blood samples were drawn into paired 75 mm × 1.5 mm heparinized micro-haematocrit capillary tubes up to three-fourth of their length. The wet end of the tubes were sealed with plasticine, and then centrifuged at 12,000 rpm for 5 min, in a haematocrit centrifuge machine. PCV levels of individual samples were determined on haematocrit reader (Hawaksly, England) and the values of packed red blood cells (RBCs) to that of the total blood volume were expressed in percentages. As mentioned, parasitological examination were conducted using the haematocrit centrifugation technique, which can detect around 50 to 200 trypanosomes/ml of blood (Desquesnes and Tresse, 1996), by placing the capillary tubes into the groove of specially designed reading chambers for HCT. The presences of motile trypanosomes were examined at the junction between the buffy coat and the plasma under the microscope.

Serology

Serum samples were tested using the card agglutination for trypanosomosis test (CATT/*T.evansi*). The CATT/*T.evansi* is a direct rapid card agglutination test, which uses formaldehyde fixed, freeze-dried trypanosomes expressing a predominant variable antigen type of *T. evansi* (RoTat 1.2) stained with Coomassie blue (Bajyana Songa and Hamers, 1988). The test was carried out as described by Verloo et al. (1998). Accordingly, 25 µl of camel serum, diluted 1:4 in CATT-buffer, was pipetted onto a reaction zone of a plastic coated test card and then added with one drop (about 45 µl) of CATT reagent. The reaction mixture was spread out by a clean stirring rod and allowed to react on a card test rotator for 5 min at 70 rpm. Blue granular deposits reveal a positive reaction visible to the naked eye (OIE territorial manual, 2010).

Data management and analysis

The data was entered into a microsoft excel spread sheet to create a database and analysis of data was made using statistical package for social sciences software version 17.0 (SPSS, v. 17.0). Prevalence was calculated for all data set as the number of infected individuals divided by the number of individuals sampled multiplied by 100. Statistical analysis was performed to determine the relationship between the two diagnostic tests using Kappa statistics, K. However, Chi- square test was used to analyze the association between surra positive camels in both tests and the assumed risk factors. Furthermore, mean PCV of parasitological positive and negative as well as serologically positive and negative camels for surra were compared using the two sample t-test. A significance level ($P < 0.05$) and confidence level (95%) was set to determine the presence or absence of statistically significant difference between the given parameters.

RESULTS

Parasitological and serological prevalence

The overall camel trypanosomosis prevalence rate in the study area was 5.15% (21/408) when haematocrit centrifugation (Woo's) technique was used, while it was 23.77% (97/408) with the card agglutination test for trypanosomosis (CATT/*T. evansi*) (Table 1). The parasitological and serological prevalence varied between the two districts and greater prevalence was recorded in Awash Fentale district than Amibara in both tests. Accordingly, parasitological prevalence rate in the Awash Fentale was 7.5% while that of Amibara was only 2.88%. Seroprevalence rate of surra was 30% for the Awash Fentale district, while it was 17.8% for Amibara (Table 1).

Comparison of parasitological and serological tests

Out of 21 camels with positive results in the parasitological test, 12 were positive using CATT/*T. evansi* test (Table 2). Nine camels that scored positive by the HCT test were negative under CATT/*T. evansi* (Table 2). Therefore, the relative sensitivity of CATT/*T. evansi* test employed in the present study was found to be 57.14% (12/21). Cohen's kappa was used to measure the concordance between the two tests and a 0.13 Kappa (K) score was found. The sore indicates a slight agreement (Everitt, 1989) between the two tests.

PCV and camel trypanosomosis

The mean PCV of parasitologically negative camels

Table 2. The relationship between parasitological and Serological tests of camel trypanosomosis study in Gabi Rasu zone, Afar Region, Ethiopia

Technique		Parasitological (HCT/Woo's)		
	Status	**Positive**	**Negative**	**Total**
Serological (CATT/*T. evansi*)	Positive	12	85	97
	Negative	9	302	311
	Total	21	387	408

Kappa (K) = 0.13

Table 3. Comparison of mean PCV of camels on the basis of parasitological and serological trypanosomosis status in Gabi Rasu zone of Afar Region, Ethiopia.

