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Research Article

Study on Prevalence of Bovine Trypanosomosis and Apparent Density of Tsetse Fly in Borecha Woreda, South-Western Ethiopia

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Abstract

Background: Animal trypanosomiasis is an economically significant disease that affects the livestock industry in Ethiopia. However, national estimates of the disease prevalence in livestock and tsetse flies are lacking, therefore a cross-sectional study aimed at determining the prevalence of bovine trypanosomosis and assessing the apparent density of tsetse flies was conducted from November to December 2019 in Borecha Woreda, South-Western Ethiopia.

Methodology: Blood samples collected from 384 randomly selected cattle and subjected to parasitological and hematological analysis. The Packed Cell Volume (PCV) value of each animal was also measured using a hematocrit reader. Standard isolation and identification procedures were performed to identify trypanosome isolates. Baited different types of traps were used for the vector survey.

Results: A total of 278 tsetse flies were collected. Only one species, namely, G. Tachnoides, was recorded from the area. The overall prevalence of trypanosomes was 5.5%. The most common trypanosome species identified were Trypanosoma congolense (57.1%). The prevalence of trypanosomes infection was not statistically significant ($p>0.05$) between Sex and Age groups. However, a statistically significant difference ($p<0.05$) was observed in the prevalence of trypanosomes with different body-conditioned animals with higher infection rates being recorded in poorly conditioned animals (16.6%). There was a statistically significant difference ($p<0.05$) in the PCV values of infected and noninfected animals.

Conclusion: In general, trypanosomosis and its vectors were found to be a great treat in cattle production activities in the study area and hence, appropriate control and prevention strategies need to be implemented in the area.

Keywords: Trypanosomosis; Tsetse fly; Livestock; Prevalence

Background

Bovine trypanosomosis is a disease that affects cattle resulting from infection with protozoa of the genus Trypanosoma transmitted primarily by the tsetse fly and by another hematophagous fly [1]. The epidemiology of this disease is determined mainly by the ecology of the tsetse flies (genus *Glossina*), which are restricted to Africa from latitude 15ºN to 29ºS. The three main species that inhabit relatively distinct environments are: *G. morsitans* usually found in savanna country, *G. palpalis* prefers areas around rivers and lakes, and *G. fusca* lives in high forest areas. All three species transmit trypanosomes, and all feed on various mammals [2]. *T vivax, T congoiense, T bruceibrucei,* and *T simiae* are the four main species responsible for African trypanosomoses, affecting virtually all domestic mammals. *T vivax* and *T congoiense* are the main pathogens of cattle [3].

African Animal Trypanosomiasis (AAT) has long been recognized as an important constraint to livestock production and a threat to food security in sub-Saharan Africa [4]. The production losses in cattle due to trypanosome infections have been estimated to be up to 20% across a range of parameters, including mortality, calving rate, draft power, meat, and milk production. A high tsetse trypanosome burden constrains the use of land for livestock production, with farmers in these areas often being more reliant on crop farming. However, trypanosomiasis also compromises crop production by reducing the availability of draft animals to plow fields and provide manure for fertilizer [5]. As a result, the agriculture revolution which is a key element in the fight against poverty and the improvement of food security in developing countries failed in tsetse infested areas of sub- Saharan Africa [6].

The disease infects various species of mammals, however, from an economic point of view, tsetse-transmitted trypanosomosis is particularly important in cattle [7]. The parasite is transmitted biologically by the tsetse fly (Glossina species) and infects animals over an area known as the 'tsetse belt', which extends approximately 10 million km2 across 37 countries in Africa, from the Sahara Desert in the North to South Africa in the south [8]. Being one of the developing countries in sub-Saharan Africa and as per different studies undertaken in the country, Ethiopia also shares the facts described

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above. According to [9], tsetse transmitted animal trypanosomosis remains as one of the largest causes of livestock production losses in Ethiopia.

