

Prevalence of haemoparasites and associated risk factors in working donkeys in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia

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Citation

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Abstract

A cross-sectional study was conducted from November 2008 to March 2009 in Adigudem and Kwiha districts of Tigray regional state with the objective of identifying the prevalence of haemoparasites and the associated risk factors in working donkeys. Blood samples were collected from a total of 400 randomly selected donkeys and examined by dark ground/phase contrast buffy coat technique and Giemsa stained blood smears. The overall prevalence of haemoparasites was 2.5% (n =10) without significant variation between the two districts ($p>0.05$). Babesia and trypanosomes were the haemoparasites observed in the donkeys with a prevalence of 1.75% (n=7) and 0.75 % (n=3), respectively. Species identification has indicated the presence of two species of Babesia: Babesia equi (71.43%) and Babesia caballi (28.6%) whereas all the trypanosomes encountered belong to the single species of Trypanosoma vivax. No significant association was observed between the prevalence of either of the two haemoparasites and the hypothesized risk factors (district, sex, age and body condition score) ($p>0.05$ for all factors). The mean packed cell volume (PCV) of trypanosome infected donkeys (20.67 ± 4.04) was significantly ($p<0.05$) lower than that of non-infected donkeys (28.68 ± 5.73); however, no significant difference was observed between babesia positive and babesia free animals ($p>0.05$). Moreover, this study has shown that 22% donkeys tested serologically were seropositive for Trypanosoma evansi and Trypanosoma equiperdum. Overall, the prevalence of haemoparasites reported in the current study is low compared with previous studies and might be associated with the study design, the season of study, low sensitivity of the parasitological technique or a better veterinary service provided in the area by a charity organization.

INTRODUCTION

The donkey is widely distributed through out Ethiopia with an estimated population of 5.2 million (Saul et al., 1997). It is most commonly found in the dry and mountainous areas (Alemu et al., 2004). Majority of the donkeys are found in the central high lands of the country including Arsi, Showa and also northern parts of Ethiopian, with highest density being in Arsi followed by Tigray and Showa. According to the Agricultural sample survey conducted during 2005/2006, the number of donkeys and mules in Tigray Region are estimated to be 387,390 and 7,900, respectively (CSA, 2006).

Despite the increase in mechanization throughout the world, donkeys are still well deserving of the name 'beasts of burden' with their inherent ability to thrive in harsh environments (in arid and semi arid areas and where roads

are poor or none existent). They are playing an important role in transportation (riding, pack transport or pulling cart), farming (tillage, threshing) and in certain countries they aid in raising water and milling (Pearson et al., 1994; Mwenya and Tandkeib, 2004).

Recurrent drought in Ethiopia resulting in increased cattle mortality has also contributed to an increase in donkey's usage as draft and pack animal both in rural and urban areas. In general, donkey has a prominent position in the agricultural system of Ethiopia especially to the resource poor communities in rural and urban areas. The low level of development of the road transport network and rough terrain of the country make the donkey the most valuable, appropriate and affordable pack animal under small holder farming system of Ethiopia (Gebreworld et al., 2004). The use of cart donkeys in door to door transport of goods also

provides urban dwellers with the opportunity of income generation (Demelash and Moges, 2006).

Despite the number, its prominent role in rural and agricultural life system of the country, the knowledge pertaining to the physiology, nutritional requirement, health problems and management system of the donkey is still limited and rarely available in the literature except the endeavor of the Donkey sanctuary since its establishment.

Even though donkeys have often been described as sturdy animals, they succumb to a variety of infectious and non infectious diseases and a number of other problems (Feseha, 1997). Donkeys harbor several protozoan and metazoan parasites. Among haemoparasitic diseases in donkeys, trypanosomiasis and babesiosis are attributed in reduction in their draughts power efficiency and even their survival (Svendsen, 1997).

