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Bovine Trypanosomosis Prevalence and Tsetse Fly Spatial Distribution in Ilu Aba Bora Darimu District, Western Ethiopia

Eyob Hirpa Tola and Yacob Hailu Tolossa

Addis Ababa University, college of veterinary Medicine and Agriculture, P.O. Box 34, Bishoftu, Ethiopia

https://orcid.org/0000-0002-5065-1042, Email: eyob.hirpa @ aau.edu.et

Abstract

Background: Bovine 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. Darimu District is one of the areas with such problems.

Methodology: Therefore, a cross-sectional study was carried out to determine the prevalence of bovine trypanosomosis and tsetse apparent density using parasitological and entomological surveys in the Darimu district of Western Ethiopia from November 2016 to March 2016. The parasitological survey was conducted on 614 randomly selected animals during the study period.

Results: The prevalence of bovine trypanosome was 8.6%, of which T. congolense was the most prevalent (71.69%) trypanosome species, followed by T. vivax (22.64%) and mixed infection with T. congolense and T. vivax (5.6%). The highest prevalence was observed in the animals with poor body condition (11.5%) and animals with anemia (10.8%). There was statistically significant variation in the prevalence of trypanosomosis among different body conditions and anemic states of the animal ($P<0.05$). There was no statistically significant difference between the sex and age groups ($p>0.05$). The entomological survey showed that the apparent density of Glossina was 4.6 flies/trap/day. fuscipes, *Glossina pallidipes, Glossina morsitans* and *Glossina tachinoides* were identified to exist in the study area. The mean PCV value of the infected animals was lower (21.4) than the mean PCV value of noninfected animals (23.5), and statistically, asignificant difference was observed between the PCV value of infected and non infected animals ($P<0.05$).

Conclusions and Recommendations: The study revealed valuable information on the epidemiology of bovine trypanosomosis in the study area. The present study shows ahigher prevalence, so implementing control of trypanosomosis with integrated approaches has paramount importance in the study sites.

Keywords: Bovine Trypanosomosis, Darimu District, Ethiopia, Tsetse fly

Introduction

Trypanosomiasis is a disease complex caused by several species of blood- and tissue-dwelling protozoan parasites of the genus *Trypanosoma.* [1-3]It is a serious disease in domestic livestock that causes a significant negative impact on food and economic growth in many parts of the world, especially in sub-Saharan Africa [4]

It is distributed over approximately 10 million km^2 of Sub-Saharan Africa between latitudes 140N and 290S [5,6.] The most important trypanosome species affecting domestic livestock in Africa are *Trypanosoma congolense*, *T. vivax*, and *T. bruicebruice*in cattle, sheep, and goats. *T, simiae* in pigs and *T.evansi* in camels [7]

Tsetse fly species are restricted to various geographical areas according to habitat, and the three main groups, named after the most common species in each group, are *fusca*, *palpalis* and *morsitans*, found in forest, riverine and savannah areas, respectively. The last two groups, because of their presence in the major livestock rearing areas, are the most from a veterinary standpoint (Urquhart, *et al*., 1991)[5].

Infected tsetses inoculate metacyclic trypanosomes into the skin of animals, where the trypanosome grows for a few days and causes localized swelling (chancres). They enter the lymph nodes and then the bloodstream, where they divide rapidly by binary fusion. In *T. congolense*infection, the organism attaches to endothelial cells and localizes in capillaries and small blood vessels. *T. brucei*and *T. vivax* invade tissues and result in tissue damage in several organs (Aiello, 1998).[7]

In tsetse-infested areas of Africa, trypanosomiasis is well recognized, and diagnosis is often based ona history of the chronic wasting condition of cattle in contact with tsetse flies. Trypanosomes can be confirmed parasitologically by demonstrating parasites in the blood of infected animals, and various techniques are available. In practice, many field programs of monitoring cattle for infection are based on routine screening of stained thick and thin blood films, thick films are examined to detect infected animals and thin films determine the species of infecting trypanosomes.[8] By buffy coat examination, the organism is well visualized.[9] Among the various tests developed, IFAT and ELISA can be used to detect circulating trypanosome antigens and antibodies (Andrews, *et al.*, 2003).[8]

There are five economically important animal trypanosome species in Ethiopia. These are *Trypanosoma congolense*, *T. vivax, T. brucei*, *T. evansi*and *T. equiperdum*.[10]The closely related *T.brucei* subspecies *T. b. rhodensience*causes human sleeping sickness. The other trypanosome species of economic importance are *T. evansi*of camel and *T. equiperdum*of horse.[11]