In Ethiopia, trypanosomosis is one of the most important diseases that limit livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of the southwest and northwest part of the country following the greater river basins of Abay, Omo, Ghibe, and Baro [10]. Currently, about 220,000km2 areas of the abovementioned regions are infested with five species of tsetse flies, namely, Glossina pallidipes, *G. morsitans, G. fuscipes, G. tachinoides,* and *G. longipennis* [10]. More than 10 million heads of cattle in Ethiopia are at risk of variable degrees of trypanosomosis at any time of the year, of which six million are tsetse borne [11]. Therefore, the main objective of this study is to determine the prevalence of trypanosomosis and the apparent tsetse fly density in the study area.

Materials and Methods

Study area

The study was conducted in 5 peasant associations (Beleka, Chulu, Mekefo, Goljo and Natero) of Borecha woreda. Borecha is found in Buno Bedele zone, Oromia regional state, southwest Ethiopia, at a distance of 507km from Addis Ababa. Geographically, it is located between 70 9' - 80 15' North latitude and 370 5'-400 00' East Longitudes, with an altitude range of 1392-2580 m.

Study design: A cross-sectional study was implemented to determine the prevalence of bovine trypanosomosis and the apparent density of tsetse flies in the area.

Study animals: The target populations were local breeds of cattle of all age groups and sexes found in each study site.

Sampling methods and sample size determination: The sample size was determined by using the standard formula for sample size determination described [12].

$$N = \frac{1.96^2 \text{ x } P_{exp}\left(1-P_{exp}\right)}{d^2}$$

Where N= sample size, Pexp= expected prevalence, d= desired absolute precision.

As there were no published studies reported in this area, the sample size was determined based on the expected prevalence of 50%, a confidence level of 95%, and 5% desired absolute precision. As a result, a total of 384 cattle were sampled from 5 different locations (villages) in the district. Systematic random sampling was carried out to examine animals for baseline parasitological surveys. To do this, owners were told to bring their animals to the sampling site for free pour-on treatment. This time the animals were systematically selected by drawing numbers on the cards distributed to the owners. These cards bearing numbers were distributed to owners on arrival to the sampling site and each owner receives a card assigned for each animal brought. During sampling, sex, age, and body condition of the animals were recorded. The age of the animals was grouped as young (<3 years) and adults (>3 years) according to the classification used by [13]. Whereas, the body condition score was categorized as poor, medium, and good, taking the middle point as a border in the 9 scale scores of as stated by [14], For the entomological survey, an abundance of tsetse flies frequency of trypanosomosis cases, altitude category, vegetation type, cattle grazing land and watering points were taken as criteria to determine the ideal habitat of tsetse flies for study site selection and 3 PAs (Beleka, Markefo AndGoljo) were selected.

Study Methodology and Procedures

Buffy coat technique and measuring of Packed Cell Volume (PCV)

Blood samples were collected from marginal ear veins using a micro-hematocrit capillary tube and sealed on one side with cristaseal. The capillary tube was then transferred to a hematocrit centrifuge and spun for 5 min at 1200 revolutions per minute. The centrifuged capillary tube was measured for PCV values on the hematocrit reader. Animals with PCV <24% were considered anemic. It was then cut 1mm below the buffy coat and the contents of the tube expressed on to a slide, mixed and covered with a 22 mm coverslip. This slide was then examined under 40 objective using phase contrast or dark field microscopy to examine for the presence/absence of motile trypanosomes [2,15].

Entomological survey: A total of 54 monoclonal traps were deployed. Traps were set at approximately 100-200 m apart. All trap positions were geo-referenced (using hand-held GPS, Garmin 48), and the altitude and vegetation type recorded. It was attempted to include different vegetation types such as Bush Land (BUL), Wooded Grassland (WGL), and Cultivated Land (CUL) for trapping. Collection of trapped flies occurred 48 h after deployment, tsetse flies in the cages were counted and identified based on their habitat and morphology to the genus and species level. Tsetse flies were sexed just by observing the posterior end of the ventral aspect of the abdomen using a hand lens. Male flies were identified by their enlarged hypopygium in the posterior ventral end of the abdomen. The apparent density of the tsetse fly was calculated as the number of tsetse catches/traps/day [4,14].

