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A Cross-Sectional Study of Equine Trypanosomosis and its Vectors in Wolayta Zone, Southern Ethiopia

Solomon Mekuria, Addisu Eyob, Alemayehu Regassa,
Abebayehu Tadesse, Berhanu Mekibib and Rahmeto Abebe
Faculty of Veterinary Medicine, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

Abstract: A cross-sectional study was conducted from October 2008-April 2009 in Humbo, Kindo koysha and Sodo zuria districts selected from Wolayta zone located in Southern Ethiopia to investigate the prevalence and species of trypanosomes infecting equines and identify the fly vectors playing a role in the transmission of trypanosomosis. Blood samples were collected from a total of 214 donkeys, 20 horses and 60 mules and examined by dark ground/phase contrast buffy coat technique and Giemsa-stained blood smears. Accordingly, *trypanosome* species were only encountered in 10.7% of the donkeys (n = 23) while none of the horses and mules examined was positive for trypanosome infection. Two species of *trypanosomes* were detected in donkeys which in order of predominance were *Trypanosoma congolense* (52.2%) and *Trypanosoma vivax* (26.1%) and mixed infection by both species was found in 21.7% of trypanosome-positive animals. Statistical analysis showed no significant association between prevalence of trypanosome infection and the district, Body Condition Score (BCS), age and sex of donkeys ($p>0.05$ in all cases). There was a highly significant difference ($p<0.0001$) in mean PCV (%) between trypanosome infected and non infected donkeys. Similarly female animals had significantly lower ($p<0.05$) mean PCV (%) than male animals. No significant difference ($p>0.05$) was observed among the mean PCVs of donkeys infected with different species of trypanosomes. The entomological survey revealed the existence of a cyclical vector *Glossina pallidipes* and other biting flies with a relative proportion of 13.2 and 86.8%, respectively. In conclusion, the prevalence of trypanosomosis obtained in the current study is generally low compared to previous studies and this might be associated with 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.

Key words: Equine, PCV, prevalence, trypanosomosis, vectors, Southern Ethiopia

INTRODUCTION

Equines play a key role in the agricultural economy of the country where poor infrastructure and very ragged topography in many parts of rural Ethiopia have made transportation by vehicle inaccessible. They are used for pack transportation, riding, carting and threshing farm cultivation among others. If equines are not available, women often have to do the same work (Mohammed, 1991). It was estimated that about 5.2 million donkeys, 2.5 million horses and 0.5 million mules exist in Ethiopia (CSA, 2005). African animal trypanosomosis is one of the major impediments to livestock development and agricultural production in Ethiopia contributing negatively to the overall development in general and to food self-reliance efforts of the nation in particular. While tsetse-borne trypanosomosis is excluding some 180,000-200,000 km^2 of agriculturally suitable land 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 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 currently very little information about equine trypanosomosis in spite of the fact that these animals contribute a lot to the economy of the country. The available scant data suggest that trypanosomosis is among the major health constraints of equine in tsetse infested areas of the country. Yimam indicated a prevalence of 21% in horses in northern Omo zone, southern Ethiopia of which 44.05% was due to *T. vivax*, 36.9% to *T. congolense* and 19.04% to *T. brucei*. Latter 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

Corresponding Author: Rahmeto Abebe, Faculty of Veterinary Medicine, Hawassa University, P.O. Box 05, Hawassa, Ethiopia

by Shelima *et al.* (2006a, b) in Humbo district, southern Ethiopia. Further more, Shelima *et al.* (2006b) have stated that trypanosomosis was claimed by farmers to be the leading health constraint of donkeys in the area. The objective of this study was therefore to assess the current prevalence of equine trypanosomosis, identify the trypanosomes species involved and investigate the distribution and density of fly vectors responsible for transmitting the disease in the study area.

MATERIALS AND METHODS

Study area: The study was conducted from October 2008-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 of 700-2950 m above sea level. It has got an average annual rain fall ranging from 450-1446 mm. The rain fall over much of the areas is typically bimodal with the major rainy season extending from June-September and the short rainy season occurs from February-April. The mean annual maximum and minimum temperature of the area is 34.12 and 11.4°C, respectively. The predominant farming system is a mixed crop-livestock production. The livestock population of Wolaita zone is estimated to be 886, 242 bovine, 117,274 ovine, 99,817 caprine, 41,603 equines and 442,428 poultry. The zone consists of 12 districts of which three (Kindo koysha, Humbo and Soddo zuria) were selected for the study based on available information that they are tsetse infested. 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 indigenous breeds of donkeys, horses and mules of all age and sex category. Equine in the area are kept under extensive husbandry system together with other livestock around villages. 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 formula given for simple random sampling technique by 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 using the oil immersion 100x objectives (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 100-200 m. During trap deployment, it was attempted to include different vegetation types like bush land, wooded grass land 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.

Statistical analysis: Data collected from each study animals and laboratory analyses were coded and entered in a microsoft excel spread sheet. 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×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.

