

## Full Length Research Paper

# A cross-sectional study of equine trypanosomosis and its vectors in Wolayta zone, Southern Ethiopia

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A study was conducted between October 2008 and April 2009 in Humbo, Kindo Koysa and Sodo Zuria districts (southern Ethiopia) to investigate the infection by trypanosomes in equines and the fly vectors present in these areas. A total of 214 donkeys, 20 horses and 60 mules were examined by a dark ground/phase contrast buffy coat technique and microscopy of blood smears. Trypanosomes were observed in 10.7% of the donkeys ( $n = 23$ ) whereas horses and mules were negative. Two *Trypanosoma* species were identified: *Trypanosoma congolense* (52.2%) and *Trypanosoma vivax* (26.1%). Furthermore, mixed infection was found in 21.7% of infected animals. No significant ( $P > 0.05$ ) association was found between prevalence and district of origin, body condition score (BCS), age or sex of the donkeys. There was a significant difference ( $P < 0.0001$ ) in mean packed cell volume (PCV) values between infected and non-infected donkeys. Female donkeys had significantly lower ( $P < 0.05$ ) mean PCV than males. There was no significant difference in the mean PCV of donkeys infected with different species of trypanosomes ( $P > 0.05$ ). Overall, the prevalence of trypanosomosis reported herein is low compared to previous studies and might be associated with the reduction in tsetse density in the study area.

**Key words:** Equine, prevalence, Southern Ethiopia, trypanosomosis, vectors.

## INTRODUCTION

In Ethiopia, there are an estimated 5.2 million donkeys, 2.5 million horses and 0.5 million mules (CSA, 2005). Equines play a key role in the agricultural economy of the country where poor infrastructure and a very rugged topography in many parts of rural Ethiopia have made transportation by vehicle inaccessible. The animals are used for pack transportation, riding, carting, and threshing farm cultivation among other things.

African animal trypanosomosis is one of the major impediments to livestock development and agricultural production in Ethiopia, contributing negatively to overall development in general and to food self-reliance efforts of the nation in particular. While tsetse-borne trypanosomosis excludes some 180,000–200,000 km<sup>2</sup> of suitable land for agriculture in the west and southwest of the country; 14 million head of cattle, an equivalent number

of small ruminants, nearly 7 million equines and 1.8 million camels are still at the risk of contracting trypanosomosis at any one time (Langridge, 1976; MoARD, 2004).

There are many well documented studies addressing the problem of bovine trypanosomosis in Ethiopia but there is very little information about equine trypanosomosis in spite of the fact that these animals contribute significantly to the economy of the country. The available data suggest that trypanosomosis is among the major health constraints of equine in tsetse infested areas of the country. A 21% prevalence of trypanosome infection has previously been detected in horses in northern Omo zone, southern Ethiopia of which 44.1% was due to *Trypanosoma vivax*, 36.9% to *T. congolense* and 19.0% to *T. brucei* (W. Yimam, Addis Ababa University, unpublished DVM thesis). Later studies in the same site reported donkey trypanosomosis with a prevalence ranging from 18.2–21% (Kanchula and Abebe, 1997; Assefa and Abebe, 2001). Similarly a 28.5% prevalence of donkey trypanosomosis has been reported by Shelima

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et al. (2006a, b) in Humbo district, southern Ethiopia. According to Shelima et al. (2006b) farmers reported trypanosomiasis as the leading health constraint of their donkeys in the area. The objective of this study was therefore, (i) to assess the prevalence of equine trypanosomiasis, (ii) identify the trypanosomes species involved, and (iii) investigate the distribution and density of fly vectors responsible for transmitting the infection in the study area.

## MATERIALS AND METHODS

### Study area

The study was conducted from October 2008 to April 2009 in three districts selected from Wolayta zone, Southern Ethiopia. Wolayta zone is located about 390 km south of Addis Ababa at an altitude ranging from 700 to 2,950 meters above sea level. It has an average annual rain fall ranging from 450 to 1,446 mm. The rain fall over much of the areas is typically bimodal with the major rainy season extending from June to September and the short rainy season occurring from February to April. The mean annual maximum and minimum temperature of the area is 34.1 and 11.4°C, respectively. The predominant farming system is a mixed crop-livestock production. The livestock population of Wolayta zone is estimated to be 886,242 bovine, 117,274 ovine, 99,817 caprine, 41,603 equines and 442,428 poultry (SNNPRSAB, 2007). The zone consists of 12 districts of which three (Kindo Koysha, Humbo and Soddoo Zuria) were selected as they are reported to be tsetse infested (Bekele et al., 2008). Overall, 9 peasant associations (PAs) were selected randomly from the three districts. PAs are the smallest administrative units in Ethiopia.