The tsetse flies in Ethiopia are confined to southern and western regions between longitudes 330 and 380 E and latitudes 50 and 120 N, which amounts to approximately 200,000 km2. Tsetse-infested areas lie in lowlands and in the river valley of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo. Out of the nine regions of Ethiopia, five (Amhara, Benishangul-gumuz, Gambella, Oromia and SNNPR) are infested with more than one species of tsetse fly.[11]

Chemotherapy and chemoprophylaxis by trypanocides are the most important aspects of the control and eradication of trypanosomes.[12] There is no vaccine against the disease, and despite intensive research, none appears likely in the near future because of the ability of trypanosomes to readily change their glycoprotein surface coat through a process called antigenic variation.[6]

Trypanosomiasis in cattle, locally referred to as Gendi, is a serious constraint on livestock production in areas of southwest Ethiopia at latitudes less than 1700 m above sea level.[13] In Ethiopia, trypanosomosis was among the factors that hinder livestock production in most settlement areas. Therefore, the objectives of the study were:

- ✓ To determine the prevalence of trypanosomosis and to identify trypanosome species infecting cattle found in the study area.
- ✓ To determine the associated risk factor for trypanosomosis.

Materials and Methods

Study Area

Darimu is located intheIllubabor Zone of the Oromia regional state, 664 km from Addis Ababa. Darimu district is located at longitude 035°15°to 035°32°E,latitude of 08°30°-08°44°N north of equator, covering 1387.97 km^2 land with altitudes ranging from 792-1800 m.a.s.l. The agroclimate of the area alternates with long summer rainfall (June-September) and a winter dry season (December-March). The mean annual rainfall of the district is 1172-1740 mm. The annual temperature in the Darimu district ranges from 18-25°C. The district is divided into two ecological zones: 46% kola and 54% Woinadega.

The main rivers in Darimu district include Golol, Guracha, Asas, Geba and many other tributaries that enter the big river, Birbir. There are many river basins and tributaries that flow to Baro Akobo. The distribution of tsetses is connected to the presence or absence of large game animals, which serve as sources of food for flies and reservoirs of infection for trypanosomes. The areas have got a number of wild animals. The commonly found buffaloes are African buffaloes (*Syncerus caffer*), Bush pigs (*Potomacherus porcus*), warthog (*Potomacherus aethiopicus*), Bush buck (*Teragelapus scriplus*), lion, kudu, Hippopotamus, crocodiles, hyena, vervet monkey, antelopes, snakes, etc. Many of these proteins serve as reservoirs of infection for trypanosomes. The areas have different vegetation: thickets, wooded grasslands and riparians. The dominant trees in both districts include *Grewia bicolor, Albizia gummifera, Carissa edulis, Cordia Africana, Phoenix reclinata,Pilostigma thonningii, Sterreospermum kunthianum, Acacia jacaranda, Juniperus procera, Erhrina abyssinca*, etc.

Study Design

A cross-sectional study conducted from November 2016 to March 2016 to determine the prevalence of trypanosomosis in the study animals.

Study Population

The cattle in the district are indigenous African Zebu breeds that are kept under traditional extensive husbandry systems with communal herding. Agriculture is the main stay of the lively hood of society, with mixed farming systems, and livestock play an integral role in agriculture. According to [14], the animal population of the district estimated to be 88,098 cattle, 42,605 sheep, 32,283 goats, 8,914 equines and 138,115 poultry.

Sample Size Determination and Sampling Methods

Purposively tsetse infested Village namely, Dedebotoro, Bena 1, 2 and 3, Metti Kerebe and Hanna Ifa were selected from the districts while the study animals were selected by using simple random sampling method by taking age, sex, and body conditions in to account according to [15,16] and all the animals in the selected areas had equal chances to be selected for this study. The number of animals required for the study was determined using the formula given by [17] for simple random sampling. The size of the sample size was determined by using a 95% confidence level, 7.1% expected prevalence and 0.05 desired absolute precision. Therefore,

$$N = \frac{1.962(0.071)\,(1-0.071}{0.0025} = 101$$

Where: n = required sample size
P_{exp}= expected prevalence
d = desired absolute precision (usually 0.05).

Therefore, 101 cattle were needed for the study; however, a total of 615 animals were sampled to increase the precision.

Study Methods and Procedures

A total of 614 samples were collected during the study period from selected Villages by using simple random sampling in each PA settlement.

