

Prevalence of Haemoparasites and Associated Risk Factors in Working Donkeys in Adigudem and Kwiha Districts of Tigray Region, Northern Ethiopia

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Abstract: A cross-sectional study was conducted from November 2008-March 2009 in Adigudem and Kwiha districts in Tigray regional state, with the objectives of identifying the prevalence of haemoparasites and the associated risk factors in working donkeys. Blood samples were collected from a total 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 found to be 2.5% (n = 10) without significant variation between the two districts ($p > 0.05$). Two genera of haemoparasites namely *Babesia* and *Trypanosomes* were observed with the prevalence of 1.75% (n = 7) and 0.75% (n = 3), respectively. Two species of *Babesia* were identified: *Babesia equi* (71.43%) and *Babesia caballi* (28.6%) while *Trypanosoma vivax* was the only trypanosome encountered during the study period. No significant association was observed between the prevalence of either of the two haemoparasites and the hypothesized risk factors (study area, 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 ($p > 0.05$). In conclusion, the prevalence of haemoparasites observed in the current study is generally low compared to previous studies. As the present study design was a cross-sectional one that only depicts a momentary picture of the infection status in the herd, a further longitudinal study that makes use of molecular techniques is recommended.

Key words: Adigudem, donkeys, haemoparasite, Kwiha, prevalence, risk factors, Ethiopia

INTRODUCTION

The donkey is widely distributed throughout 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 Ethiopia with highest density being in Arsi followed by Tigray and Showa. According to the Agricultural sample survey conducted during 2005/2006, the number of donkey 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 of 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) in farming (for tillage, assist in threshing) and in certain countries they aid in raising water and milling (Pearson *et al.*, 1999; 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 animals both in rural and urban areas. In general, donkey has 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 animals 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 donkey has often described sturdy animals, they succumb to a variety of infectious

and non infectious diseases and a number of other problems (Feseha, 1997). Donkeys harbor several protozoa 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 of donkey hemoparasites (Kanchula and Abebe, 1997; Assefa and Abebe, 2001; Shelima *et al.*, 2006; Abebe and Wolde, 2010) and they are all on trypanosomiasis and in tsetse infested areas namely in North omo, Wolayita and Northwest Ethiopia. In this regard well documented information about haemoparasite of donkeys in most of the geographic areas is scanty and is not strong enough to execute effective control measures. Therefore, the current study was contemplated with the objective of estimating the prevalence of haemoparasites in donkeys and identifying the associated risk factors.

MATERIALS AND METHODS

Study area: The study was conducted from November 2008-March 2009 in Adigudem and Kwiha districts of Tigray regional state. Adigudem is located at 13°14'50"N and 39°-53'E with an elevation of 2100 m.a.s.l. (IFPRI, 2006). It is found 32 km a long side the main high way from Mekelle to Addis Ababa. 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. and found 8 km away from Mekelle, the capital city of Tigray Regional state. Both Adigudem and Kwiha 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 650 mm and varies considerably between years and is characterized by unpredictable drought (Corbeels *et al.*, 2000).

Study population: Donkeys provide power source for draught and packing in the study areas. 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 the animals' manure and wasted feed are not regularly cleaned. The available donkey's feed resource in these areas consists of natural pasture, concentrates and crop residues. In the study area, donkeys comprise indigenous breeds and managed in a traditional extensive way (CSA, 2006).

Study design and sampling method: A cross sectional study was used to address the objectives of the study.

The study animals were selected randomly from those donkeys presented to Tigray Donkeys Health and Welfare Project (DHWP) mobile clinics and also from market places. Donkeys of all age groups and both sexes were included in the study.

The sample size was determined by using the formula given for simple random sampling methods by Thrusfield (1995). Expected prevalence was used based on the recent work done by Shelima *et al.* (2006) in Humbo district (28.5%). Thus a total of 400 donkeys (206 from Adigudem and 194 from Kwiha) which can represent the target population were randomly selected and included in the study.

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 2 years were classed as young (n = 57), those in range of 2-10 years were classed as adult (n = 303) and those beyond 10 years were classed as old (n = 40).

Prior to blood sample collection, Body Condition (BC) of all sampled donkeys was assessed and recorded by using the BC scoring method suggested by NEWC (2005). Hence, grades of A, B and C were given accordingly for good, moderate and poor body condition, respectively.

Parasitological and hematological examination: Blood samples were collected directly from ear veins using heparinized microhaematocrit (capillary) tube and then centrifuged for 5 min at 15,000 rpm. The packed cell volume 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* and *Trypanosoma* species were identified by morphological characteristic using thin smear (Murray *et al.*, 1977). Degree of anemia was estimated by using PCV reading set by Knottenbelt (2005) who reported a normal range of 30-40%.

Serological investigation: Blood sample needed for serological investigation of *Trypanosoma equiperdum* and/or *Trypanosoma evansi* was 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 then shipped to the Faculty of Veterinary Medicine, Debre zeit, Ethiopian. However, due to lack of sufficient serological kit, only 50 randomly selected serum samples were examined.