### Study animals and sampling strategy

The study animals were local breeds of donkeys, horses and mules of all ages and sex category. Equine in the area are kept under an extensive husbandry system together with other livestock. A cross-sectional study design was employed to achieve the objective of the study. The sample size for the study was determined by using the simple random sampling technique (Thrusfield, 1995). Expected prevalence was calculated by taking the average prevalence of two previous studies (Kanchula and Abebe, 1997; Shelima et al., 2006a). Accordingly, a total of 294 animals comprising 214 donkeys, 20 horses and 60 mules were selected for the study. The age of the selected animals was determined by dentition (Crane, 1997) and the body condition status of the animals was assessed based on the criteria of NEWC (2005) and scored as '1' (poor), '2' (moderate), '3' (good), '4' (fat) and '5' (obese).

### Parasitological examination

Blood samples were collected directly from the ear veins of the study animals into heparinized capillary tubes. The blood samples were examined by the capillary micro-hematocrit centrifugation method to estimate the packed cell volume (PCV) as an indicator of anemia. After determination of the PCV, the buffy coat (BC) was examined by dark ground/phase contrast microscope (Murray et al., 1983) for the detection of trypanosomes in the blood. For the purpose of species identification, a thin blood smear was prepared from the BC for those samples that were positive on BC examination and stained with Giemsa stain and examined under a microscope (Murray et al., 1983).

### Entomological survey

During the study period a total of 24 NG2U traps baited with cow urine, acetone, and octenol were deployed in the three districts included in the study. The traps were set at approximate intervals of 100 to 200 m. During trap deployment, it was attempted to include different vegetation types like bushland, wooded grassland, and cultivated land. Traps were allowed to stay at the deployment sites for a period of 24–72 h before collection. Caught tsetse flies and other biting flies were counted, identified and sexed. Fly identification was based on the morphological features described by Soulsby (1982).

### Statistical analysis

Data collected from each study animal and laboratory analysis were coded and entered in a Microsoft Excel spreadsheet. All statistical analyses were performed using STATA-9 software (Stata Corp. 4905 Lake way drive College Station, Texas 77845, USA). The point prevalence was calculated for all data as the number of infected individuals divided by the number of individuals sampled  $\times 100$ . The association between prevalence of trypanosome infection and different study variables (district, age, sex and BCS) was analyzed by univariate logistic regression analysis, whereas one-way analysis of variance (ANOVA) was used to examine the differences in mean PCV (%) between trypanosome positive and negative animals, districts, male and female animals and different BCSs. In all the analyses, the confidence level was held at 95% and  $P < 0.05$  was required for significance.

## RESULT

### Prevalence of trypanosome infection and species identified

Results from the BC and Giemsa stained blood smear examination in donkeys are given in Table 1. Of the 214 donkeys examined, 23 animals (10.7%) were found to be infected with different trypanosome species while none of the 20 horses and 60 mules examined were positive for trypanosomes. Two species of trypanosomes were identified in the study districts: *T. congolense* in Humbo and Kindo Koysha districts and *T. vivax* in all three districts. Overall, *T. congolense* (52.2%) was the predominant species encountered. *T. vivax* and mixed infection by both species was observed in 26.1% and 21.7% of the infected animals, respectively.

The district of sampling, BCS, age and sex did not have significant influence on prevalence ( $P > 0.05$ , Table 2).

### Hematological findings

The results of the analysis of the mean PCV with the different risk factors are given in Table 3. The mean ( $\pm$ SD) PCV value in donkeys was  $32.2 \pm 4.3\%$ . Infected donkeys ( $28.7 \pm 3.6$ ) had significantly ( $P < 0.0001$ ) lower PCV than non-infected donkeys ( $32.6 \pm 4.2$ ). Taking a PCV value of 30–46% as a normal value (Knottenbelt, 2005), 60.9% of the infected and 24.6% of the non-

**Table 1.** Prevalence of trypanosome infection and *Trypanosome* species identified in donkeys in the study districts.

District	No examined	No positive	Prevalence (%)	<i>Trypanosome</i> spp (%)		
				<i>T. congolense</i>	<i>T. vivax</i>	Mixed infection
Humbo	104	8	7.7	62.5	25	12.5
Kindo Koysha	85	13	15.3	53.8	15.4	30.8
Soddo Zuria	25	2	8.0	0	100	0
Overall	214	23	10.7	52.2	26.1	21.7

**Table 2.** Univariate logistic regression analysis of the prevalence of trypanosome infection with assumed risk factors in donkeys.

