

Survey For The Determination Of Prevalence Of Piroplasmosis In Working Donkeys Of Central Ethiopia

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Abstract: The current cross-sectional study was conducted from November 2009 to April 2010 to determine the prevalence of piroplasmosis in working donkeys in three districts of East shoa namely, Boset Ada and Dugada Bora Administrative Zone of Oromia Region. Investigation for the presence of piroplasmosis for this study was conducted in three districts of central Ethiopia where occurrence of piroplasmosis is previously not reported. A total sample of 400 (male = 181 and female =219) donkeys with different sexes, ages and body conditions scores were investigated for the presence or absence of piroplasma by screening the using thin blood smears and staining them with Diff Quick stain for the detection of piroplasma out of the 400 blood samples examined qualitatively, a total donkeys 78 (19.5%) were relatively found to be positive for piroplasmosis. Among these T. equi has been found with relatively a higher prevalence of 18.25% (73) B. caballi 4.25% (17), total infection rate of 22.5% (90) and mixed infections 3.0%(12) were positive. From this result, there were no significant difference ($p>0.05$) in the prevalence of piroplasmosis between sexes and body conditions. On the other hand, there were significant differences ($p<0.05$) in the prevalence of piroplasmosis between age and study districts. The result of this study showed that piroplasmosis was a common health problem of working donkeys in the study districts. Among the two species of piroplasma (T. equi and B caballi), T. equi is more prevalent and might cause severe health and welfare problems of working donkeys in the study districts. Accordingly, due emphasis should be given particularly in prevention and control of this disease.

[Tesfie D, Tamrat H, Assefa Z. **Survey for the Determination of Prevalence of Piroplasmosis in Working Donkeys of Central Ethiopia.** *Rep Opin* 2018;10(9):23-29]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). <http://www.sciencepub.net/report>. 4. doi: [10.7537/marsroj100918.04](https://doi.org/10.7537/marsroj100918.04).

Key words: Central Ethiopia, Diff Quick staining, working donkeys, Piroplasmosis Prevalence, Qualitative.

1. Introduction

Ethiopia, located in East Africa, is prominently an agricultural country with over 85% of its population engaged in agricultural activity. The country has adverse agro-ecological zones which contributes to the evolution of different agricultural production systems. Animal agriculture forms an integral part of agricultural production in almost all ecological zones of the country (Tegegne and Crawford, 2003).

The number of equine in Africa is in the range of 17.6 millions comprising 11.6 million donkeys, 2.3 million mules, and 3.7Million horses. According to recent reports, Ethiopia has an approximately 5.2 million donkeys (Pearson et al., 1997), and the domestic donkey of Ethiopia traces its ancestor to the wild asses found in Egypt, Sudan and Ethiopian namely *Equusasinusafricanus* and *Equusasinussomalicus* this represents more than 55% of the national equine species which is again equivalent to 11.4% of the world and 37.4% of the African donkeys, population: with this figure the country stands not only the biggest in Africa but also

the second largest in the world next to china (Feseha *et al.*, 1991).

According to the present regional classification of Ethiopia, 97% of donkeys are found in three regions: 44% in Oromia, 34% in Amhara and 19% in Tigray regional state. This shows that the; largest number of donkeys is found in the central part of Ethiopia which makes the basis for the three districts, where this study was conducted (Feseha, 1998).

The donkey has spent hundreds of years being used by man but, despite this, in the past little attempt has been made to study any aspect of equine (Svendsen, 1997). It is still one of the most important draught animals existing in millions and playing a key role in the agricultural economy of most under developing countries in the form of pack, transportation, carting, threshing, farm cultivation, riding, and meat and milk production for human consumption (Oppugn, 1979; Fielding, 1987). Recurrent drought in Ethiopia resulting in increased cattle mortality has also contributed to an increase in donkey usage as a draft and pack animals in both rural and urban area.

In countries like Ethiopia, where modern transportation and communication services are poorly developed, the natural choice rests on the use of draft animals as well as social functions. This still, remains true even in Ethiopian context (Feseha et al., 1991) they are also kept for providing manure for both energy and soil fertility (Wilson, 1991).