In Ethiopia, there are only few published reports about donkey hemoparasites and all the available data are on trypanosomiasis and restricted to only some tsetse infested areas of the country (Kanchula and Abebe, 1997; Assefa and Abebe, 2001; Shelima et al., 2006; Abebe and Wolde, 2010). In this regard well documented information about donkey haemoparasites in most geographical areas of the country is scanty and not strong enough to plan a control strategy. Therefore, the current study was contemplated with the objective of estimating the prevalence of haemoparasites and identifying the associated risk factors in the selected two districts of Tigray regional state, Northern Ethiopia.

MATERIALS AND METHODS

STUDY AREA

The study was conducted from November 2008 to March 2009 in Adigudem and Kwiha districts of Tigray regional state, Northern Ethiopia. Adigudem is located at 13° 14' 50"N and 39° to 53° E with an elevation of 2100 m.a.s.l. (Atlas of the Ethiopian Rural economy, 2006). Kwiha, the second site, is located at 13° 20' 50"N and 39° 32' 38" E with an altitude of 2247 m.a.s.l. Both districts have a cool tropical semiarid climate with mean annual temperature of around 18 °C. The areas are affected by high wind velocity. The mean annual rainfall is about 650mm and varies considerably between years and is characterized by unpredictable drought (Corbeels et al., 2000).

STUDY POPULATION

In the study area, donkeys comprise indigenous breeds and

managed in a traditional extensive way. They are mainly used for draught and pack work types. Most of the owners keep their donkeys in open housing system that does not protect them from extreme weather conditions. The donkeys were housed in stone paved floors without bedding and their manure and wasted feed were not regularly cleaned. The available feed resource for donkeys in these areas constituted natural pasture, concentrates and crop residues.

STUDY DESIGN AND SAMPLING METHOD

A cross sectional study was used to achieve the objective of the study. The study animals were selected randomly from those donkeys brought to the Tigray Donkeys Health and Welfare Project (DHWP) mobile clinics. Donkeys of all age groups and both sexes were included in the study. The sample size for the study was determined by using the simple random sampling technique (Thrusfield, 2005). The expected prevalence was taken as 28.5% based on a recent study (Shelima et al., 2006). Thus, a total of 400 donkeys (206 from Adigudem and 194 from Kwiha) which can represent the target population were selected and included in the study. These animals were drawn from a population of donkeys brought to the aforementioned DHWP mobile clinics by applying a lottery method as follows: First all the donkeys in the population were numbered from 1 to N. These numbers were written on the small slips of paper. The slips were placed in a bowl and thoroughly mixed. Then, a blind-folded researcher was allowed to select the required number of samples. Population members having the selected numbers were included in the sample

The age of the selected donkeys was determined using the incisor eruption times and wear (Crane, 1997). Donkeys were grouped into three age categories: donkeys under two years were classed as young (n=57), those in range of two to ten years were classed as adult (n=303) and those beyond ten years were classed as old (n=40).

Body condition scoring (BCS) of the donkeys was performed based on the criteria of NEWC (2005). Hence, grades of A, B and C were given accordingly for good, moderate and poor BCS, respectively.

PARASITOLOGICAL AND HEMATOLOGICAL EXAMINATION

Blood samples were collected directly from the ear veins using heparinized microhematocrit capillary tubes and then centrifuged for 5 minutes. The PCV was determined by haematocrit reader and the color of the plasma was

simultaneously checked and recorded. The capillary tubes were then cut using a diamond pencil 1 mm below the buffy coat and the contents of the capillary tube were expressed on clean glass slide, mixed and covered with cover slip. Thin smears were prepared directly from the ear vein and also from the buffy coat and fixed with methanol and stained with Giemsa. Both the wet and stained smears were systematically examined for the presence of haemoparasites (Coles, 1986; Urquhart et al., 1996). Babesia species were identified based on morphological characteristic described by Soulsby (1982). Identification of trypanosome species was carried according to Uilenberg (1998). The degree of anemia was estimated by using PCV reading set by Knottenbelt (2005), who reported a normal range of 30-40%.

SEROLOGICAL INVESTIGATION

Blood samples needed for serological investigation of *Trypanosoma equiperdum* and/or *Trypanosoma evansi* were collected aseptically from jugular vein into 10 ml plain vacutainer tube. The tubes were then placed on a level ground at 45° to facilitate separation of serum. The serum was then decanted to another sterile test tube, labeled and then packed properly into an ice box and shipped to the Faculty of Veterinary Medicine located at Debre Zeit. However, due to lack of sufficient serological kits, only 50 randomly selected serum samples were examined.