Sample collection

Blood samples were collected in heparinized microhematocrit tubes after puncturing the ear vein using a lancet, and then one end of the tube was sealed with crystal sealant. On the other hand, for theentomological survey, baited monopyramidal traps were deployed at 100-200 meter intervals.

Parasitological study

Blood samples were collected randomly from cattle of seven PAs into heparinized microhaematocrit tubes (capillary tubes). After piercing the ear vein using a lancet, one end (the heparinized) of capillary tubes was sealed with crystal sealant and centrifuged at 12000 rpm for 5 minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces as a buffy coat. Then, the PCV was calculated using haematocrit reader. The capillary tubes were then broken just below the buffy coat usinga diamond pen, expressed on a microscope slide and covered with a cover slip. Then, the parasites were examined under ax40 microscope objective to detect the presence of the parasites using the dark background buffy coat technique [18]. Trypanosome species were identified according to their morphological descriptions as well as movement in wet film preparations provided by [19].

Entomological study

A total of 78 baited different types of traps, including bioconical, monoconical and monopyramidal traps, were deployed along suitable tsetse habitats to assess the apparent densities, distributions and species of tsetse flies and other biting flies involved in the transmission of trypanosomosis. All traps were baited with acetone, octanol (1-3-octanol) and cow urine filled in separated bottles and deployed at an interval of 100-200 meters. The coordination of each trap was recorded using a GPS. The vegetation type, prominent features within a100 meter radius and canopy of each trap were recorded. After 48 hours of trap deployment, the cages were collected, and captured flies were identified and sexed according to morphological characteristics and counted.

Data Analysis

The collected data were entered into the Microsoft Excel office and transported to statistical analysis software SPSS version 20 for statistical analysis. The prevalence of trypanosome infection was calculated as the number of positive animals as examined by Buffy coat divided by the total number of animals examined at a particular time multiplied by 100. The chi-square(x^2) test was used to determine the association between explanatory variables and the prevalence of trypanosomosis. In all analyses, a confidence level of 95% and a p value-value of 0.05 were used for statistical testing of significance. Finally, the density of the fly population was calculated by dividing the number of flies caught by the number of traps deployed and the number of days of deployment and expressed as fly/trap/day.

Ethical Approval and Consent to Participate

Animal handling ethics were assessed and approved by the Wollega University Research Ethics Approval Board Committee in accordance with the Declaration of Helsinki. The best practice guidelines for veterinary care were followed, and the owners provided informed consent for their animals to be used in this study.

Results

Parasitological Findings

Parasitological studies were conducted in seven Villages found in Darimu district, namely, Dade Botoro, Wachale, Bena1, Bena2, Bena3, MettiKerebe and Hanna Ifa.In total, 614 animal bloodsamples were collected for trypanomosis investigation and trypansome species identification. Out of 614 animals, 53 were positive for trypanomosis. Therefore, the overall prevalence of Trypanomosis in the study was 8.6% (53/614). The highest 16.7% (9/54) and the lowest 4.8% (8/167) prevalence were recorded in Mettikerebe and Wachale, respectively. There was no statistically significant (P>0.05) variation inthe prevalence of trypanosomosis among the different Villages indicated in Table 1.

Table 1: Prevalence in different Village.

PAs name	Positive	Negative	Total	Prevalence	X^2 (p value)
Dade botoro	9	87	96	9.4%	9.609(0.14)
Wachale	8	159	167	4.8%	
Bena 1	10	85	95	10.5%	
Bena 2	8	64	72	11.1%	
Bena 3	3	57	60	5.0%	
Mettikerebe	9	45	54	16.7%	
Hanna ifa	9	45	70	8.6%	
Total	**53**	**561**	**614**	**8.6%**	

T. *congolense*was the most prevalent trypanosome species at 71.69% (38/53), followed by T. *vivaxat* 22.64%. (12/53), while mixed infection of T. *congolense* and T. *vivaxwas* seen only in three Villages, namely, Wachale, bena 2 and Mettikarebe. The overall prevalence of mixed infection was 5.6% (3/53) for the three mentioned Villages. Prevalence by different species of trypanosomes indicated in Table 2.

Table 2: Prevalence by different species of trypanosomes.