Donkeys have been completely neglected and omitted from the national livestock development programmers'. This is because the contribution of donkeys, power in the agricultural systems and their role in production is not yet well recognized and magnified (Fielding, 1987). In Ethiopian context, especially in the marginal lands of the country, donkeys are good vehicles and will remain as the main means of transport animals in the coming decades. This is because in addition to the mountainous and rugged features of the country that causes difficulty in motor road construction, there is insufficiency of all weather road accessibility and/ or even lack of infrastructure in many parts of the country having better topography (Feseha et al., 1991). The increasing human population, demands for transport of good to and from far, remote areas, and construction activities around the town are making donkeys highly demand animals (Mengistu, 2003).

Some of the advantages of power animals over machines in the context of Ethiopian economy are: their low initial cost, their suitability for small unit with limited output, the fact that they are self perpetuating if both males are used, the concomitant facts that they do not require spare parts and thus their running costs are low: and finally that there is no foreign exchange elements involved (Wilson, R.T.1976).

The treatment services provided to these species of animals have been far below the one given to other species of animals. This can be due to age- old erroneous concept that when donkeys do get sick they are quick to die and probably because they are not provider of meat and milk (Yoseph et al., 2001) .

Due to minimum management attention given to donkeys, particularly in countries like Ethiopia, they are prone to a number of diseases. Donkeys in Ethiopia, at least in the Donkey Health and Welfare project (DHWP) operation site, are subjects to a variety of health disorders including multiparasitism, back sores, and other wounds, hoof problems, ophthalmic problems, colic various infectious disease such as strangle, Tetanus, Epizootic Lymphangitis, African Horse sickness (AHS), piroplasmosis, etc, (Getachew et al., 2002) Among these, piroplasmosis is known to be most important protozoan disease of donkey in Ethiopia that lowers protection efficiency of these animals (kebere, 1998). This organism invades erythrocytes and subsequently cause haemolysis or

break down of Red Blood cells (RBCs) leading to general weakness and even mortality of the animals (Abuladze, 1982).

Piroplasmosis is virulent inoculable and non-contagious infectious disease that affects most domestic and wild mammals. It is tick-borne protozoan infections of donkeys. The causative agent is a protozoal parasite of the genus *Babesia* that is obligatory in nature and is transmitted after cyclic development in ticks. Equine piroplasmosis results from infection by the protozoa *Babesiacaballi* and *theileriaequi* (formerly *Babesiaequi*). Both organisms belong to the phylum Apicomplexa and order piroplasmida. *Tequi* is thought to have a wider distribution and tends to cause more severe disease than *B. caballi*. Equine piroplasmosis affects Horse, Mules, Donkeys and zebras. The latter are considered to be an important reservoir of infection in Africa. It is endemic in many tropical and subtropical regions of the world including parts of Africa and is believed to be one of the major constraints to the international movement of equines (Mira, 1989).

Babesiacaballi and *Theileriaequi* are transmitted by ticks which become infected when they ingest parasites in the blood of infected equids. *B. caballi* is a large species which occur as pairs, pyriform and measure 2.5µm to 4µm in length and the angle formed by the organism is acute while *Tequi* is relatively small, only 2µm long and the merozoites in the erythrocytes are rounded or, most often, pyriform and the angle formed is obtuse (soulsby, 1982).

Approximately 14 species of ticks in the genera *Dermacentor*, *Hyalomma* and *Rhipicephalus* can be vectors for these organisms. The incubation period of piroplasmosis is 12-19 days when it is caused by *T. equi*, and 10-30 days when it is caused by *B. caballi*. True equine piroplasmosis is extremely serious (mortality 20-50% and convalescence is always long (Mira, 1989).

When compared with other domestic animals, there is very little or no formal study conducted on piroplasmosis of working donkeys in Ethiopia as a whole and this issue has made the information on this disease in working donkeys very scant.

Therefore, the objectives of this study were to:

- Collect baseline data on piroplasmosis of working donkeys.
- Determine the prevalence of donkeys' piroplasmosis in selected districts of central Ethiopia.
- Determine the potential risk factors that may influence its occurrence and finally.

2. Materials And Methods

2.1. Study districts

The study was conducted in central Ethiopia in three districts namely low (Boset) mid (Ada'a) and

relatively high (Dugda Bora) altitudinal zones from November 2009 to April 2010. These sites were previously selected as a working area by the mobile and sastenariy clinic of the Donkey Health and Welfare project (DHWP) based on their high equine population and poor economic status of the owner.