STATISTICAL ANALYSIS

Data collected from the study animals and laboratory analyses 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, TX 77845, USA). The association between prevalence of haemoparasites and the study variables (district, age, sex and BCS) was analyzed by Chi-square (χ^2) test of independence, whereas student's t-test was used to examine the differences in mean PCV between trypanosome/babesia positive and negative animals. In all the analyses, the confidence level was held at 95% and $p < 0.05$ was required for significance.

RESULTS

PARASITOLOGICAL FINDINGS

From the total of 400 donkeys examined in the two districts, 10 animals were found to be infected with Babesia or Trypanosoma. Therefore, the overall prevalence of haemoparasites was 2.5%. On a genus level, the prevalence was 1.75% for Babesia and 0.75% for Trypanosoma (Table

1). The species of Babesia identified were Babesia equi (71.4%) and Babesia caballi (28.6%) while all the trypanosomes encountered were belong to the single species of Trypanosome vivax (Table 2).

Figure 1

Table 1: Prevalence of haemoparasites in working donkeys in the two study districts

District	No. donkeys sampled	Babesia spp		Trypanosoma vivax		Overall infection	
		No. positive	Prevalence (%)	No. positive	Prevalence (%)	No. positive	Prevalence (%)
Adigudem	206	4	1.94	2	0.97	6	2.91
Kwiha	194	3	1.55	1	0.52	4	2.06
Total	400	7	1.75	3	0.75	10	2.5

Figure 2

Table 2: and species identified in working donkeys of the study area

Genus	Species	No. Positive	Relative prevalence (%)
Babesia	Babesia equi	5	71.4
	Babesia caballi	2	28.6
Trypanosoma	Trypanosoma vivax	3	100

The results of statistical analysis of different risk factors with Babesia spp and Trypanosoma vivax infections are given in Table 3 and Table 4, respectively. No significant variations were observed in the prevalence of both Babesia spp and Trypanosoma vivax infections in donkeys between the two study districts ($p > 0.05$). Observation of the BCS of the donkeys showed that from the 400 donkeys examined, 94 (23.5%), 203 (50.75%), and 103 (25.75%) animals were in good, moderate and poor conditions, respectively. Babesia spp were detected in donkeys with moderate and poor BCS whereas Trypanosoma vivax was found only in donkeys with poor BCS. Nevertheless, the association between Babesia spp or Trypanosoma vivax infections and BCS was not found to be significant ($p > 0.05$ in both cases). Age of the animals was not significantly associated with the prevalence of the haemoparasites ($p > 0.05$). All the animals infected with Babesia spp or Trypanosoma vivax were above two years of age. As with other factors, the prevalence of both Babesia spp and Trypanosoma vivax infection was not significantly associated with the sex of the animals ($p > 0.05$).

HEMATOLOGICAL FINDINGS

The mean PCV value of all donkeys tested was $28.6 \pm 5.76\%$. There was no significant ($p > 0.05$) variation in mean PCV between babesia infected and free animals. In contrast, animals infected with Trypanosome vivax had a significantly ($p < 0.05$) lower mean PCV than those non-infected. Using a

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PCV value of 30-46% as a normal value (Knottenbelt, 2005), about 90% of parasitaemic and 43.85% of non parasitaemic donkeys were found to be anemic (Table 5).

