

## Prevalence and Risk Factors for Donkey Babesiosis in and Around Debre Zeit, Central Ethiopia

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**Abstract:** The cross-sectional study was conducted with the objectives to determine the prevalence of babesiosis in donkeys and associated risk factors responsible for the occurrence of the disease. Blood samples from 384 randomly selected donkeys were examined using parasitological methods and the PCV value by using Hematocrit reader. Parasitological examination of blood smears revealed babesia parasites in 12 (3.13%) donkeys. An attempt was also made to identify the species of *Babesia* involved and 2 species were identified with prevalence of *B. caballi* 1.04% (n = 4) and *B. equi* 2.08% (n = 8). Major risk factors were assessed on the occurrence of babesiosis. There was a significant difference ( $p < 0.05$ ) in the occurrence of babesiosis due to body condition. About 75% (n = 9) of the donkeys with poor body condition were positive for *Babesia* species. The babesia infection rate in different sexes and age was not significantly different ( $p > 0.05$ ). The study revealed that tick infestation resulted significance difference ( $p < 0.05$ ) in the occurrence of babesiosis and tick transmitted donkey babesiosis is an important disease. Statistical analysis of the data derived showed significant correlation between red urine and anemia (low PCV) with prevalence of babesia with  $r = 0.8$ ,  $r = 0.75$ , respectively. The prevalence of babesia was decreased while an increased mean PCV and decreased red urine. Finally, each result was discussed accordingly and recommendations were forwarded to undertake the socio-economic importance of donkeys and the perspectives for future research and development activities.

**Key words:** Babesiosis, body condition, prevalence, risk factors, tick, perspectives

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### INTRODUCTION

Ethiopia has about 5,421,895 donkeys (CSA, 2009) or 32% of all the donkeys in Africa and 10% of the world population (FAO, 1994). Majority of them are found in the central highlands of the country including Arsi and Showa and also Northern parts of Ethiopia like Tigray (Mekibib *et al.*, 2010). 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 endeavour of the Donkey sanctuary since its establishment (Mekibib *et al.*, 2010). 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 (Mekibib *et al.*, 2010). 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 (Mekibib *et al.*, 2010). *Babesia* species are

intraerythrocytic protozoal organisms (piroplasm) spread by arthropods (ticks, biting flies), trans-placentally and by blood transfusions. *B. equi* and *B. caballi* infect horses and other equids in tropical and subtropical areas worldwide (McGavin and Zachary, 2007). Babesiosis may cause both intravascular and extra vascular hemolysis and is also associated with a wide range of other clinical signs. The wide variety of clinical signs is due to variations in pathogenicity of the organisms and susceptibility of the host. Infection is highly virulent strains may cause severe multi-systemic disease. Babesiosis is distributed worldwide and its prevalence is directly related to the distribution of tick capable of transmission (Radostits *et al.*, 2000). The principal significance of *B. equi* is impact on the international movement of horses. Several countries including the United States, restrict the entrance of horses that are serologically positive for *Babesia* species (Radostits *et al.*, 2000). In Ethiopia, the presence of equine babesiosis was first reported by NVI at Debre Zeit and Feseha Regassa at Bahir Dar veterinary laboratory. In the study undertaken by Nuria Yassen and Feseha Gebreab in 1993, out of 582 equine species (348

donkeys, 106 mules and 128 horses) examined, 116 (19.93%) of which 60 donkeys, 27 mules and 29 horses were found positive for babesiosis. *B. equi* (86.2%) were widely distributed than *B. caballi* (13.8%) (Abebe and Wolde, 2010). 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 and little is about babesiosis (Abebe and Wolde, 2010; Mekibib *et al.*, 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 this study is undertaken with the objectives to estimate the prevalence of babesiosis and the species of babesia in donkeys in the study area and to determine the major risk factors responsible for the occurrence of the disease.