Data analysis

During the data analysis, the row data obtained from parasitological and entomological examination results were inserted into Micro-Soft excel spreadsheets to create the database and transferred to Statistical Package for Social Science (SPSS) version 20.0 software program for data analysis. Descriptive statistics was used to determine the prevalence of trypanosomosis in cattle and Chi-square test (χ^2) was used to assess the associated trypanosome infection and risk factors or variables (age, sex, body condition, and origin/peasant association). Student t-tests were used to compare the mean PCV of the study animals. In all analyses, the Confidence Interval Level (CI) was 95% and P-value <0.05 was considered as the significance.

Results

Parasitological findings

Out of 384 cattle examined, 21(5.5%) were found to be infected with trypanosomes. On Peasant Association (PAs) basis, the highest prevalence (7.9%) was recorded in Markefo, but there was no significant difference (P.0.05) among the selected PAs (Table 1). With regard to the prevalence in terms of trypanosome, the prevalent species was *T. congolense*, (2.9%) followed by *T. vivax*, (1.6%) and

Table 1: Prevalence of trypanosomosis in the study PAs.

PAs	Examination result		Total	prevalence	X^2	P-value
	Negative	Positive				
Beleka	73	4	77	5.19%	1.27	0.87
Chulu	74	3	77	3.90%		
Goljo	73	4	77	5.19%		
Markefo	70	6	76	7.90%		
Natero	73	4	77	5.19%		
Total	363	21	384	5.50%		

Table 2: Distribution of parasite species in the study area.

Parasite species	Frequency (N=21)	Percent
T.brucei	2	0.5
T.congolenc	11	2.9
T.congolenceT.vivax	2	0.5
T.vivax	6	1.6
Total	21	5.5

T. brucei (0.5%). The proportion of trypanosome species was 52.3% (11/21) *T. congolense*, 28.6% (6/21) *T. vivax*, 9.5% (2/21) *T. brucei* and the rest 9.5% was mixed (*T. congolence* and *T. vivax*) (Table 2).

Prevalence of trypanosomosis according to age, sex, and body condition

The prevalence of trypanosomosis was only slightly higher in females (5.88%) as compared to male animals. However, the difference was not statistically significant ($P>0.05$) (5.19%) (Table 3). Regarding the age groups, higher prevalence was observed in adult animals greater than 3 years old and the variations in prevalence between the different age groups were also not statistically significant ($P>0.05$) (Table 4). The prevalence of trypanosomosis among different body condition scores was found to be statistically significant ($P<0.05$) and the highest prevalence was in recorded poor conditioned animals, followed by good and medium body conditioned animals with 16.6%, 7.6% and 2.7% prevalence respectively as indicated in (Table 3). The mean PCV value of the infected animals was considerably lower (22.62±3.074 %) compared to noninfected animals (24.73±3.317 %). There was a statistically significant difference ($P<0.05$) in the PCV values of infected and non-infected animals (Table 4).

The entomological survey result of this study revealed that a total of 278 tsetse flies was caught by deploying 54 mono pyramidal traps that were collected after 48 hours of deployment bringing the apparent density of 2.6 fly/trap/day. Only one Glossina species namely Glossina tachinoides was encountered in the current study (Table 5).

Discussion

According to the present study, the overall prevalence of bovine trypanosomiasis in the study area was found to be 5.5 %. This result was higher than the reports by different authors from another tsetse belt areas of the country (Ethiopia) such as [13,16,17] who reported a prevalence of 4.43%, 2.10% and 4.25% from Arbamich Area, Amhara region Northwest, Ethiopia and Ilubabor Zone, Southwestern Ethiopia, receptively. In contrast to this, very higher than the present reports like 27.5% and 23% were reported by [18,19] in Arba Minch, Southern Ethiopia, and western Ethiopia, Metekel district, respectively. The variation among these reports might be due to the difference in the management system and disease control, agricultural activities in the study areas, the season of the study period, the development of drug resistance, which suggests the requirement of integrated research work to identify problems to enhance the effectiveness prevention and control approaches of trypanosomosis challenges in this study area in particular and at a country level in general.

The current research work result has shown that out of 21 animals infected by trypanosomes, the proportion of the trypanosome species was, 52.3% (11/21) *T. congolense*, 28.6% (6/21) *T. vivax*, 9.5%(2/21) *T. brucei* and the rest 9.5% was mixed (*T.congolence+T.vivax*) (Table 2), indicating that the predominant species causing bovine trypanosomosis was T. congolense and this finding agreed with previous reports in Ethiopia reported by different authors [16,20,21]. The reason for the predominance of *T. congolonse* infection on cattle maybe due to the high number serdomes of *T. congolonse* as compared to *T. vivax* and the development of a better immune response to *T. vivax* by the infected animal as stated by [22,23].

