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Journal of Veterinary Medicine and Research

Research Article

Epidemiological Status and Vector Identification of Bovine Trypanosomosis in Lalo-Kile District of Kellem Wollega Zone, Western Ethiopia

Abebe Olani[1]* and Dagim Bekele[2]

[1]*Department of Parasitology, National Animal Health Diagnostic and Investigation Center, Ethiopia*

[2]*Livestock development and Fisheries, Lalo-Kile distrct, Kellem Wollega zone, Ethiopia*

***Corresponding author**

Abebe Olani, Department of Parasitology, National Animal health Diagnosis and Investigation Centre, Sebeta, Ethiopia, Tel: 251-913176665; Email: abebenaol@gmail.com

Submitted: 26 January 2016

Accepted: 26 April 2016

Published: 28 April 2016

ISSN: 2378-931X

Keywords

- Bovine
- Trypanosomosis
- Buffy coat
- Lalo-Kile and tsetse flies

Abstract

Across-sectional study was conducted from Nov, 2013 to May, 2014 to assess the prevalence of bovine trypanosomosis and apparent density of tsetse flies in seven peasant associations of Lalo-Kile district of Kellem wollega zone, Western Ethiopia. The overall 7.78% prevalence of bovine trypanosomosis was recorded from 836 blood sample collected from selected animals using Buffy coat method. *Trypanosoma congolense* was the dominant species 36 (55.38%), while the low infection was mixed infection of *Trypanosoma Congolense* and *trypanosome vivax* 2 (3.07%). The highest prevalence 36(17.64%) of the disease was recorded in Merfo peasant association while the lowset 1(0.7%) was recorded in Kutala-Lube association. The mean packed cell volume (PCV) was 21.95% and 24.47% in parasitamic and aparasitemic animals, respectively. There were statistically significant difference ($P<0.05$) in prevalence of the disease between sexes and higher prevalence rate 26(9.42%) and 48(8.75%) in poor body condition scores and with > 3 years ages, respectively. Overall an apparent density of the flies was 4.3 f/t/d by using Monopyramidal, Biconical and Ngu traps. It indicated that, *G. fuscipes fuscipes, G. pallidipes* and *G. morsitance submorsitance* were tsetse flies species caught. Generally, this survey shows that frequent despite control strategy is implemented; trypanosomosis is still a core problem for livestock production in the study area, therefore, integrated and sustainable vector control approaches should be practiced.

INTRODUCTION

Ethiopia has enormous livestock resource with a total contribution of 15% gross domestic product (GDP) and 33% to agriculture output. Currently estimate of Livestock population shows that there are 41.5 million heads of cattle, 41 millions of sheep and goat, 5.8 millions equine, 1 million camels and over 52 million poultry [1]. Despite the large animal population, productivity in Ethiopia is low and even below the average for most counties in eastern and Sub- Saharan Africa counties, due to poor nutrition, reproduction insufficiency, management constraints and prevailing animal diseases [2].

Trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. Trypanosomosis is a complex disease caused by unicellular parasite (Genus: Trypanosoma) found in the blood and other tissue of vertebrate including cattle (Livestock), wildlife and people [3]. The most important trypanosoma species in Ethiopia are *Trypanosoma congolense*, *Trypanosome vivax* and *Trypanosome brucei* in cattle, sheep and goat, *Trypanosoma evansi* in camels and *Trypanosoma equiperdium* in horses [4]. Tsetse transmitted animal trypanosomosis still remain as one of the largest cause of livestock production losses in Ethiopia.

Tsetse flies in Ethiopia are confined to South Western and North West region between a longitude 33° and 38° E and latitude of 5° and 12° N [4]. Five species of *Glossina* (*G.m.submorsitance, G.Pallidipes, G. tachinodies, G. fuscipes fuscipes* and *G. longipennies)* has been recorded in Ethiopia [5]. All species of Glossina transmite trypanosomes in various mammals and also biting flies may act as mechanical vectors, but their significant in Africa is still undefined [6]. Lalo-Kile district is potentially a productive

place for agricultural activity and raise live stock.

Unfortunately the area is infested with medium to high tsetse transmitted trypanosomosis. Therefore, the objective of the study was

- To determine the prevalence of bovine Trypanosomosis
- To identify vector species and their apparent density
- To assess the risk factors associated with the disease and collecting baseline data to control the vectors.