## MATERIALS AND METHODS

**Study area:** The study was conducted in and around Debre Zeit, East Showa zone, south east of Ethiopia from October 2008 to March 2009. Debre Zeit is located 45 km south East of Addis Ababa. The area is located 9°N and 4°E longitudes at an altitude of 1850 m.a.s.l in the central highland of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June-September). The dry season extends from October-February. The mean maximum and minimum temperature of the area is 30.7 and 8.5°C, respectively and a mean relative humidity of 61.3%.

**Study population:** In the present study donkeys of all ages and both sexes were examined for the presence of *Babesia* species. The animals used in this study were donkeys presented to Donkeys Health and Welfare Project (DHWP) and Society for the Protection of Animals Abroad (SPANNA) clinics at Debre Zeit.

**Sampling method and sampling size:** The sampling strategy was simple random sampling method. The sample size was decided by using 95% level of confidence interval, expected prevalence was 50% with desired absolute precision of 5%. Sample size was calculated using the formula of (Thrusfield, 2005). As a result, a total of 384 donkeys were sampled.

**Study design:** A cross-sectional investigation of donkey babesiosis was carried out in and around Debre Zeit from October 2008 to March 2009. Blood samples were collected from marginal ear vein of each animal using capillary tube for smear preparation and PCV

determination. Samples were examined for the presence of babesia by Giemsa stain. *Babesia* species was identified by morphological characteristic using thin smear and anemia was estimated by PCV (Urquhart *et al.*, 1996; William, 2001).

**Study methodology:** The study was based on 384 animals which were from in and around Debre Zeit. During sampling of animals for this study the donkey's history such as the presence of tick infestation, poor body condition, hemoglobinuria or pale mucus membrane were well noted.

**Collection of blood and preparation of smears:** Thin blood film preparation was made from the ear veins puncture of each examined animal. Before blood collection, the area of puncture was cleaned, hair removed and disinfected with 70% alcohol. Smaller ear veins were punctured and the first drops of blood were taken for thin smear preparation. Blood samples were also collected from Jugular veins of the same donkeys in heparinized vacutainer tubes for subsequent determination of Packed Cell Volume (PCV) (Urquhart *et al.*, 1996; William, 2001).

**Body condition score:** The body condition of all sampled animals were assessed and recorded during physical examination of the animal. Grades of A-C were given for good, moderate and poor body condition, respectively. The scoring was based on the method suggested by NEWC (2005).

**Data analysis:** The data collected during the study period were stored in excel spreadsheet for statistical analysis. The data were analyzed by the statistical software called SPSS version 16. Chi-square ( $\chi^2$ ) was used to test the possible existence of relationship between risk factors and positivity and  $p < 0.05$  indicated significant association.

## RESULTS AND DISCUSSION

All the 384 donkeys were examined for blood parasites. Blood smear were made and stained with Giemsa. The overall prevalence of babesiosis was found to be 3.13% ( $n = 12$ ). Total 2 species, i.e., *B. equi* and *B. caballi* were identified with prevalence of 2.08% ( $n = 8$ ) and 1.04% ( $n = 4$ ), respectively (Table 1). Out of the sampled donkeys, 53.1% ( $n = 204$ ) were categorized as having poor body condition, 39.1% ( $n = 150$ ) of them moderate and 7.8% ( $n = 30$ ) with good body condition. Donkeys with poor and moderate body condition were moderately infected where as donkeys with good body condition were infested in a lesser degree (Table 2). In

this study, age, tick infestation, red urine and PCV were assumed to be risk factors for the occurrence of babesiosis and analyzed to see the existence of any significant association between the prevalence of the disease and the risk factors. Age was supposed to have some association with the occurrence of the disease. The prevalence of babesia in the different age groups was variable. However, significant difference in prevalence was not observed in the four age groups, although prevalence was highest in older animals (Table 3).

It was worth enough to look the impact of sex on the prevalence of babesiosis. The study found that the prevalence in females was 3.8% and in male 2.6%. No significant difference was observed in prevalence of babesiosis between female and male donkeys which means males and females are equally affected with babesiosis irrespective of sex (Table 3). The study showed that significant association between the prevalence of babesiosis and tick infestation ( $p < 0.05$ ). The prevalence was 6.45% in tick infested donkeys (Table 3). There was marked correlation ( $p < 0.05$ ) ( $r = 0.8$ ) between donkeys infected with babesia and the presence of hemoglobinuria (Table 4).