Risk factors	No examined	No positive	Prevalence (95% CI)	OR (95% CI)	P-value
<b>District</b>					
Humbo	104	8	7.7	1	
Kindo Kosha	85	13	15.3	2.1 (0.9– 5.5)	0.104
Soddo Zuria	25	2	8.0	1.0 (0.2– 5.2)	0.959
<b>BCS</b>					
Good	28	1	3.6	1	
Moderate	127	11	8.7	2.6 (0.3 – 20.7)	0.378
Poor	59	11	18.6	6.2 (0.8 - 50.6)	0.089
<b>Age group</b>					
≤ 1	32	2	6.3	1	
>1	182	21	11.5	1.96 (0.44 - 8.8)	0.381
<b>Sex</b>					
Female	73	8	11.0	1	
Male	141	15	10.6	1 (0.4 – 2.4)	0.943

infected animals were found to be anemic. There was a significant ( $P = 0.031$ ) association between mean PCV and sex: female donkeys had a significantly lower mean PCV than male animals. However, the mean PCV was found to be independent of BCS, district and age ( $P > 0.05$  in all cases). No significant difference ( $P > 0.05$ ) was observed in mean PCV among animals infected with *T. congolense*, *T. vivax* and mixed species (Table 4).

### Entomological survey findings

Results of the entomological survey carried out in the three districts revealed that from a total of 24 traps deployed, a total of 242 flies were caught (Table 5). Of these, 32 (13.2%) were *Glossina* spp. and the rest 210 (86.8%) were other biting flies of the genera *Stomoxys*, *Tabanus* and *Haematopota*. Furthermore, all of the *Glossina* caught were identified as *Glossina pallidipes* and were caught in Humbo and Kindo Koysha districts only. The *Glossina* species was absent from Soddo Zuria.

### DISCUSSION

The overall point prevalence (10.7%) of trypanosome infection recorded in donkeys in this study is generally low when compared with previous reports of 18.2–28.5% in the same and/or nearby zones (Kanchula and Abebe, 1997; Assefa and Abebe, 2001; Shelima et al., 2006a, b). This could be due to a reduction in tsetse density as a result of increased agricultural activities and tsetse control interventions being carried out by governmental and non-governmental organizations in the area.

The finding that *T. congolense* is the most prevalent trypanosome species in donkeys in this study is in agreement with previous reports (Assefa and Abebe, 2001; Shelima et al., 2006b). However, the present finding is not in agreement with another report (Yimam, Addis Ababa University, unpublished DVM thesis; Kanchula and Abebe, 1997) in which *T. vivax* was reported as the predominant species. *T. congolense* has also been reported as the major species in other equine trypanosomosis studies from Kenya (Nudungu et al., 1998) and Gambia (Mattioli et al., 1994; Faye et al.,

**Table 3.** Analysis of the association between mean PCV and the hypothesized risk factors using one-way ANOVA

Risk factors	No examined	Mean PCV (%)	Std. Dev.	F	P-value
<b>Trypanosome infection</b>					
Negative	191	32.6	4.1		
Positive	23	28.7	3.6	19.42	0.0000
<b>Body condition</b>					
Good	28	32.0	3.9		
Moderate	127	32.6	4.2		
Poor	59	31.5	4.5	1.45	0.238
<b>District</b>					
Humbo	104	31.6	4.3		
Kindo kosha	85	32.9	4.2		
Soddo zuria	25	32.3	4.3	2.02	0.136
<b>Age group</b>					
≤ 1	32	31.6	4.2		
>1	182	32.3	4.3	0.72	0.396
<b>Sex</b>					
Female	73	31.3	4.4		
Male	141	32.7	4.2	4.73	0.031

**Table 4.** The mean PCV (%) of donkeys infected with different species of trypanosomes as analyzed by one-way ANOVA.

Species	Mean PCV (%)	Std. Dev.	F	P-value
<i>T. congolense</i>	29.3	2.4		
<i>T. vivax</i>	28.8	5.4		
Mixed infection	27	3.9	0.67	0.523

**Table 5.** Results of the entomological survey presented by study districts.

District	No of traps used	Trapping days	<i>Glossina pallidipes</i>		Other biting flies <sup>b</sup>
			No	FTD <sup>a</sup>	No
Humbo	10	3	5	0.17	68
Kindo Kosha	6	1	27	4.5	82
Soddo Zuria	8	2	-	-	60
Total	24		32		210

<sup>a</sup> Flies/trap/day. <sup>b</sup> *Tabanus*, *Haematopota* and *Stomoxys*.