Camel trypanososmosis status	No. of observation	Mean PCV	Std. error	P-value
HCT+	21	20.71	0.585	0.000
HCT-	387	24.27	0.184	
CATT+	97	23.48	0.36	0.064
CATT-	311	24.27	0.20	

HCT+: parasitologically positive, HCT-: parasitologically negative, CATT+: serologically positive, CATT-: serologically negative

(24.27 ± 0.18) was significantly higher ($p < 0.05$) than that of parasitologically positive camels (20.71 ± 0.58). Serologically negative camels had a mean PCV of 24.27%, which was not significantly different from that of positive camels (23.48%) (Table 3).

Camel trypanosomosis and assumed risk factors

Categorical comparison of the prevalence of trypanosoma evansi between study districts, age groups and sex is shown in Table 4. There was significant difference ($p < 0.05$) in camel trypanosomosis prevalence between the two study districts. Higher trypanosome infection was recorded in Awash Fentale than Amibara district, both parasitologically and serologically. Age-wise analysis revealed that, there was significant difference in parasitological and serological prevalence between the two age groups, where higher infection rate was recorded in Adult (> 4 years) than in young (< 4 years) camels. With regard to sex, although parasitological and serological prevalence were relatively higher in female camels than males, these differences were not statistically significant.

DISCUSSION

The 5.15% overall parasitological prevalence of camel trypanososmosis recorded in this study is comparable with the investigations made by Abebe (1991), Kassa et al. (2011) and Tadesse et al. (2012), who reported 6.54, 4.4, and 3.5% prevalence of *T. evansi* in camels, respectively, from different parts of Ethiopia. A study conducted in Somalia also showed 5.3% prevalence of *T. evansi* (Dirie et al., 1989). However, the present result is lower than the findings of previous workers who reported a prevalence of 12.1% (Hagos et al., 2009) and 10.5% (Megersa, 2010) in Ethiopia, 8.3% (Swai et al., 2011) in Tanzania and 13.72% (Shah et al., 2004) in Pakistan.

Lower prevalence rate of the present finding might be due to the variations in the ecology of the study areas and seasons of the year when the study was conducted. It is clear that season has direct effect on the distribution of biting flies, which are responsible for the mechanical transmission of *T. evansi* (Luckins, 1988). The current study was conducted during the dry season when the biting fly population is low. Furthermore, local epidemics of surra occur where conditions are favorable for the spread of infection with *T. evansi*, such as when many animals are stabled together or close herded and particularly when the biting fly population is abundant, commonly during the wet season (Luckins, 1988). Although the present study was conducted during dry season in both districts, significantly higher parasitological and serological prevalence was recorded in Awash Fentale district compared to Amibara. The higher prevalence observed in Awash Fentale district may be linked to the ecological conditions of the district where there are numerous animal watering points and the existence of big and medium sized trees and shrubs (Abule et al., 2007) along with a year round river called

Table 4. The effect of Study district, Age and Sex on Parasitological and Serological prevalence of camel trypanososmosis in Gabi Rasu zone, Afar Region, Ethiopia.

Risk Factor	Group category	No. of camels examined	Technique					
			Parasitological (HCT/Woo's)			Serological(CATT/T.evansi)		
			No. of positive	Prevalence (%)	P- value	No. of positive	Prevalence (%)	P- value
Study district	Amibara	208	6	2.88	0.035	37	17.79	0.004
	Awash Fentale	200	15	7.5		60	30.00	
Sex	Male	43	1	2.33	0.376	6	13.95	0.110
	Female	365	20	5.48		91	24.93	
Age	Young	96	1	1.04	0.037	15	15.63	0.032
	Adult	312	20	6.41		82	26.28	