MATERIALS AND METHODS

Study Area

The study area is located in Oromia regional state, Kellem Wollega zone and lies at 035^0 15 to $035^0$26 E longitudes and $08^0$45 to $08^0$59 latitude and north of equator. Altitude of the area ranges from 500 to 1800 m.a.s.l. Distance of the district from zonal is 120 km. The climatology alternates with long summer rain fall (June- Sep), short rainy seasons (March-April) and winter dry seasons (December-February). The district has 32^0c maximum temperature and 15^0c minimum temperature and 1000mm to 1500mm Rain fall. The district has a total area of 40,382 hector and 97% Kola and 3% Woyena Dega agro-ecology. The study was conducted in 7 peasant associations (PAs), namely Amara Kucho, Merfo, Dera Jarso, Kutala Lube, Wayu Dibaba, Medale Kalisa and Bile Buba. There are river basins which flow throughout the year from the district to Birbir River system, namely Kile River, Buba River and Guluf River other seasonal rivers which are tributers of Kile and Birbir Rivers are also found. The different vegetation type which are found in the district, include *combratum Spp, pillistigama thonningi*, *Acacia Spp,* and *ficas sycomors*. Wild games like buffalos, Bush pig, Kudu, warthog, hippo, and crocodiles are the most commonly found in the study area. Agriculture is the main stay of livelihood of people with a mixed farming system and livestock plays an integral role for agriculture [7].

Study animals: Study animals were zebu cattle kept under extensive traditional husbandry condition. The animals graze the communally owned pasture land throughout the year. They are managed under the same agro-ecology without any additional supplementary feedings. In the study area human population is estimated to be 7,797 and the livestock population of bovine is 81723, equine 10159, Caprine 2134 and ovine 27378 and [7]. The study was conducted on 836 local breed cattle selected from seven peasant associations in the district. Of these animals, 109 were from Amhara-Kucho, 102 were from Merfo, 110 were Dera-Jarso, 104 were from Kutala- Lube, 178 were from Wayu-Dibaba, 140 were from Medale-Kalisa and 94 were from Bile-Buba. The origin, sex, age and body condition score of the animals were explanatory variables used to associate with prevalence rate.

Study Design: Cross-sectional study was conducted to determine the prevalence of bovine trypanosomosis and apparent density of vectors (tsetse population and other biting flies).

Sample size determination: The sampling method applied was simple random sampling. The sample size was calculated at 50% prevalence with the expected precision at 5% and at 95% confidence interval. The required sample size was 384 animals; however a total of 836 animals were sampled to increase the precision [8].

$$N = \frac{a^2_{exp}(1-p_{exp})}{d^2}$$

$$= \frac{(1.96)^2(0.5)(1-0.5)}{(0.05)^2}$$

Where

n = the required sample size

P = the expected prevalence

d = desired absolute precision

a = constant at 95% confidence level

Study methodology

Entomological Survey: For the entomological study, tsetse flies and other flies were collected from selected sites of the study area. The altitude levels, Peasant Associations, numbers of traps, tsetse species caught, other biting flies, days and vegetation types were recorded during the sampling period. The flies were caught with Monopryamidal, Biconical and Ngu traps baited with acetone, octenol and cow urine [9]. In the selected sites of the study area, about 80 baited traps were deployed at 200-250 meters interval at side of rivarian and woody grass land and kept in position for 48 hours. During trapping, acetone and octenol was dispensed from open vials through an approximately, 'O'-sized hole while cow urine from open bottles into which a quarter of tissue paper was used. All odors were placed on the ground about 30cm upwind of the trap. The underneath of each pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The coordinates of each trap position were recorded with a Global Positioning System (GPS). The different fly catches in each trap were counted and identified; the species of tsetse flies and other biting flies were identified based on their morphological characteristics such as size, color and wing venation structure [10].

Parasitological Survey: To determine the prevalence of bovine trypanosomosis, cross sectional parasitological survey was conducted. Blood sample was collected by puncturing of the marginal ear vein of each animals with a lancet and drawn directly in to heparinized capillary tube and centrifuged with capillary haematocrite centrifuge for 12000 rpm(revolution per minute) for five minute and examined for trypanosomes using the Buffy coat technique (BCT) [11]. The Packed Cell Volume (PCV) measurement of trypanosomes was done by the dark ground Buffy coat technique [12]. Positive were further processed for thin blood smear for confirmation of trypanosome species using their morphological characteristic [13] with Giemsa technique. A sample was considered positive for trypanosomosis when trypanosome was detected in the Buffy coat. Trypanosomes were usually found in or just above Buffy coat layer. So, capillary tube was cut using a diamond tipped pen 1mm below the red blood cells and 3mm above to include the plasma. The content of the capillary tube was expressed on to the slide, homogenized on to a clean glass and covered with a 22x22mm cover slip. The slide

was examined under 40x objective and low eye pieces for the movement of the parasite. The species were identified based on the characteristic morphology of trypanosome [13]