The prevalence of trypanosomosis was only slightly higher in females (5.88%) as compared to male animals. However, the difference was not statistically significant ($P>0.05$) (5.19%) (Table 3). Suggesting that sex may not be an important risk factor. Nearly similar results

Table 3: Prevalence of Trpanosomosis on different factors.

Factors		Diagnostic result		Total	Prevalence (%)	X^2	P-value
		Positive	Negative				
Sex	Male	219	12	231	5.19	0.08	0.77
	Female	144	9	153	5.88		
	Total	363	21	384	5.5		
Age	<3 year	69	3	72	4.16	0.29	0.59
	≥3 year	294	18	312	5.76		
	Total	363	21	384	5.5		
Body condition	Poor	53	11	64	16.6	21.54	≤0.001
	Medium	286	8	294	2.7		
	Poor	24	2	26	7.6		
	Total	363	21	384	5.5		

Table 4: The mean PCV value of parasitemic and aparasitemic animals.

Examination Result	No	Mean	SD	SE	95% CI	T-test	P-Value
Negative	363	24.73	3.32	0.174	.65-3.57	2.842	0.005
Positive	21	22.62	3.07	0.671	.67-3.54		

Table 5: Entomological survey results.

PAs	No of traps	No of fly	Species	Sex		Total FTD
				Female	Male	
Beleka	18	91	*G. tachinoides*	66	25	
Goljo	18	88	*G.tachinoides*	71	17	2.6
Markefo	18	99	*G. tachinoides*	77	22	
Total	54	278		214	64	

between sexes in this study may be due to similar exposure of the groups. This result was in agreement with the previous researches reported by [24-26].

Regarding the age groups, higher prevalence was observed in adult animals greater than 3 years old, with a prevalence of 5.76% and 4.16% for greater than and less than 3 years old animals respectively, but the variation in prevalence between the two age groups was not statistically significant (P>0.05) (Table 4). This finding is similar to the result of [27]. This may be due to more exposure of adult animals to vectors of trypanosomes.

The prevalence of trypanosomosis among different body condition scores were also evaluated and found to be statistically significant ($p<0.05$) and the highest prevalence was in recorded poor conditioned animals, followed by good and medium body conditioned animals with 16.6%, 7.6% and 2.7% prevalence respectively as indicated in (Table 5). This result was in agreement with other reports such as [12,28,29]. The reason behind this is the finding maybe due to the reduced performance of the animals as a result of lack of essential nutrients and poor management by the animal owner or the observed emaciation and weight loss might be caused by the disease itself as trypanosomosis is a chronic disease according to [1], on the other hand, the variation between the medium and good body condition could be due to the large sample size grouped under medium body condition.

The mean PCV value of the infected animals was considerably lower (22.62±3.074 %) compared to noninfected animals (24.73±3.317 %). There was a statistically significant difference (P<0.05) in the PCV values of infected and noninfected animals (Table 3). Similar to the finding were reported by different authors such as Tewelde [30] in western Ethiopia, Desta [6] in upper Dedesa valley of Ethiopia and [13] in the Amhara region, Northwest Ethiopia. Therefore, in this study, it was demonstrated that PCV could be considered as a good indicator of trypanosomal infection in the absence of other anemia causing diseases as it has also been stated by other authors [31]. The aparasitemic cattle with PCV<24% in the current study might be either due to the low sensitivity of diagnostic (buffy coat) techniques employed in chronic cases of trypanosomiasis or could be due to other factors like poor nutrition and other diseases particularly parasitic diseases which cause anemia [15,32].