Statistical analysis: Data collected from each study animal 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 association between prevalence of haemoparasites and the study variables (study area, age, sex and BCS) was tested by 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 AND DISCUSSION

Parasitological findings: The overall prevalence of haemoparasites in both study sites was found to be 2.5%. Two genera of haemoparasites namely *Babesia* and *Trypanosoma* were identified with a prevalence of 1.75 and 0.75%, respectively (Table 1). Two *Babesia* species i.e. *B. equi* and *B. caballi* were identified with the relative prevalence of 71.4 and 28.6%, respectively while only one species of *trypanosome* i.e., *Trypanosome vivax* was identified (Table 2).

The results of statistical analysis of different risk factors with *Babesia* sp. and *trypanosome* sp. infections are shown in Table 3 and 4, respectively. Both *Babesia* and *Trypanosome* sp. infection in donkeys did not show significant variation between the two study districts ($p > 0.05$).

Observation of the Body Condition Score (BCS) of the donkeys showed that from the 400 donkeys examined, 94 (23.5), 203 (50.75) and 103 (25.75%) were in good, moderate and poor conditions, respectively. All donkeys infected with haemoparasites were in poor and/or moderate body conditions. *Babesia* sp. were detected in donkeys with moderate and poor BCS

whereas, *Trypanosome* sp. were found only in donkeys with poor BCS. No haemoparasite was detected from donkeys with good body condition. However, no significant association was observed between both *Babesia* and *Trypanosome* sp. infections and BCS ($p > 0.05$ in both cases). With respect to the age of the animals, all animals infected with both *Babesia* and *Trypanosome* sp. were above 2 years age and no infection was detected in those below 2 years of age. Among animals infected, no significant difference ($p > 0.05$) was observed in the prevalence of infection between animals <10 years and >10 years of age for *Babesia* and *Trypanosome* sp. although it was higher in older animals. As with other factors prevalence of both *Babesia* and *Trypanosome* sp. 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±5.76%). There was no significant ($p > 0.05$) variation in mean PCV between *Babesia* infected and *Babesia* free animals while animals infected with *Trypanosome* sp. had a significantly ($p < 0.05$) lower mean PCV than those non-infected. Using a 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).

Serological analysis: Of the total of 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.

In Ethiopia considerable study has been carried out on bovine haemoparasites but there is very little study carried out on haemoparasites of equine particularly in donkeys. The reports made so far on the problem are fragmented and hence a very little or no information is available on the subject matter in the donkeys.

The prevalence of donkey babesiosis (1.75%) and trypanosomosis (0.75%) observed in this study is generally very low when compared to previous reports from different parts of the country. Kebera has reported babesiosis in 10.3% of the 358 donkeys observed in three different areas of Central

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

District	No. of donkeys sampled	<i>Babesia</i> sp.		<i>Trypanosoma</i> sp.		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	2.50

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

Type of haemoparasite	Species	No. positive	Relative prevalence (%)
Babesia	<i>Babesia equi</i>	5	71.4
	<i>Babesia caballi</i>	2	28.6
Trypanosoma	<i>Trypanosoma vivax</i>	3	100.0

Table 3: Results of Chi-square analysis of different risk factors with *Babesia* sp. Infection in donkeys

Risk factors	No. examined	No. positive	Positive (%)	χ^2	p-value
District					
Adiqudem	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	-	-	-	-
2-10 years	303	5	1.65	-	-
>10 years	40	2	5.00	3.49	0.18
Sex					
Male	245	5	2.04	-	-
Female	155	2	1.29	0.31	0.58

Showa. The prevalence of donkey trypanosomosis reported from other parts of the country is 12-21% in north Omo Zone (Kanchula and Abebe, 1997; Assefa and Abebe, 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 donkey health and welfare project with babesiocidal and trypanocidal drugs. Secondly, it could also be due the study design employed as a cross-sectional study depicts only a momentary picture of the infection status in the herd. Furthermore, the investigator competency to detect the haemoparasites and the diagnostic capability of the parasitological technique used may be the other possible reasons. 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).

In species level, a greater proportion of donkeys were infected with *Babesia equi* (71.4%) than *Babesia caballi* (28.6%). This finding confirms a previous study by Nuria who reported *Babesia equi* (86.2%) as a widely distributed species than *Babesia caballi* (13.8%) in Bahir dar and its surroundings. It 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).