Data Management and Analysis: Data collected from vector fly and trypanosome infection survey was entered in to Ms excel spread sheet program to create data base. For the analysis of data statistical software program (SPSS 20.0) was used. Data collected on PCV values was analyzed by Independent Sample t-test to compare the mean PCV values of parasitaemic and a parasitaemic animal. In all cases differences between parameters were tested for significance at probability levels of 0.05. The risk factors like sex, age and body condition score were compared by using chi-square test. Prevalence (counting positive per total number of cattle examined) for trypanosomosis data and apparent density of tsetse flies were used to analyze flies trapped per trap per day.

RESULTS

Entomological survey

A total of 688 tsetse flies, 11 *tabanus* and 106 *Stomoxys* were caught from the seven selected peasant associations during study period. The overall apparent density of tsetse flies was 4.3 f/t/d. Three tsetse species have been identified. 379(55.08%) were *Glossina fucipes fuscipes*, 307(44.6%) were *Glossina pallidipes* and 2(0.29%) were *Glossina morsitance submorsitance*. From overall the study sites, the highest (9.4 f/t/d) and no tsetse catch in Bile-Buba and Kutala-Lube peasant associations, respectively. From total tsetse flies trapped females occupied larger proportion and out of 688 tsetse flies caught, 492(62.79%) flies were female while the rest 256(37.2%) were male as indicated in (Table 1). During this study 300(6.5 f/t/d), 287(3.99 f/t/d) and 101(2.53 f/t/d) tsetse flies were caught by Bioconical, Monopyramidal and Ngu traps, respectively (Table 2).

Parasitological Findings

The overall prevalence of bovine trypanosomosis in the study area was 7.78%. The prevalence of bovine trypanosomosis in each peasant association was determined to be 17.64% in Merfo, 16.36% in Dera-Jarso, 6.38% in Bile-Buba, 6.17% in Wayu-Dibaba, 5.5% in Amhara-Kucho and 0.7% in Kutala-lube. Among those seven peasant associations, Merfo peasant association showed the highest prevalence rate (17.64%) and the lowest being in Kutala-Lube (0.7%) as shown in (Table 3). *T.congolence* was dominant species with a proportion of 36(55.38%), followed by *T.vivax* 27(41.53%) and *T. congolence, T. vivax* mixed infection 2(0.7%). There was statistically significant difference (P<0.05) in prevalence of infection between sexes and higher prevalence rate of 9.42% and 8.72 % in poor body condition score and with >3 years of age, respectively (Table 5).

Hematological Findings

The mean PCV value for the parasitemic cattle was 21.95+6.3 SD while the mean PCV value for the aparasitaemic cattle was 24.47+4.6 SD. There was statistically significant difference (P<0.05) in mean PCV value between parasitaemic and aparasitaemic cattle.

DISCUSSION

The present study revealed that from a total of 836 randomly selected cattle's in the study area, 65 (7.78%) of the animal were positive for trypanosomes. Similar findings of 7.1% from Darimu district [14] and 6.77% from Quara district [15] were reported. But this is lower than previous report, 20.4% in Wolyta and Dawero Zone of Southern Ethiopia [16], 16.9% in Sayo, district, kellem Wollega, Western Ethiopia [17] and 29% prevalence in Gawo-Dale, West Oromia [18]. On other hand, the current study result was higher than 4.86% prevalence in Didesa distict, Oromia Region [19] and 4.43% from Arbaminch [20] were reported. This study shows that, *T.congolence* was dominant species with a proportion of 36 (55.38%), followed by *T.vivax* 27 (41.53%) and *T. congolence*, *T. vivax* mixed infection 2(0.7%). This results in agreement with the previous work in Sayo district of kellem Wollega, Western Ethiopia [17] that sated the predominance of *T.congolense* infection in cattle as compared to *T. vivax* and may due to the development of better immune response to *T. vivax* by infected animal. Moreover, the most prevalent trypanosome species in tsetse infested area of Ethiopia are *T. congolense* [21].