The entomological survey results of this study revealed that a total of 278 tsetse flies was caught by deploying 54 mono-pyramidal traps that were collected after 48 hours of deployment, bringing the apparent density of 2.6 flies/trap/day. Only one Glossina species namely *Glossina tachinoides* was encountered in the current study. The negative result of the present study for dominant savanna species such as *Glossina morsitans* And *Glossina pallidipes* which are frequently reported in previous studies performed following the Dedesa river basin and other parts of the country might be due to excessive expansion of agricultural activities and the accompanied clearing of areas that were assumed to ideal habitats for these Glossina species. Of the total trapped flies, 77% (214/278) were females in (Table 5). This finding is in agreement with Leak [24] reported that in an unbiased sample females would comprise 70- 80% of the mean populations. The higher the population of females may be attributed to the fact that they live longer lifespan [33].

Conclusion and Recommendation

The present study revealed that despite the ongoing vectors and disease control operations, tsetse is still existed in a considerable amount in the studied district and trypanosomosis remains an impediment to livestock health and production. Based on the above conclusion, the following recommendations are forwarded:

- Effective, strong, sustainable, and community-based prevention and control strategies of trypanosomosis need to be designed and implemented.
- Awareness creation in the community to enhance their understanding especially on uncontrolled use of anti trypanosomosis drugs which could be an obstacle to the operation of effective control and finally, eradication tsetse flies and make the land free for agriculture and livestock production.
- Further researches directed at the discovery of a better diagnostic techniques suitable for the local condition should be encouraged to effectively monitor the efficacy of the control measures being implemented.

Limitations of the Study

The isolated *trypanosome* species were not molecularly characterized due to the unavailability of these resources in the laboratory facility in our country.

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References

1. Urquhart G, Armour J, Duncan J, Dunn A, Jennings F. Veterinary Parasitology 2nd edition. Blackwell Publishing, Scotland. 2006.
2. Merck Veterinary Manual. Kahn and Line ed, (9th edition), Wiley Publishers, Merck and Co., Inc: White house station, USA. 2005; 18-35.
3. Radostitis OM, Gay CC, Hinch KW, Cliff PDC. Disease associated with Trypanosomes. In: Veterinary Medicine, Textbook of disease of cattle, horses, pigs and goats10th ed. Elsevier, UK. 2007; 1531-1554.
4. Pattec. A continental plan of action for the eradication of Tsetse and trypanosomosis. The OAU pathway for the PATTEC. Pan African Tsetse and trypanosomosis, Global Veterinaria, Eradication campaign. 2000.
5. Swallow BM. Impacts of trypanosomiasis on African agriculture. PAAT Technical and Scientific Series (FAO). 2000.

6. Desta M. Trypanosome infection rate of Glossina morsitans and trypanosomosis prevalence in cattle in upper Didessa valley western Ethiopia. Int J Curr Microbiol App Sci. 2014; 3: 378-388.

7. Meberate A, Menjeta B, Vreysen M, Bencha B, Woldyes G. The distribution and relative abundance of tsetse flies in the Southern Rift valley of Ethiopia. OAU/SCTRS publication. 2000; 120: 202-213.

8. Ilemobade A. Tsetse and trypanosomosis in Africa: The challenges, the opportunities. OnderstepoortJo of Vet Res. 2009; 76: 35-40.

9. Langridge WP. Tsetse and trypanosomosis survey of Ethiopia. Ministry of overseas development UK. 1976; 1-40.

10. Abebe G. Trypanosomosis in Ethiopia. Review Article. The Biological Society of Ethiopia. J bio Soci. 2005; 4: 75-121.

11. Lemecha HM. "Trypanosomosis research and control in Ethiopia: an overview," in Proceedings of the 8th Annual Conference of the Ethiopian Veterinary Association. 1994; 7-12.

12. Fayisa G, Mandefro A, Hailu B, Chala G, Alemayehu G. Epidemiological Status and Vector Identification of Bovine Trypanosomiosis in Didesa District of Oromia Regional State, Ethiopia. International Journal of Nutrition and Food Sciences. 2015; 4: 373-380.

13. Ayana M, Tesfaheywet Z, Getnet F. A cross-sectional study on the prevalence of bovine Trypanosomosis in Amhara region, Northwest Ethiopia. Livestock Research for Rural Development. 2012; 24.