Village	Tested Animal	*Trypanosoma congolense,*	*Trypanosomavivax*	Mixed	Total	Prevalence	Chi square (X^2)
Dade botoro	96	6	3	0	9	9.4%	17.7
Wachale	167	5	2	1	8	4.8%	
Bena 1	95	7	3	0	10	10.5%	
Bena 2	72	5	2	1	8	11.1%	
Bena 3	60	2	1	0	3	5.0%	
Mettikerebe	54	7	1	1	9	16.7%	
Hanna ifa	70	6	0	0	6	8.6%	
Total	614	38	12	3	53	8.6%	

Hematological Findings

The normal PCV value of zebu cattle was 24 to 46% according to Blood and Radostits (2007) [20],but in this study, the mean PCV value of all examined animals was 23.36%, which is below the normal range. The mean PCV value of the infected animals was lower (21.4%) than the mean PCV value of no infected animals (23.5%), and the variation in PCV value and prevalence of trypanosomosis were statistically significant ($P<0.05$).

Table 3: Apparent tsetse density in different Villages.

PAs	No. Trap	M	F	Total	F/t/d
Dade Botoro	20	92	138	230	5.75
Wacale	19	10	12	22	0.58
Bena 1	10	71	86	157	7.85
Bena 2	9	90	103	193	10.72
Mettikerebe	10	35	76	111	5.53
Hanna Ifa	10		1	1	0.05
Total	78	301	420	714	4.6

Entomological findings

A total of 714 tsetse flies were caught by employing 78 traps during the study period from six different Villages. The apparent density of Glossina in the study area was 4.6 (f/t/d). Four tsetse species have been identified. The proportions of G.*fuscipes*, G.*pallidipes*, G.*morsitans* and G. *tachinoides* were 38.14%, 35.09%, 18.31% and 8.46%, respectively. Of the 714 tsetse flies captured, 416(58.26%) flies were females, while the remaining 298(41.73%) flies were males (Table3). The apparent density of each Glossina species was (1.8 f/t/d), (1.6 f/t/d), (0.8 f/t/d), and (0.4 f/t/d) for G.*fuscipes*, G.*pallidipes*, G.*morsitans*and G. *tachinoides*, respectively. (Table 4).Overall, the highest (10.72 f/t/d) and lowest (0.58 f/t/d) tsetse fly densities were recorded in Bena 2 and Wachale, respectively. Apparent tsetse density in different Villages indicated in Table 3.Apparent density of each Glossina species indicated Table 4.

Table 4: Apparent density of each Glossina species.

	Dade B.	Wacale	Bena 1	Bena 2	Metti.K	Hanna. I	Total	f/t/d
No. traps	20	19	10	10	9	10	78	
Gp		2	105	70	76	1	253	**1.6**
Gm	1			96	34		132	**0.8**
Gf	193	13	44	24	1		275	**1.76**
Gt	36	7	8				54	**0.4**
Total	230	22	157	193	111	1	714	**4.6**
f/t/d	**5.8**	**0.58**	**7.85**	**10.72**	**5.5**	**0.05**	**4.6**	**4.6**

Gm=Glossina morsitans sub morsitans, Gp=Glossina pallidipes, Gt=Glossina tachinoides, Gf= Glossina fuscipes.

Prevalence according to Age, Sex, and Body conditions

The prevalence of trypanosomosis was higher in female (9.1%) animals than in male (7.9%) animals. However, the difference was not statistically significant (P> 0.05). The highest prevalence was observed in old animals greater than five years old (11.0%), and the variation in prevalence between the different age groups was not statistically significant (P> 0.05). Statistically significant variation was observed in the prevalence of trypanosomosis (P<0.05) among those animals with different body conditions, and the highest prevalence was observed in the animals with poor body conditions (11.5%) Prevalence according to host risk factor indicated in Table 5.

Table 5: Prevalence according to host risk factor

Variable	No Of Animal	Positive	Prevalence	x^2 (P Value)
Sex				
Male	253	20	7.9%	0.29(0.59)
Female	361	33	9.1%	
Age				
<2 year	117	10	8.5%	1.99(0.37)
2 ≤ 5 year	315	23	7.3%	
>5 year	182	20	11.0%	
Body Condition				
Good	94	40	11.5%	8.24(0.016)
Medium	171	8	4.7%	
Poor	349	5	5.3%	

Discussion

The overall Trypanosomosis prevalence in Darimu district was 8.6%, and the present study finding was lower relative to previous research work in study area reports, such as the average seasonal incidence of trypanosomes by [20] 21.66%, 10%, 13.79% and 17.24% during the late rainy, dry, early and wet seasons, respectively, for theBirbir and Barovalies of Ethiopia. Again,a higher prevalence of 12.41% was recorded in other parts of Ethiopia by [21%] in the Metekel and Awi zones of northwest Ethiopia, 23.0% in Daremello district, southwestern Ethiopia by[22] and 7.1% in selected Villages of Darimu district[23], and 9.1% and 15.1% in MadaTalila and Gudina WachoKebeles of HewaGelan, Western Ethiopia[24].