Figure 3

Table3: Chi-square analysis of different risk factors with spp infection in donkeys

Risk factors	No examined	No positive	% positive	χ^2	p-value
District :					
- Adigudem	206	4	1.94		
- Kwiha	194	3	1.55	0.09	0.76
Body condition score:					
- Good	94	-	-		
- Moderate	203	4	1.97		
- Poor	103	3	2.91	2.54	0.28
Age:					
- <2 years	57	-	-		
- 2 to 10 years	303	5	1.65		
- >10 years	40	2	5	3.49	0.18
Sex :					
- Male	245	5	2.04		
- Female	155	2	1.29	0.31	0.58

Figure 4

Table 4. Chi-square analysis of different risk factors with infection in donkeys

Risk factors	No examined	No positive	% positive	χ^2	p-value
District :					
- Adigudem	206	2	0.97		
- Kwiha	194	1	0.52	0.28	
Body condition score:					
- Good	94	0	-		
- Moderate	203	0	-		
- Poor	103	3	2.91	-	-
Age:					
- <2 years	57	0			
- 2 to 10 years	303	2	0.66		
- >10 years	40	1	2.5	3.49	0.18
Sex :					
- Male	245	1	0.41		
- Female	155	2	1.29	0.99	0.58

Figure 5

Table 5: Analysis of the association between haemoparasites and mean PCV in donkeys using t-test

Type of haemoparasite	No of animals	Mean PCV (%)	Std. Dev	t	p-value
<i>Babesia</i> spp					
- Infected	7	24.54	4.04		
- Non-infected	393	28.69	5.77	1.88	0.06
<i>Trypanosoma vivax</i>					
- Infected	3	20.67	4.04		
- Non-infected	397	28.68	5.73	2.41	0.02

SEROLOGICAL ANALYSIS

Of the total 50 sera collected from randomly selected donkeys and sent for serological detection of antibodies against *Trypanosoma equiperdum* and *Trypanosoma evansi* by using CATT/T.evansi, a direct card agglutination test, 11 (22%) samples were found to be seropositive.

DISCUSSION

The prevalence of both *Babesia* spp (1.75%) and *Trypanosoma vivax* (0.75%) infection observed in this study

is generally very low when compared with previous reports from different parts of the country. The prevalence of babesiosis in donkeys was reported to be 10.3% in a previous study conducted in three different areas of Central Showa (Kebere, 1998, unpublished). On the other hand, previous studies of donkey trypanosomosis have reported a prevalence of 12 - 21% in north Omo Zone (Yimam, 1993, unpublished; Kanchula and Abebe, 1997; Assefa and Getachew, 2001), 28.5% in Humbo, Wolayita zone (Shelima et al., 2006) and 6.3% in Northwest Ethiopia (Abebe and Wolde, 2010). The observed difference in prevalence between the present and previous studies might be associated with the better veterinary services provided in the area by the Tigray DHWP with babesiocidal and trypanocidal drugs. Secondly, it could also be due the study design employed as a cross-sectional study depicts only a specific period of the infection status in the animals examined. The diagnostic capability of the parasitological technique used might be another possible reason. The traditional light-microscopic examination of thin blood smears can be difficult in the case of carrier animals where presence of parasites is scant, and even in acute cases at the onset of the disease (Nagore et al., 2004). The low prevalence of haemoparasites in this study might also be associated with the season of study. The current study was carried out from November 2009 to March 2010, a period known to be dry in Ethiopia. A previous trypanosomosis study in the country has shown seasonal variations in the prevalence of *Trypanosoma vivax* infection with values higher between June and November (rainy season) than in the other months correlated with biting fly density (Rowlands et al., 2001). Similarly, Sinshaw et al. (2006) have recorded a high population density of biting flies in the rainy season than in the dry and stated that biting flies require a wet habitat for multiplication and larval growth.

Trypanosoma vivax was the only trypanosome species identified in the current study. This species was differentiated based on the morphological features described by Uilenberg (1998) as follows. The parasite had a free flagellum. Its length, including the free flagellum, varied from 18 to 26 μ m. The kinetoplast was large and terminal. The nucleus was centrally placed, but the bulk of the cytoplasm was found in the posterior part. The posterior extremity was swollen and blunt and the undulating membrane was inconspicuous. Since the study area is known to be tsetse-free, the occurrence of this species could be due mechanical transmission by biting flies. It has been

established that in the absence of appropriate tsetse flies, *Trypanosoma vivax* can be transmitted mechanically between infected and susceptible donkeys (Radostits et al., 2007). The present finding is consistent with that of a previous study (Addisu, 2009, unpublished) in which *Trypanosoma vivax* was detected to be the only species in a district where tsetse flies were not caught. This parasite has also been reported as the predominant species even in the tsetse infested areas of the country (Yimam, 1993, unpublished; Kanchula and Abebe, 1997).