Awash River. As compared to the result obtained through the parasitological test (5.15%), the serological test showed higher prevalence (23.77%). This is in agreement with the findings of Hagos et al. (2009), who reported higher serological prevalence (24.9%) of camel trypanosomosis than its parasitological (12.1%) complement, in Bale zone, Ethiopia. Delafasse and Doutouin (2004) also reported a parasitological prevalence of 5.3% using Buffy coat technique (BCT) and a serological prevalence of 30% using CATT test, in Chad. The higher seroprevalence compared to the parasitological result recorded in the present study could be due to the fact that demonstration of trypanosomes in blood is quite unreliable since large proportions of infections (50 to 80%) in the field do not develop detectable level of parasitaemia (Killick Kendrick et al., 1968). This is because, infection with trypanosomes in camels is usually in chronic form during which they exhibit very low parasitaemia. Furthermore, the inability of CATT/*T. evansi* test to distinguish current from cured infection (Luckins and Mehlitz, 1978), as detectable level of antibodies can still be found in self cured animals or after treatment with trypanocidal drugs (Desquesnes et al., 1999), might also explain the higher prevalence difference under the two tests. Although CATT was sensitive in identifying 86 latent/aparasitaemic infections, the test was unable to detect 9 of 21 (42.86%) patent/parasitaemic infections (Table 2). The CATT/*T. evansi*, a direct agglutination test, is the most widely applied test and has a proven record of reliability for different host species, such as buffaloes and camels (Gutierrez et al., 2000; Holland et al., 2002).

The test is based on the native variant surface glycoprotein (VSG) of the predominant variable antigen type (VAT) RoTat 1.2 of *T. evansi* (Bajyana and Hamers, 1988). A high sensitivity (86 to 100%) of CATT test was reported from different geographical regions of the world (Bajyana and Hamers, 1988; Gutierrez et al., 2000; Verloo et al., 2000; Abdel-Rady, 2008). However, sensitivity of CATT/*T. evansi* RoTat 1.2 in the present study was found to be 57.14%. This lower sensitivity of CATT test recorded in the present study is in agreement with previous studies in Kenya who reported 65.5% (Ngaira et al., 2003) and 68.6% (Njiru et al., 2004) sensitivity. Similarly, Hagos et al. (2009) reported 72% sensitivity of CATT/*T. evansi* from Ethiopia. A lower sensitivity or a high false negative result of CATT test in the present study might result from the following likely scenarios. First, a non RoTat 1.2 *T.evansi* isolates (*T. evansi* type B) might have existed from camels of the study area; because, a number of *T. evansi* type B isolates has been reported not to express the RoTat 1.2 VAT and serological tests based on RoTat 1.2 of *T. evansi* remained negative in Kenya (Ngaira et al., 2003; Ngaira et al., 2005).

Second, other trypanosoma species (*Trypanosoma vivax*), might be the other possible isolates from camels of the present study area because, it is necessary to take into consideration the various trypanosoma species present in a given area (OIE territorial manual, 2010). Therefore, an emphasis is necessary to address the problem of diagnosis of *T. evansi* in the region.

It is also important to note that serological tests need to be validated and standardized, if they are to be suitable for correct identification of infected animals; cross evaluation in different laboratories is thus required. The explanations given for false negative results of CATT test in the present study may assist future studies to improve the test accuracy.

The significantly higher mean PCV of parasitologically negative camels than the positive ones, observed in the present study, is in agreement with the reports of Tadesse et al. (2012). This suggests that anaemia was the major clinical finding of surra. The situation in serological test was different; showing no significant difference in mean PCV of serologically negative camels and that of seropositive ones. This might be due to the limitation of CATT test to distinguish antibodies due to active infection from those of cleared or past infections, as previously suggested by Luckins and Mehlitz (1978). Therefore the PCV values of cured camels from surra (past infections) which are serologically detected as positive, are not significantly different from seronegative ones (Bengaly et al., 2001) and highly reduced PCV values occur when trypanosome parasites were detectable in blood.

Age significantly influences the parasitological and serological prevalence, where a higher infection rate was recorded in adult camels compared to the young ones. This finding is in general agreement with Dia et al. (1997), Gutierrez et al. (2000), Atarhouch et al. (2003) and Tadesse et al. (2012), who reported a tendency for infection rate to increase with age. This could be due to larger scale movement, which increases the risk of infection in adult camels (Delafosse and Doutoum, 2004; Bhutto et al., 2010), heavy stress on adult male camels being used for transportation of goods and their possible poor management (Shah et al., 2004) as well as stress associated with pregnancy and lactation in adult female camels (Bhutto et al., 2010).

Conclusion

The present study provides useful baseline data on the prevalence of camel trypanososmosis in the study area, and the results indicated that camel trypanosomosis is prevalent in Awash Fentale district. Considering the widespread existence of the disease and its significant impact on camel productivity, detailed epidemiological studies should be carried out on the seasonality of the disease and its vectors in order to establish integrated vector and parasite control strategies.

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Conflict of interest

The authors declare that they have no competing interests.

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