2.1.1. *Ada'a district*

The capital town of the district is Debre-zeit which is located 45 kms south east of Addis Ababa: located 90N and 40 E at an altitude of 1550 meter above sea level. It receives an annual rainfall of 1151.6mm with a mean maximum and minimum temperature of 30.7°C and 8.5 °C, respectively, and a mean relative humidity of 61.3% (NMSA, 2003).

The donkey population of the district is estimated to be 46,222 (NMSA, 2003). The donkey population of the district is estimated to be 46,222 (MOA, 2004).

2.1.2. *Boset district*

The capital town of the district is welenchity which is located 120-124 km east of Addis Ababa. The total land coverage is 124, 160 hectares. The altitude range from 1200-1800 meters above sea level and the annual rainfall range from 550mm to 700mm. The average daily maximum and minimum temperatures are 43°C and 28°C, respectively (NMSA, 2003). The population of donkey in the district is estimated to be about 31,181 donkeys (MOA, 2004).

2.1.3. *Dugda Bora district*

It is located 124 Kms south of Addis Ababa. the altitudinal range is 1800 ms to 2020 ms and the mean annual rainfall is 750mm. The average daily minimum and maximum temperatures are 22°C and 28°C, respectively (NMSA, 2004). The total land coverage is 146,800 hectares. The donkey population is estimated to be 12,900 (MOA, 2004).

2.2. Study Animals

Donkeys coming to the Donkey Health and Welfare project (DHWP) mobile clinic make the study population. A total of 400 donkeys from the three selected areas were subject to qualitative microscope examination primarily to detect the parasites and, eventually to determine the prevalence of equine piroplasmosis. The group of animals investigated was working donkeys of mixed ages, sexes and body conditions scores such donkeys are brought to the mobile click for strategic deforming, vaccination and also seeking medical assistance against other clinical disease conditions.

The farmers in the selected peasant association were informed in advanced on the importance of the study and the need to present their donkeys on specific visit dates and places. The ages of the selected donkeys were determined by dentition (Kahn, 2005) and body condition scores were subjectively estimated based on the guides published by (Svenden, 1997). Donkeys were then grouped in to three age categories.

Donkeys under two years of age were amassed as young those in range of two ten years classed as adult and beyond ten years were classed as old these age classes were based on age of first work, productive age and the life span of Ethiopian donkeys (Svenden, 1997; Yoseph et al., 2001 and Ayele, 2006).

2.2. Study design and sample size

The type of the study was cross-sectional with simple random sampling technique which was conducted from November 2009 to April 2010 to establish the prevalence of donkey piroplasmosis in central Ethiopia, East shoa zone of oromia region, namely, Ada, a, Boset and Dugda Bora districts. Blood samples were collected from both apparently healthy and clinically diseased donkey with no discrimination of ages, body conditions scores sexes and color. For this particular study, the sample size was determined for a definite precision and level of confidence, and calculated as follows (Thrusfield, 1995).

Since there was no previous estimated prevalence of piroplasmosis in the selected areas, 50% prevalence was expected, desired absolute precision 5% and 95% confidence interval were used to determine the minimum sample size.

$$\begin{aligned} \text{i.e. } n &= 1.96^2 p \exp (1-p \exp) / d^2 \\ n &= 1.96^2 \times 0.5 (1-0.5) / 0.05^2 \\ n &= 384 \approx 400 \end{aligned}$$

Where, n=required sample size

P exp =expected prevalence

d= desired precision

1.96= Z-value for the confidence levels.

For the study, a total of 400 donkeys were included from the three study districts.

2.2.1. Sampling method

Blood sample was collected cross-sectionally with simple random sampling technique from a total of 400 donkeys. There was no discrimination of ages, body condition scores, sexes and color in the sampling process. Blood samples for making of the smears were taken directly from the ear vein of each donkey with strict sanitation. The hair was removed by using paris of scissors or scalpel blades and the sites disinfected with alcohol or savlon and left for some time to dry. The smaller ear vein was punctured and, as much as possible, the first drop of blood was taken and smears were made soon using glass slides. It was air dried and fixed with methanol in the field (Mira, 1989/. Finally, it was stained with Diff Quick stain solution after transport to the Donkey Health and Welfare project (DHWP) laboratory in Debre-zeit.

2.2.2. Microscopic examination

The blood which was collected from each donkey was subject to microscope examination. The smears were done according to the producer to the producer of thin blood smear with Diff Quick stain method and then examined under high magnification

(100x) with oil immersion, and parasites identified by their sizes and morphological features.