With respect to *Babesia*, a greater proportion of donkeys were infected with *Babesia equi* (71.4%) than *Babesia caballi* (28.6%). This finding is in agreement with a previous study (Nuria, 1992, unpublished) reporting *Babesia equi* (86.2%) as a widely distributed species than *Babesia caballi* (13.8%) in Bahir dar and its surroundings. *Babesia equi* infection in donkeys has also been reported from other tropical and subtropical countries like India, Brazil and Arabian countries (Kumar et al., 2002). The present finding is also inline with Soulsby (1982) stating that *Babesia equi* has a possibly much wider distribution than *Babesia caballi*. Contrary to the current finding, the predominance of *Babesia caballi* over *Babesia equi* has been reported in Turkey (Acici et al., 2008). *Babesia equi* is known to be more virulent and tends to cause a fulminating parasitaemia (Gerstenberg et al., 1998).

Ixodid ticks of the genera *Hyalomma*, *Dermacentor* and *Rhipicephalus* have been identified as vectors for the transmission of either *Babesia equi* or *Babesia caballi* protozoa to natural host (Soulsby, 1982). However, *Boophilus* species were the most common ticks frequently encountered on the body of donkeys in the current study. This is consistent with a previous study (Feseha, 1993, unpublished) in which *Rhipicephalus* and *Boophilus* species were reported to be the major vectors of equine babesiosis in the specific zone.

In agreement with previous studies (Addisu, 2009, unpublished; Abebe and Wolde, 2010), age and sex of the animals did not have significant influence on the prevalence of haemoparasites. Similarly, no significant variation was found in the prevalence of both *Babesia* spp and *Trypanosoma vivax* infection between the two districts covered by the study. This could be due to similar agro-ecological conditions and equal veterinary attention given by the Tigray DHWP for the two sites.

The mean PCV of *Trypanosoma vivax* infected donkeys was significantly lower than non-infected ones. The detection of anemia (indicated by a lowered PCV) in infected donkeys in this study is in agreement with other studies of donkey trypanosomosis (Dhollander et al., 2006; Shelima et al., 2006; Pinchbeck et al., 2008; Abebe and Wolde, 2010). However, the difference in mean PCV between *Babesia* infected and free donkeys was not statistically significant and this is in agreement with the report of Kebera (1998, unpublished). Using the PCV value range 30-46% as a normal (Knottenbelt, 2005), 43.85% of *Trypanosoma vivax* free donkeys were found to be anemic. The degree of anemia observed in non-infected animals could possibly be attributed to the compound effects of poor nutrition and concurrent helminthes infection.

Although the number of samples used was small, the serological study revealed that 22% sampled donkeys were seropositive for *Trypanosoma equiperdum* and *Trypanosoma evansi*. Lack of sufficient serological kit was the limiting factor to test large number of donkeys. This serological test (CATT/*Trypanosoma evansi*) uses a standard antigen and proved to be a good test for equine trypanosomosis, whether the causative agent is *Trypanosoma evansi* (surra) or *Trypanosoma equiperdum* (dourine) (Claes et al., 2003). Microscopic examination has a low sensitivity particularly for the detection of *Trypanosoma equiperdum*, which is considered to be a tissue parasite rather than a blood parasite (Burn et al., 1998).

In conclusion, this study has revealed the existence of both *Babesia* spp (*Babesia equi* and *Babesia caballi*) and *Trypanosoma vivax* in donkeys in the study area. The detection of *Trypanosoma evansi* and *Trypanosoma equiperdum* antibodies in the blood of serologically tested donkeys is also of note. The prevalence of haemoparasites observed in the current study is generally low. However, the study design used, season of the study and the low sensitivity of the parasitological test employed should be considered in drawing conclusions from this work. Therefore, a further study using more sensitive tests in combination with vector survey need to be conducted in different seasons and agro ecological zones in order to generate more complete data on the epidemiology of donkey haemoparasites in the Tigray regional state.

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