Table 1: Over all prevalence of Babesiosis in donkeys

Total tested	Babesia species found	No. positive	Prevalence (%)
384	<i>B. equi</i>	8	2.08
	<i>B. caballi</i>	4	1.04
		12	3.13

Table 2: Body condition score versus Babesiosis

Cases	No. of donkeys	Body condition score No. (%)			$\chi^2$ (p-value)
		Good	Moderate	Poor	
Babesia positive	12	0 (0.0)	3 (25.0)	9 (75.0)	8.9 (0.020)
Babesia negative	372	30 (8.2)	147 (39.5)	195 (52.4)	-
Total	384	30 (0.0)	150 (0.0)	204 (0.0)	-

Table 3: Prevalence of Babesiosis in relation to age group

Risk factor	Classification	No. examined	Babesia positive	Prevalence (%)	$\chi^2$ (p-value)
Age	<2	8	0	0.00	1.7247 (0.422)
	2-5	106	1	0.94	
	6-10	137	4	2.92	
	>10	133	7	5.26	
Sex	Female	158	6	3.80	0.4010 (0.527)
	Male	226	6	2.65	
Tick infestation	Positive	124	8	6.45	6.6946 (0.010)
	Negative	260	4	1.54	

Table 4: The correlation between red urine and packed cell volume with the babesiosis

Risk factor	Classification	No. examined	Babesia positive	Prevalence (%)	$\chi^2$ (p-value)	r
Red urine	Positive	21	3	14.30	9.1404 (0.003)	0.80
	Negative	363	9	2.50		
PCV level	Low (<30%)	116	10	8.62	16.6946 (0.000)	0.75
	Normal (30-40%)	184	1	0.54		
	High (>40%)	84	1	1.19		

r = Pearson's correlation coefficient

Packed cell volume values were measured for each animal examined at study area and found that babesiosis had strong correlation with the occurrence reduced packed cell volume ( $r = 0.75$ ). The lower PCV due to Babesia infection was pronounced in females 3.8% and male 2.6%. Total mean PCV, mean PCV in parasitologically positive and negative donkeys have been determined as being 24.4, 19.1 and 24.3%, respectively. But the difference in PCV value between parasitaemic and aparasitaemic animals was statistically significant ( $p < 0.05$ ) (Table 4).

The prevalence of tick transmitted donkey babesiosis observed in this study was 3.13%. The result was in agreement with the research done by Mekibib *et al.* (2010) who found prevalence of 1.75% ( $n = 7$ ) in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia but not in agreement with previous study conducted by Kebere (1998) on tick of donkeys and observation on babesiosis and PCV in 3 different areas of Showa Central, Ethiopia, who reported prevalence of 10.3% ( $n = 37$ ) in donkeys. In former studies, donkey babesiosis accounted for 18% of the cases confirmed by Segwagwe *et al.* (1999) and 53 (34.6%) *B. caballi* was detected in 5 provinces of Black sea, region of Turkey by Acici *et al.* (2008). These results indicated that donkey babesiosis was less prevalent in the present study area (3.13%). The lower prevalence recorded in the current study might be due to regular use of chemoprophylactic drugs.

In addition, donkeys usually are tethered around homestead where ticks do not exist because of human activity. Another possible reason for the low prevalence recorded in the present study could be attributed to the fact that the survey was under taken during the dry season (October-December) in the area where the vector population was significantly low, hence in association with the decrease in tick population during the dry season

the prevalence rate was expected to decrease. In species level out of 12 positive result *B. equi* (66.6%) had high prevalence than *B. caballi* (33.3%). This was totally agrees with the previous studies conducted by Nuria and Feseha (1993) in Bahir dar and its surroundings. *B. equi* (86.2%) were widely distributed than *B. caballi* (13.8%) and Mekibib *et al.* (2010) who found *Babesia equi* (71.43%) and *Babesia caballi* (28.6%) but it was different with the report by Chahan *et al.* (2006) in Western Xinjiang China found the prevalence of *B. equi* and *B. caballi* infection, 9 (9.6%) and 36 (38.7%) of the 93 samples.