2.3. Data Analysis

Prevalence was defined as the proportion of donkeys positive for piroplasmosis by blood smear examination to the total number of donkeys examined, which was expressed in percent. SPSS 15.0 software was used to analyze the data. Variation of prevalence (data) among or between age group, sex group, areas, and body condition scores were determined by Pearson's chi-square (χ^2) regarded statistically significant if ($p < 0.05$).

3. Results

3.1. Microscope examination

During the study period, a total of 400 (181 = male and 219 = female) donkeys from three districts were examined for piroplasmosis using blood smear with Diff Quick stained.

Out of 400 donkeys examined 78 (19.5%) were found to be positive for donkey piroplasmosis. The

overall prevalence of *T. equi* and *B. caballi* was 73 (18.25%) and 17 (4.25%) respectively, with total infection rate 90 (22.5%) 35 (19.34%) male donkeys and 38 (17.35%) female donkeys were found positive for *T. equi*. 9 (4.97%) male donkeys and 8 (3.65%) female donkeys were found positive for *B. caballi*. Mixed infection was detected in 7 (3.67%) male and 5 (2.28%) female working donkeys which were indicated in Table 3. There were no significant differences ($p > 0.05$) in the prevalence of donkey piroplasmosis (both *B. caballi* and *T. equi*) was significantly different ($p < 0.000$) between the study districts.

There were no significant difference ($p > 0.05$) in infection rates between different body condition scores but there was significant ($p < 0.05$) between the study districts and age group. Sex group and different BCS donkeys are equally susceptible to the disease. As far as parasitaemia per microscopic field of observation concerned, for piroplasmosis, the slides show different parasitized erythrocytes.

Table 1. Relative prevalence of *T. equi* and *B. caballi* in working donkeys of the three study areas.

Study Districts	Total examined	No. of positives		
		<i>T. equi</i>	<i>B. caballi</i>	mixed infection
Boset	139	22 (15.83%)	4 (2.88%)	3 (2.16%)
Dugda Bora	57	5 (8.77%)	1 (1.75%)	1 (1.75%)
Ada'a	205	46 (22.44%)	12 (5.85%)	8 (3.9%)
Total	400	73 (18.25%)	17 (4.25%)	12 (3.0%)

Table 2. Relative prevalence of *T. equi* and *B. caballi* in working donkeys by sex.

Sex	Total examined	No. of positives		
		<i>T. equi</i>	<i>B. caballi</i>	mixed infection
Male	181	35 (19.34%)	9 (4.97%)	7 (3.87%)
Female	219	38 (17.35%)	8 (3.65%)	5 (2.28%)
Total	400	73 (18.25%)	17 (4.25%)	12 (3.0%)

Table 3. Relative prevalence of *T. equi*, and *B. caballi* in working donkeys by age.

Age	Total examined	No of positives		
		<i>T. equi</i>	<i>B. caballi</i>	mixed infection
Young	5	0 (0.0%)	0 (0.0%)	0 (0.0%)
Adult	238	37 (15.55%)	9 (3.78%)	6 (2.52%)
Old	157	36 (22.93%)	8 (5.09%)	6 (3.82%)
Total	400	73 (18.5%)	17 (4.25%)	12 (3.0%)

Table 4. Relative prevalence of *T. equi* and *B. caballi* in working donkeys by BCS.

BCS	Total examined	No of positives		
		<i>T. equi</i>	<i>B. caballi</i>	mixed infection
Poor	185	37 (2.0%)	7 (3.78%)	6 (3.24%)
Medium	211	35 (16.59%)	10 (4.74%)	6 (2.84%)
Good	4	1 (25%)	0 (0.0%)	0 (0.0%)
Total	400	73 (18.25%)	17 (4.25%)	12 (3.0%)

Discussion

In Ethiopia, considerable study has been done on bovine piroplasmiasis but there is no study carried on piroplasmiasis of donkeys except the report (Huria, 1992) from Bahir Dar and its surrounding area. During the study period, a total of 400 (181= male and 219= female) donkeys were examined for Parasitaemia in a dry season from November 2009 to April 2010. Out of these, 19.5% donkeys were found to be positive for piroplasmiasis. In this trial, it was revealed that donkeys harbor both species of Babesia and the proportion of *T. equi*, 18.25% prevalence was found to be positive which is relatively greater than that of *B. caballi*, 4.25% indicating that both species of Babesia (*T. equi* and *B. caballi*) are present in donkeys considered in the current study. The possible infection of *T. equi* is generally might be server and wider than *B. caballi* (solusby, 1982). This might be due to the carrier states of the host for long period of time if infected once by *T. equi* and causes reinfection.