14. Nicholson MJ, Butterworth MH. A guide to condition scoring of zebu cattle. ICCA, Addis Ababa, Ethiopia. 1986; 45.

15. Murray M, Murray PK, McIntyre WIM. An improved parasitological technique for the diagnosis of African trypanosomosis. Trans R Soc Trop Med Hyg. 1977; 71: 325-326.

16. Teka W, Terefe D, Wondimu A. Prevalence study of bovine trypanosomosis and tsetse density in selected villages of Arbaminch, Ethiopia, Journal of Veterinary Medicine and Animal Health. 2012; 4: 36-41.

17. Kumela L, Delesa D, Senbeta T, Mohamed K, Mulisa M. Prevalence of Bovine Trypanosomosis and Vector Distributions in Chewaka Settlement Area of Ilubabor Zone, Southwestern Ethiopia. Advances in Biological Research. 2016; 10: 71-76.

18. Abraham Z, Tesfaheywet Z. Prevalence of Bovine Trypanosomosis in Selected District of Arba Minch, SNNPR, Southern Ethiopia. Global Veterinaria. 2012; 8: 168-173.

19. Terzu D. Seasonal dynamics of tsetse and trypanosomosis in selected sites of Southern Nation, Nationalities and peoples Regional State, Ethiopia. Msc thesis, Addis Ababa University, Faculty of Veterinary Medicine, DebreZeit, Ethiopia. 2004.

20. Abebe G, Jobre Y. "Trypanosomiasis: a threat to cattle production in Ethiopia," Revue deMedecine Veterinaire. 1996; 147: 897-902.

21. Duguma R, Tasew S, Olani A, Damena D, Alemu D, Mulatu T, et al. Spatial distribution of Glossina sp. And Trypanosoma sp. in south-western Ethiopia. Parasit Vectors. 2015; 8: 430.

22. Leak SGA. Tsetse Biology and Ecology; their role in the epidemiology and control of trypanosomosis. CAB international Walling ford (UK). 1999.

23. MacLennan KJR. Tsetse transmitted trypanosomosis in relation to the rural ecology. Wld Animal Rev. 1970; 36: 69-92.

24. Adane M. Survey on the prevalence in and around Bahir Dar. DVM Thesis, Addis Ababa University, faculty of veterinary medicine, Deber Zeite, Ethiopia. 1995.

25. Abebayehu T, Eset H, Berhanu M, Rahmeto A, Solomon M. "Mechanically transmitted bovine trypanosomosis in Tselamity wereda,Western Tigray, Northern Ethiopia," Agricultural Journal. 2011; 6: 10-13.

26. Tafese W, Melaku A, Fentahun T. "Prevalence of bovine trypanosomosis and its vectors in two districts of East Wollega zone, Ethiopia," The Onderstepoort Journal of Veterinary Research. 2012; 79: E1-E4.

27. Gemtessa T, Dera KL. Study on Prevalence of Bovine Trypanosomosis in Dale Wabera District, Kellam Wollega Zone, Western Ethiopia. Int J Anim Sci. 2017; 1: 1002.

28. Girma K, Meseret T, Tilahun Z, Haimanot D, Firew L. Prevalence of Bovine Trypanosomosis, its Vector Density and Distribution in and Around Arbaminch, Gamogofa Zone, Ethiopia. Acta Parasitological Globalis. 2014; 5: 169-176.

29. Tewelde N. Study on the occurrence of drug resistant trypanosomes in cattle in the farming in tsetse control areas FITCA Project in western Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia. 2001.

30. Marcotty T, Simukoko H, Berkvens D, Vercruysse J, Praet N, Van Den Bossche P. Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia, Preventive Veterinary Medicine. 2008; 87: 288-300.

31. Afework Y, Clause PH, Abebe G, Tilahuen G, Mehliz D. Multiple drug resistance trypanosome congolonsce population in village cattle of metekel district, North west Ethiopia. Acta Trop. 2000; 76: 231-238.

32. Lehane MJ. The Biology of Blood-Sucking in Insects, second edition, Liverpool School of Tropical Medicine, Cambridge University Press, The Edinburgh Building, Cambridge, UK. 2005.

33. IAEA, Workshop on Strategic Planning of Area Wide Tsetse and Trypanosomiasis Control in West Africa May, 21-24, 2001, Ouagadougou, Burkinafaso. 2002.