RESULTS AND DISCUSSION

Prevalence of trypanosome infection and species identified: The results of BC and Giemsa stained blood smear examination in donkeys are shown in Table 1. Out 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 was

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 the 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.

Table 2 shows the results of univariate logistic regression analysis of the prevalence of trypanosome infection with district, BCS, age and sex of the animals. Among the study districts, the highest prevalence was recorded in Kindo kosha (15.3%) than Sodo zuria (8%) or Humbo (7.7%) but the difference was not statistically significant (p>0.05). Similarly, animals with poor BCS had the highest prevalence (18.6%) compared with those with good (3.6%) and moderate (8.7%) BCS but the variation was not significant (p>0.05). On the other hand, the prevalence was relatively higher in animals >1 year of age than those ≤1 year old however the difference was not significant (p>0.05). It was also observed that the infection was independent of sex (p>0.05).

Hematological findings: The mean (±SD) PCV value of all donkeys tested was 32.2±4.3%. The results of analysis of mean PCV with different factors are shown in Table 3. The mean PCV of infected donkeys (28.7±3.6) was significantly (p<0.0001) lower than that of non-infected donkeys (32.6±4.2). Using a PCV value of 30-46% as a normal value (Knottenbelt, 2005), 60.9% of the infected and 24.6% of the non-infected animals were found to be anemic. There was a significant (p = 0.031) association between mean PCV and sex in that 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

Table 1: Prevalence of trypanosome infection and species of trypanosomes identified in donkeys in the study districts

				Trypanosome sp. (%)		
District	No. of examined	No. of positive	Prevalence (%)	*T. congolense*	*T. vivax*	Mixed infection
Humbo	104	8	7.7	62.5	25.0	12.5
Kindo kosha	85	13	15.3	53.8	15.4	30.8
Soddo zuria	25	2	8.0	0.0	100.0	0.0
Overall	214	23	10.7	52.2	26.1	21.7

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

Risk factors	No. of examined	No. of positive	Prevalence (95% CI)	OR (95% CI)	p-value
District					
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
BCS					
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
Age group					
≤1	32	2	6.3	1	
>1	182	21	11.5	1.96 (0.44-8.8)	0. 381
Sex					
Female	73	8	11.0	1	
Male	141	15	10.6	1 (0.4-2.4)	0.943

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

Risk factors	No. examined	Mean PCV (%)	SD	F	p-value
Trypanosome infection					
Negative	191	32.6	4.1		
Positive	23	28.7	3.6	19.42	0.0000
Body condition					
Good	28	32.0	3.9		
Moderate	127	32.6	4.2		
Poor	59	31.5	4.5	1.45	0.238
District					
Humbo	104	31.6	4.3		
Kindo kosha	85	32.9	4.2		
Soddo zuria	25	32.3	4.3	2.02	0.136
Age group					
≤1	32	31.6	4.2		
>1	182	32.3	4.3	0.72	0. 396
Sex					
Female	73	31.3	4.4		
Male	141	32.7	4.2	4.73	0.031

(p>0.05 in all cases). Table 4 demonstrates the test for differences in mean PCV among animals infected with different species of trypanosomes. Accordingly, no significant difference (p>0.05) was observed in mean PCV among animals infected with *T. congolense*, *T. vivax* and mixed species.

Entomological survey findings: The results of entomological survey carried out in the three districts are shown in Table 5. It was shown that from 24 traps deployed in 3 districts, a total of 242 flies were caught. Of these, 32 (13.2%) were *Glossina* sp. and the rest 210 (86.8%) were other biting flies of the family Stomoxys, Tabanus and Haematopota. Further more, all Glossina caught were identified to be *Glossina pallidipes*. This species was caught from Humbo and Kindo koysha districts and absent from Soddo zuria whereas the other biting flies were caught from all the three districts.

The overall point prevalence (10.70%) 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). As all these studies used the buffy coat method of diagnosis, the observed differences in prevalence between the present and previous studies cannot be methodological but might be due to 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. Furthermore, the competency of the investigator to detect trypanosomes may also play a part. The finding that *T. congolense* is the most prevalent trypanosome species in donkeys in this study is in agreement with previous reports of Assefa and Abebe (2001) and Shelima *et al*. (2006b). However, it is inconsistent with reports of and Kanchula and Abebe (1997) in which *T. vivax* was reported to be the predominant species.