The study was conducted in the dry season and, hence, the prevalence of piroplasmiasis might be higher in wet season especially at the beginning of the rainy season which favors the multiplication of the vectors (Mira, 1989). There were no significant difference ($p>0.05$) in the prevalence of piroplasmiasis between different sex groups. This shows that sex seems have no effect on the prevalence and, hence, both sexes are equally susceptible and exposed to this disease.

The overall comparative prevalence of *T. equi* and *B. caballi* in the study districts were Ada, a 24.39% Boset 16.55% and Dugda Bora 8.77% with significant difference in these values ($p<0.05$). This significant difference might be due to the difference in the epidemiology and climatic conditions such as attitude, rainfall, temperature and humidity of the study districts. The relative high prevalence in the study districts may be, in part, attributed to the presence of conducive ecological factors for the tick vector and the parasites. In this study, there were significant differences ($p<0.05$) in the prevalence of *T. equi* between different age groups. Young 0.0% adult 15.5% and old 22.93% were found to be positive. Also for *B. caballi* there was significant difference ($p<0.05$) between different age groups in the study areas. This indicates that age groups do vary for the prevalence of piroplasmiasis in working donkeys and, hence, both age groups are not equally susceptible to the disease as well. Probably, the pathogenicity of the disease could be severing in old donkeys compares to young and adult animals because young animals are less susceptible to piroplasmiasis due to passive protection resulting from maternal antibodies received with the

colostrums and the presence of thymus that appears to ensure non-specific protection against piroplasmiasis (Mira, 1989).

During this study, donkeys were examined with different body condition score but there was no a significant differences ($p>0.05$) in the prevalence of donkeys between different body condition scores. This indicates that different body conditions cores have no effect the prevalence of piroplasmiasis in donkeys and, hence, both donkeys which have different body condition scores were equally susceptible to the disease. This might be due to the transmission nature of the disease which is not an opportunistic rather it is transmitted by the vectors.

When compared with findings of Huria (1992), who reported a prevalence of donkey piroplasmiasis as being 19.24% in Bahir Dar and its surrounding areas, the present finding of is 19.5% prevalence is in agreement for donkeys living in central Ethiopia. Statically variation analysis has indicated that there was no significant variation between the prevalence of piroplasmiasis from both finding which had similar prevalence and this might be due to their similarity in agro-ecological and the climatic condition of both areas were favor the multiplication of vectors and the parasite to be infect their host and cause a disease.

Conclusions And Recommendations

Donkeys play great importance in the Ethiopian economy, particularly in the farming system, transportation and other activates. Despite their importance, very little study has been done on their health, management and feed aspects. Therefore, much more attention is needed to their health, welfare and use as a whole.

As shown in this work, piroplasmiasis constitutes an important constraint due to the diversity of climatic and ecological conditions in which the donkey live and are used for the development of the vector species in general. The prevalence was higher in these study districts of donkeys because the climatic condition favors the multiplication of vectors.

However, the attention given to the disease in the country, so far was not satisfactory in that there were perhaps little or no attempts made to study the epidemiology of the disease in the area. Strategic treatment with appropriate, effective and broad spectrum anthelmintic should be practiced at the beginning of rainy season when the vector is expected to be favored and multiplication increase. And thus, increasing the rate of infection with the piroplasma is in question.

Based on the above conclusive remarks the following recommendations are given:

❖ Detailed local epidemiological study on the seasonal dynamics of infection in a particular area needs to be varied out and this information, apart from addressing the problem from the host's side will also help in reducing vectors.

❖ An integrated and cost effective control strategy in cooperating strategic anthelmintic treatment against the parasite and other feasible control measures including vector control have to be used to ensure a satisfactory degree of control in the long run.

❖ Field veterinarians and stockowners should be aware of the importance and burden of the disease in donkeys.

❖ Further serological study should be conducted.

Acknowledgements:

We would like to thank Addis Abeba University, School of Veterinary Medicine for letting us Survey for the Determination of Prevalence of Piroplasmosis in Working Donkeys of Central Ethiopia.

We wish also to express our profound gratitude to personnel of the School of Veterinary Medicine, who assist during study period and suggest valuable comments.

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9/25/2018