Thus, the study revealed that the result was lower than the average seasonal incidence of trypanosomes reported by [18], which was 21.66, 10, 13.79 and 17.24% during the late rainy, dry, early and wet seasons, respectively, for theBirbir and Barovalies of Ethiopia.

The lower prevalence of trypanosomosis in this study might be related to low tsetse distribution and low fly–animal contact in the area due to the ongoing parasite and vector control programs practiced in the area by NTTICC. However, the results indicate that trypanosomes are still of much concern and represent a major obstacle to livestock production in the study area.

T.congolense was the predominant species (71.69%) in the study areas compared to other species of trypanosomes. This is in agreement with the previous results of [18] for Birbir and Baro valies of Ethiopia (65.6%); [24] Frat Adanhegn Village 62.5%; [21] In selected Darimu district Village 82.61%. [25] in the Ghibe valley, southwestern Ethiopia, 84% also showed higher results for *T.congolense*. It was found that in most cases, the prevalence of *T. congolense* in cattle was higher than that in*T. vivax* when specific tsetse areas were considered separately because investigations were sometimes made after the cattle were treated with trypanocidal drugs such as diminazeneaceturate. After such treatments,*T. congolense* predominates over *T. v*ivax in prevalence [26].

According to Getachew [27,]*T. congolense* and *T.vivax* are the most prevalent trypanosomes that infect cattle in tsetse-infested and tsetse-free areas of Ethiopia, respectively. These results suggest that the major cyclical vectors or Glossina species are more efficient transmitters of *T. congolense* than *T. vivax* in east Africa[28] Host's reaction to *T. vivax* may be more adverse than to *T. congolense* because *T. congolense* is more virulent to cattle than *T. v*ivax [21].

The prevalence of bovine trypanosomosis was studied according to the sex of cattle, and significant variation was not observed (P > 0.05). This might be because of an equal chance of exposure to the parasite. This result is in agreement with previous studies[21,29,30] [31.] In the present study, sex was not found to be a risk factor. This might be because both sexes have virtually similar exposure to flies in grazing areas. There was a higher prevalence of the disease (11.0%) in older animals >5 years old than in younger animals (8.5% less than 2 years old and adults (7.3% 2≤5 years old). The difference observed in the prevalence of trypanosomosis among the age groups was not statistically significant (P>0.05). In addition, [31] in the Ghibe valley indicated that suckling calves were not allowed to go out with their dams until they were weaned off. Young animals are also naturally protected to some extent by maternal antibodies [32] [33]. This could result in a low prevalence of trypanosomes that was observed in calves

Trypanosome infection in animals with poor body conditions was significantly higher (P<0.05) than that in animals with good body conditions. On theone hand, the disease itself results in progressive emaciation of the infected animals; nevertheless, on the other hand, non infected animals under good body conditions have good immune status and can respond to any foreign protein better than non infected cattle with poor body conditions, which can be immune compromised due to other diseases or malnutrition, since malnutrition and concurrent

infections depress immune responsiveness in some cases [33].

The mean PCV value of the infected animals was found to be significantly lower (21.4%)than the mean PCV value of non infected animals (23.5%). This result is similar to the results obtained by [33-35] Taking a PCV value of 24 to 46% as normal for zebu cattle,[38] 73.6% of the parasitemic and 57.2% aparasitemic animals registered PCV values less than 24%. These factors are likely risks for both parasitaemic and non parasitaemic animals. Therefore, the difference in the mean PCV value between parasitemic and aparasitemic animals indicates that trypanosomosis is involved in reducing the PCV values in the infected animals. This suggests that even though anemia is characteristic of trypanosomosis, other factors can also cause reduced PCV, yet some trypanosome-infected animals can also keep their PCV within the normal range for a certain period of time. Therefore, while diagnosing trypanosomosis on the basis of PCV, one should take various anaemia-causing agents into consideration.

Conclusions and Recommendations

The study revealed that *T. congolense and T. vivax* were the prevailing species of trypanosomes in the study area. In relation to host risk factors, the prevalence of bovine trypanosomosis was highest in animals with poor body condition. Finally, bovine trypanosomosis is an important disease and a potential threat affecting the health and productivity of cattle in the district. Hence, necessary attention should be given to this disease to improve livestock production and agricultural development in the area.

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