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

Trypanosome species	Mean PCV (%)	SD	F	p-value
Trypanosoma congolense	29.3	2.4	-	-
Trypanosoma vivax	28.8	5.4	-	-
Mixed infection	27.0	3.9	0.67	0.523

The present finding is also in accordance with equine trypanosomosis reports from Kenya (Nudungu *et al*., 1998) and Gambia (Mattioli *et al*., 1994; Faye *et al*., 2001; Dhollander *et al*., 2006). *Glossina pallidipes* was the only species of tsetse detected in the study area and this finding confirms earlier observations of 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. This finding also supports earlier observations of Langridge (1976) who stated that the savanna tsetse flies (*G. m. submorsitans* and *G. pallidipes*) are more efficient transmitters of *T. congolense* than *T. vivax* in the east Africa. *T. congolense* was not detected in donkeys from Soddo zuria district. This is most likely due to the absence of appropriate fly vector in the district. During entomological survey tsetse flies were not caught from Soddo zuria district and thus, the *T. vivax* infection observed in the district resulted 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 quite in 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 a sudden death. *T. congolense* and *T. vivax* usually cause more chronic infections with progressive anemia and weakness (McLennan, 1970; Mattioli *et al*., 1994).

The prevalence of trypanosome infection reported in Kindo koysha district was almost twice that of the other two districts although the difference was not significant. This parasitological finding matched the entomological survey result. As shown in Table 4, the apparent fly density for *G. pallidipes* was higher in Kindo koysha than Humbo district (4.5 vs. 0.17) while no Glossina was observed in Soddo zuria. Kindo koysha is located at the verge of Omo River which is one of the tsetse belts whereas, Humbo is currently intervened by a project called Southern Tsetse Eradication Project (STEP). Similar finding has also been reported from a study in Gambia where trypanosome prevalence was higher in horses and

Table 5: Results of entomological survey presented by study districts

			Glossina pallidipes		
District	No. of traps used	Trapping days	No.	FTD[a]	Other biting flies[b] (No)
Humbo	10	3	5	0.17	68
Kindo kosha	6	1	27	4.50	82
Soddo zuria	8	2	-	-	60
Total	24	-	32	-	210

[a]Flies/trap/day, [b]Tabanus, Haematopota and Stomoxys

donkeys in areas with high tsetse challenge compared with sites with relatively few flies. Animals with poor BCS had the highest prevalence than those with good or moderate BCS but the difference was not significant. Similarly, the lowest mean PCV was recorded in poor conditioned animals. Body condition is an indication of nutritional status with poorer scores corresponding to poorer nutritional intake and/or greater metabolic needs (Vatta *et al*., 2002). This study was conducted in the dry season where there is an extreme shortage of feed for livestock in Ethiopia due to a deterioration of grazing both in quantity and quality. Therefore, in the dry season, animals are not even able to meet their maintenance requirements and lose a substantial amount of weight. It is a well known fact that poor nutrition lowers the resistance and resilience of the animal thus enhancing the establishment and the severity of parasitosis.

The mean PCV% of infected donkeys was significantly lower than non-infected ones. Using the PCV value range from 30-46% as a 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 (lowered PCV) in trypanosome infected donkeys in this study is quite 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 poses difficulty to associate the low PCV observed in this study with trypanosomosis. According to observations of Shelima *et al*. (2006a), trypanosomosis exerted a weak and non-significant mean PCV depression in the absence of concurrent nematode infection but a highly significant depression 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 asses the net effect of trypanosomosis on PCV. There was no difference observed in the occurrence of trypanosome infections in male or female donkeys. Similarly, the difference in prevalence of trypanosome infection between donkeys older and younger than 1 year was not significant, although there were more older animals with trypanosome infections than young donkeys.

These findings are consistent with previous reports from Gambia (Secka, 2003; Dhollander *et al*., 2006). The mean PCV was significantly lower in female than male donkeys. The reason for this is not clearly known and thus, requires further investigation. 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. Other than the small sample size, this 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.

On the other hand, 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. The other possible explanation for this could be the fact that farmers value donkeys less than horses and mules and consequently, do not take them to veterinary clinics.

CONCLUSION

This study revealed that *T. congolense* is the most important trypanosome species in donkeys in the area and *G. pallidipes* is the only cyclical vector of it. The detection of horse flies (Tabanus) and stable flies (Stomoxys) which are important mechanical vectors of *T. evansi* may suggest the existence of this species in equine in the area. The prevalence observed in this study is generally low however, the study design used should be taken into account as a cross-sectional study depicts only a momentary picture of the infection status in the herd. The diagnostic capability of the buffy coat method is also another factor to be considered because the diagnosis of trypanosomosis by direct parasitological techniques is feasible 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, a good parasitological diagnosis is rather difficult (Rae and Luckins, 1984). Pinchbeck *et al*. (2008) reported that the sensitivity of buffy coat method was very low (20%) relative to Polymerase Chain Reaction (PCR)-based diagnosis. Whereas, the buffy coat technique has a threshold of detection of around 1×10^{2} parasites mL^{-1}, PCR based methods can detect as few as 1-20 trypanosomes mL^{-1} (Pinchbeck *et al*., 2008). Thus, more sensitive techniques such as serology or PCR should be used for the effective diagnosis of the disease. Therefore, a further study that makes use of such techniques and includes entomological survey needs to be conducted in different seasons and agro-ecological zones so as to generate a complete data set on the epidemiology of equine trypanosomosis in the area.

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