During the study period, the prevalence of bovine trypanosomosis was assessed between sexes, body condition scores and age of the animals and there is significant difference (P<0.05) between sexes. Among 65 trypanosome positive animals, 44(10.4%) of them were male animals and 21(5.22%) of them were female animals. The higher infection rate in male may be attributed to stress factors related to work where animals are

Table 1: Apparent density of flies in different PA's in Lalo-Kile district of Kellem -Wollega zone, western Ethiopia.

PA	No of trap	Tsetse Spp. caught								Biting flies	
		G.m submorsitance		*G.pallidipes*		*G.f fucipes*		Total	f/t/d*	*Tabanus*	*Stomoxys*
		M	F	M	F	M	F				
Amara-Kucho	10	0	0	1	1	0	0	2	0.1	2	10
Merfo	10	0	0	11	12	29	18	70	3.5	0	15
Dera-Jarso	10	2	0	34	51	0	0	87	4.35	5	35
Kutala-Lube	10	0	0	0	0	0	0	0	0	0	5
Wayu-Dibaba	10	0	0	0	1	0	0	1	0.05	0	2
Medale-Kalisa	10	0	0	53	91	3	5	152	7.6	0	26
Bile-Buba	20	0	0	12	40	204	120	376	9.4	4	13
Total	80	2	0	111	196	236	143	688	4.3	11	106

With* f/t/d fly per day per trap

Table 2: mean tsetse fly caches by three traps during the study period.

S/N	Trap type	No. Trap Deployed	*G.m.Submorsitance*		*G.pallidipes*		*G.f.fucipes*		Total	F/T/D
			M	F	M	F	M	F		
1	Biconical	24	0	0	55	60	131	54	300	6.5
2	Monopyramidal	36	2	0	41	72	96	76	287	3.99
3	NGU	20	0	0	15	64	9	13	101	2.53
	Total	80	0	0	111	196	236	143	688	4.3

With M male and F female

Table 3: Overall prevalence of Bovine trypanosomosis in different PA's of Lalo-Kile district of Kellem -Wollega zone, western Ethiopia.

PA	Number of animal examined	Infected animals	*Non* Infected animals	*Trypanosome spp.* identified				Prevalence (%)
				T.c	*T.v*	*T.b*	Mixed	
Amara-Kucho	109	6	103	1	5	0	0	5.5
Merfo	102	18	84	12	6	0	0	17.64
Dera -Jarso	110	18	92	13	5	0	0	16.36
Kutala -Lube	104	1	103	0	1	0	0	0.7
Wayu-Dibaba	178	11	167	6	5	0	0	6.17
Medale -Kalisa	140	6	134	2	3	0	1	4.86
Bile- Buba	94	6	88	2	2	0	1	6.38
Total	836	65	771	36	27	0	2	7.78

With *T.c Trypanosoma congolense, T.v Trypanosoma vivax* and mixed *Trypanosoma congolense, T.v Trypanosoma vivax*

Table 4: Distribution of Trypanosomosis species in different peasant associations.

Peasant association	*Trypanosome species* (n=65)			Total
	T.c	*T.v*	Mixed	
Merfo	12(18.48%)	6(9.2)	0(0.0)	18(27.6)
Dera-Jarso	13(20%)	5(7.6)	0(0.0)	18(27.6)
Bile-Buba	2(3.0)	2(3.0)	1(1.5)	5(7.6)
Wayu-Dibaba	6(9.2)	5(7.6)	0(0.0)	11(16.9)
Amhara-Kucho	1(1.5)	5(7.6)	0(0.0)	6(9.2)
Medale-Kalisa	2(3.0)	3(4.6)	1(1.5)	6(9.2)
Kutal-Lube	0(0.0)	1(1.5)	0(1.5)	1(1.5)
Total	36(55.38)	27(41.5)	2(3.0)	65(100)

With *T.c Trypanosoma congolense, T.v Trypanosoma vivax* and mixed *Trypanosoma congolense, T.v Trypanosoma vivax*

Table 5: Prevalence of Trypanosomosis in relation to sex, body condition score and Age of the animals.

Sex	Examined	Positive	*T.c*	*T.v*	*T.c+T.v*	Prevalence Rate %	x^2	P. value
Male	434	44	23	19	2	10.14	8.138	.004
Female	402	21	13	8	0	5.22		
Good	148	9	3	6	0	6.08	1.77	.412
Medium	412	30	20	8	2	7.28		
Poor	276	26	13	13	0	9.42		
<2 years	43	1	0	1	0	2.32	2.957	.228
2-3 years	243	16	10	5	1	6.58		
>3 years	550	48	26	21	1	8.7.2		
Total	836	65	36	27	2	7.8		

With *T.c Trypanosoma congolense, T.v Trypanosoma vivax and mixed Trypanosoma congolense, T.v Trypanosoma vivax*

used for drought purpose and they have to walk long distance in areas where there is a high risk of tsetse challenge.