Table 4: Results of Chi-square analysis of different risk factors with *Babesia* sp. Infection in donkeys

Risk factors	No. examined	No. positive	Positive (%)	χ^2	p-value
District					
Adiqudem	206	2	0.97	-	-
Kwiha	194	1	0.52	0.28	-
Body condition score					
Good	94	0	-	-	-
Moderate	203	0	-	-	-
Poor	103	3	-	-	-
Age					
<2 years	57	0	-	-	-
2-10 years	303	2	0.66	-	-
>10 years	40	1	2.50	3.49	0.18
Sex					
Male	245	1	0.41	-	-
Female	155	2	1.29	0.99	0.58

Table 5: Analysis of the association between haemoparasites and mean PCV in donkeys

Type of haemoparasites	No. of animals	Mean PCV (%)	SD	t	p-value
<i>Babesia</i> sp.					
Infected	7	24.54	4.04		
Non-infected	393	28.69	5.77	1.88	0.06
<i>Trypanosome</i> sp.					
Infected	3	20.67	4.04		
Non-infected	397	28.68	5.73	2.41	0.02

It is known that different tick species are responsible for transmission of babesiosis. The common known vectors include the genera of Dermacentor, Rhipicephalus and Hyalomma (Soulsby, 1982). In the current study, *Boophilus* species were the most common ticks frequently encountered on the body of donkeys in both districts. This is consistent with a previous study conducted by Feseha in which *Rhipicephalus* and *Boophilus* species were reported to be the major vectors of equine babesiosis in the specific zone. *Trypanosoma vivax* was the only *Trypanosome* species identified in the current study. Since the study area is tsetse-free, it was probably caused by mechanical transmission through 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). In tsetse infested areas of Ethiopia, donkey trypanosomosis has been reported to be cause by tse-tse vectors such as *Glossina morsitans submorsitans* (Abebe and Wolde, 2010) and *Glossina pallidipes*.

Thee current study showed no statistically significant difference in the prevalence of both *Babesia* and *Trypanosoma* sp. infection between male and female donkeys, though male donkeys were much more frequently infected than their female counterparts. This finding implies that both male and female donkeys are equally susceptible and exposed to infection and the relatively higher prevalence observed in male donkeys

may be attributed to the extensive use of males. In both study areas, male donkeys are usually used for transportation of salt and other heavy material like wood, coal and agricultural products far away from their home which potentially pose a heavy work load and stress condition as well as the more chance of exposure to fly vectors as they cross fly infested areas along their way of long travel.

In the present study, statistically significant variation in the prevalence of Babesia and Trypanosome infection was not observed between the two districts covered by the study (Adigudem and Kwiha). This could be attributed to the same agro-ecology and equal veterinary attention given by the Tigray donkey health and welfare project for the two sites.

Analysis of the data on the age basis revealed that donkeys <2 years of age were free from haemoparasite infection and donkeys between 2 and 10 years had relatively lower infection rate than those over 10 years of age, although the difference was not significant. Such variation in the susceptibility may be associated with the acquired maternal immunity that can protect foals up to 6 months of age from haemoparasite infection. But such colostral immunity regresses gradually as donkeys get older and older. Unlike older or functionally mature age groups, young donkeys were usually confined around human settlement where there is smoke which potentially repel flies from the surrounding and protect them from mechanical vectors. Moreover, they are not mature enough to work as pack animal and travel long distance where there is risk of getting the mechanical flies.

The results of the current study showed that the prevalence of haemoparasite was higher in donkeys with poor BCS than those with moderate BCS, although the difference was not significant and no infection was observed in those animals with good BCS. This could be explained from two angles. The poor body condition observed could be the result of the haemoparasites because emaciation is usually considered as the feature of haemoparasite disease particularly trypanosomosis. However, the reason for poor body conditions could also be nutritional factors such as starvation and some other wasting diseases. On the other hand, the high prevalence of haemoparasitism observed in poor body condition could be associated with the poor immune defense in such group of animals.

The mean PCV of Trypanosome infected donkeys was significantly lower than non-infected ones. The detection of anemia (lowered PCV) in Trypanosome infected donkeys in this study is quite in agreement with many 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 Babesia free donkeys was not statistically significant and this was in agreement with the report of Keber. Using the PCV value range 30-46% as a normal (Knottenbelt, 2005), 43.85% of Trypanosome 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.

Though the number of samples used for serological study is small, 22% sampled donkeys were found to be seropositive for *Trypanosoma equiperdum* and *Trypanosoma evansi*. 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). Such detection power difference observed between microscopic detection of the parasites and serology is mainly associated with the low sensitivity of microscopy particularly for the detection of *Trypanosoma equiperdum* which is considered to be a tissue parasite rather than a blood parasite (Burn *et al.*, 1998).

CONCLUSION

In conclusion the prevalence of haemoparasite observed in the current study is generally very low; however, it should not be overlooked as the infected donkeys may serve as a potential reservoir of infection for the mechanical vectors and consequently, the infection may circulate in the population. For better implementation of practically and economically feasible control and prevention strategies, the magnitude of the problem should be clearly elucidated by further studies preferably with a wider study areas and serological or molecular biology techniques. The latter have proved very useful for the detection and identification of many haemoparasite species like the *Theileria/Babesia* group (Caccio *et al.*, 2000).

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