2001; Dhollander et al., 2006). *Glossina pallidipes* was the only species of tsetse detected in the study area and our findings agree with earlier observations by Bekele et al. (2008) in the same area. The predominance of *T. congolense* infection in the current study suggests increased contact of donkeys with this tsetse vector. Langridge (1976) stated earlier that the savanna tsetse flies (*G. m. submorsitans* and *G. pallidipes*) are more

efficient transmitters of *T. congolense* than *T. vivax* in East Africa. The fact that *T. congolense* was not detected in donkeys from Soddo Zuria district is most likely due to the absence of the appropriate fly vector in the district as we did not catch any tsetse flies from Soddo Zuria district. Only *T. vivax* infection was observed in the Soddo district from mechanical transmission by other biting flies.

Mixed infection with *T. congolense* and *T. vivax* was observed in 21.7% of the positive animals. This is in good agreement with the 20.6% report of mixed infection by Assefa and Abebe (2001). The absence of *T. brucei* in the current study might be explained by the high virulence of the species in equine. The development of disease due to *T. brucei* is often acute in equines, and can cause sudden death. On the other hand *T. congolense* and *T. vivax* usually cause more chronic infections with progressive anemia and weakness (McLennan, 1970; Mattioli et al., 1994).

The mean PCV% of infected donkeys was significantly lower than non-infected ones. Taking the PCV value range from 30 to 46% as normal (Knottenbelt, 2005), 60.9% of the infected and 24.6% of the non-infected animals were found to be anemic. The detection of anemia (indicated by a lowered PCV) in trypanosome infected donkeys in this study is in agreement with other studies of donkey trypanosomosis (Dhollander et al., 2006; Shelima et al., 2006a; Pinchbeck et al., 2008). However, the observation of anemia in 24.6% of the non-infected donkeys and the fact that other diseases of parasitic origin could also produce anemia makes it difficult to associate the low PCV observed in this study directly with trypanosomosis. According to observations by Shelima et al. (2006a), trypanosomosis exerted a weak and non-significant mean PCV reduction in the absence of concurrent nematode infection but a highly significant reduction when the two parasites occurred together. In the current study, the animals were not screened for gastrointestinal or hemoparasites during the study period. It is, therefore, essential that other anemia producing parasites are identified and their effects known in order to assess the net effect of trypanosomosis on PCV.

Female donkeys had a significantly lower mean PCV than male donkeys. This finding is in agreement with that of Shamaki et al. (2009).

Contrary to other African studies which reported significantly higher rates of infection in horses than donkeys (Faye et al., 2001; Dhollander et al., 2006), all the mules and horses examined in this study were found to be negative for trypanosome infection. Although the sample size is too small to be conclusive this observation might be due to less contact of mules and horses with the tsetse vectors as they are usually kept around villages unless needed for transportation. In contrast, donkeys are the major pack animals in the area and often travel long distances crossing a high tsetse challenge areas during the day time when fly activity is high and consequently, are more exposed to tsetse flies than mules and horses.

## Conclusion

This study has revealed that *T. congolense* is the most important *trypanosome* species in donkeys in the area

and that *G. pallidipes* is the only cyclical vector for the same. The detection of horse flies (*Tabanus*) and stable flies (*Stomoxys*), which are important mechanical vectors of *T. evansi*, is also of note. The prevalence observed in this study is generally low. However, the study design used should be seen only as a cross-sectional study covering a specific period of the infection status in the animals examined. The diagnostic ability of the buffy coat method is also another factor to be considered in drawing conclusions from this work since the diagnosis of trypanosomosis using direct parasitological techniques is feasible only in the acute state of the illness when the blood is colonized by a large number of parasites. In the chronic state of the illness, which is characterized by low parasitemia, the parasitological diagnosis technique is not be suitable (Rae and Luckins, 1984). Pinchbeck et al. (2008) have also reported that the sensitivity of the buffy coat method is very low (20%) relative to polymerase chain reaction (PCR)-based diagnosis. Whereas the buffy coat technique has a threshold of detection of around  $1 \times 10^2$  parasites/ml, PCR based methods can detect as few as 1–20 trypanosomes/ml (Pinchbeck et al., 2008). Thus, more sensitive techniques such as serology or PCR should be used for the effective diagnosis of infection. Therefore, a further study using these alternative techniques in combination with entomological surveys need to be conducted in different seasons and agro-ecological zones in order to generate more complete data on the epidemiology of equine trypanosomosis in the region.

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