It was found that body condition could create significant difference in babesia infection. In this study donkeys that were shown to be parasitologically free of babesia had a better body condition score compared to those that were positive for babesia 75% of the donkeys in the latter group were categorized in the poor body condition score group while in those that were negative 52.4% of the donkeys were placed in the poor category. This was also in agreement with the study by Nuria and Feseha (1993). However, Mekibib *et al.* (2010) found no significant difference in babesia infection due to difference in body condition.

Previous study conducted by Kebere (1998) the effect of babesiosis on PCV and body condition, 62.2% of animals infected with babesia were placed in poor category but babesia negative animals categorized in poor body condition was 24.7%. All in all positive correlation has been shown between high tick burden, high in parasitologically positive donkeys and poor body condition. Body condition is the result of the cumulative effect of nutrition and management conditions. Animals on a good plane of nutrition have been shown to withstand the deleterious effects of ecto and endo parasites. Age dependent prevalence observation conducted showed that donkeys in the age group of <2 years were zero (0%). Age between 2-10 years relatively lower infection rate but above 10 years of age affected the most, up to 5.26%. Such variation in the susceptibility may be associated with the acquired immunity protecting the younger ones from babesia infections during their early period of life. But such colostral immunity waning gradually afterwards as donkeys get older.

The study found that there was no significant difference in the prevalence of babesiosis due to difference in age and sex. This is inline with previous studies Abebe and Wolde (2010) age and sex of the animals did not have significant influence on the prevalence of haemoparasites. Similarly, Mekibib *et al.* (2010) found no significant variation in the prevalence of both *Babesia* sp. and *Trypanosoma vivax* infection between the two districts covered by the study.

Tick infestation resulted significant variation in the occurrence of babesiosis and considered as risk factor. 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, 1994) in which *Rhipicephalus* and *Boophilus* species were reported to be the major vectors of equine babesiosis in the specific zone.

The status of anemia was assessed in the parasitologically positive and negative donkeys on the assumption that all other variables were the same for both groups of donkeys. Normal PCV values considered as references was that of Feseha (1994) in Ethiopia with value of 31-40, 32-38 and 30-36%, respectively. In this study, 30.3% of the total examined donkeys were found to be anemic and out of the 12 parasitologically positive donkeys, 83.3% were found to be anemic while only 27.6% of parasitologically negative donkeys were also recorded as anemic. Degree of anemia in parasitemic donkeys as measured by PCV was lower than aparasitemic and the variation was statistically significant ( $p < 0.05$ ) this was in agreement with former studies conducted by Kebere (1998). However, Mekibib *et al.* (2010) found the difference in mean PCV between *Babesia* infected and free donkeys was not statistically significant.

## CONCLUSION

In the present study, the prevalence of babesiosis in donkeys was found to be low. Parasitologically positive donkeys had low PCV and poor body condition score category. *Babesia equi* and *Babesia caballi* were identified as the species responsible for donkey babesiosis with greater prevalence of *Babesia equi*. Those donkeys with overt clinical signs (red urine, tick infestation, poor body condition and pale mucous membrane) had strong relationship with babesiosis. Sex and age had no significant relationship with the prevalence of the disease.

## RECOMMENDATIONS

In order to alleviate the existing problem and to promote the status of the donkey dependent people living in these areas, the following recommendations were forwarded:

- Regular strategic prophylactic treatment and establishment of veterinary clinic should be enhanced in the control of babesia parasites
- Extension workers and farmers should be trained in the management and utilization of donkeys
- Further research should be conducted to elucidate the impacts and epidemiology of tick borne disease using immunological methods to implement better control measure against ticks and tick borne diseases of donkeys

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