In this study, the occurrence of the disease in their different body condition scores (Good, Medium and Poor) animals, shows that no statistical significance (P>0.05) variation. The prevalence of trypanosomosis in those animals with poor body condition was higher than those in good and medium body condition. Similar findings were reported in Abay (Blue Nile) base areas of Northwestern, Ethiopia [22] in Bure district, western Ethiopia [23]. On another hand disagreement with the study in Metekel and Awi zone of North West Ethiopia [24]. Obviously, the disease itself result in progressive emaciation of infected animals; never less, non infected animals under good condition have well developed better immune status that can respond to any foreign protein better than those non infected cattle with poor body condition which can be immune compromised due to other disease or malnutrition, since malnutrition and concurrent infections depress the immune responsiveness in some cases [25].

In this study, age wise analysis revealed that there was no significance difference (P>0.05) in prevalence between age groups. Higher infection rate 48 (8.72%) was observed in animals with >3 years of age and lower infection rate was observed in animals with <2 years of age. Similar result were reported [26,27]. This could be associated to the fact that older animals travel long distance for grazing and draught as well as harvesting crops in tsetse challenge areas [28], in Ghibe valley indicated that suckling calves don't go out with their dams but stay at home until they are weaned off. Besides, young animals are also naturally protected to some extent by maternal antibodies [29] and tsetse flies are attracted significantly by odor of large animals and animals that showed less defensive behavior according to [30,31].

The present study indicated that the difference between mean PCV values of parasitaemic and aparasitaemic cattle of the study area was significant (P<0.05). This result was in agreement with the previous work done in Sayo district [17] and in Awi zone, West Ethiopia [24]. The mean PCV value of parasitaemic animal's 21.95%+6.33 SD and aparasitaemic animals was 24.47%+4.56 SD and agreement with the report at Awi zone, North West Ethiopia [18]. The difference in mean PCV between parasitaemic and aparasitaemic animals indicated that, trypanosomosis involves in reducing the PCV values in infected animals. This result was also in agreement with previous report as anemia is the classical sign of the disease pathogen city, the low PCV in parasitaemic animal's could have contributed in reducing the mean PCV for cattle [32,33].

The risk of trypanosomosis is also influenced by apparent density and type of vector prevailing in the area. The overall apparent tsetse flies density 4.33 f/t/d was recorded during the study and similar report from Didesa district [19] and in Sayo district [17]. These findings lower than the previous report 14.97 f/t/d from Arbaminch [20] and 11.9 f/t/d from Hewa-Gelan district, Oromia region, west Ethiopia [34]. The relative low level of tsetse population in present study may be due to the intervention like deployment of insecticide impregnated targets and insecticide treated livestock under taken in the area by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC). The result also higher than the previous report 1.15f/t/d for tsetse in East Wollega zone [35], 2.83 f/t/d from Bench Maji zone [36] and 1.35 f/t/d in southern rift valley of Ethiopia [37].

Regarding the sexes, composition of the female tsetse flies constitute 62.79% and this was in agreement with females tsetse flies comprise 70.80% of the mean population from Southern Rift Valley of Ethiopia[38]. *Glossina fuscipes, G.pallidipes* and *G.m.sub morsitance* were the major species of tsetse flies caught, respectively.

CONCLUSION AND RECOMMENDATIONS

The present study indicated that Trypanosomosis is one of the most important constraints for livestock production in the area. Thus, strategic control of bovine Trypanosomosis including integrated and sustainable vector control should be strengthened to improve livestock production and agriculture development in the area.

ACKNOWLEDGMENTS

The Authors are grateful to the National Tsetse and Trypanosomosis Investigation and Control Center, Bedelle (NTTICC) for providing the required budget and logistics for this study. The cooperation's of Lalo-Kile district, Livestock development and Fisheries and cattle herd owners of the study area are highly acknowledged.

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Cite this article

Olani A, Bekele D (2016) Epidemiological Status and Vector Identification of Bovine Trypanosomosis in Lalo-Kile District of Kellem Wollega Zone, Western Ethiopia. J Vet Med Res 3(2): 1045.