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Epidemiological Investigation of Bovine Trypanosomosis and Its Vector Apparent Densities in Yayo District Illuababora Zone, Western Oromia, Ethiopia

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Abstract

The cross-sectional study was carried out from January to May, 2015 to assess prevalence of bovine trypanosomosis and apparent density of its vectors in Yayo district Iluababora zone of western Oromia. In current study 408 cattle were randomly selected for the study of bovine trypanosomosis prevalence in the district. Blood sample were collected from ear vein of cattle drawn into heparinized capillary tube then centrifuged and buffy coat techniques and thin blood smear were used to identify species of trypanosomes. This revealed that overall prevalence 7.4%, 95% CI: 5.1 - 10.0% with the highest rate of infection of *T. congolonse* (66.7%) than *T. vivax* 8(26.7%) and mixed infection 2(6.7%). Prevalence of trypanosomosis higher in female cattle 8%, 95% CI: 5.0 – 11.6%, old cattle 25%, 95% CI: 0.0 – 62.5% and poor body condition score cattle 9.3%, 95% CI: 3.7 – 14.8% and they were insignificantly associated with the bovine trypanosomosis prevalence. Bovine trypanosomosis prevalence was higher in aneamic 26(7.54%), poor body condition score 10(9.26%), female 21(8.11%) and old cattle 28(7.49%) and all the risk factors are insignificantly associated with trypanosomosis prevalence. The mean PCV of the infected cattle, male cattle and medium body condition score cattle were 17.767%, 95% CI: 15.96 – 19.58%, 20.274%, 95% CI: 19.78 – 20.77%, 20.09%, 95% CI: 19.53 – 20.65% lower than their perspective, respectively. In study 45 monopyramidal traps used to assess apparent density of tsetse flies and other biting flies in the district were *G. pallidipes* and *G. f. fuscipes* caught with overall apparent density of 142.41 FTD and other biting flies Tabanus, Stomoxys and Heamatopota. This indicates that how much tsetse flies and trypanosomosis challenge the livelihood of farmers. Therefore, chemoprophylaxis and prophylactics techniques and vector control techniques should be implemented.

Keywords: Prevalence; Risk factors; Bovine Trypanosomosis; Apparent densities; Yayo district

Introduction

Bovine trypanosomosis is disease that results in scarcity of animal production and productivity in sub-Saharan African countries which include western and southwestern parts of Ethiopia. It covers over 10 millions of square kilometers of potentially productive land [1,2]. Vector borne trypanosomosis is excluding some 180,000–200,000km^2 of agriculturally suitable land in the west and southwestern parts of the country; leaving 14 million of cattle, equivalent number of goat's and more than 7.5 million equines and 2.3 million of camels are at risk of contracting trypanosomosis [3].

Trypanosomosis is one of the diseases that are caused by flagellated protozoan parasites which belong to the genus *Trypanosoma*. *Trypanosoma* is a unicellular parasite found in the blood and other tissues of vertebrates including livestock, wild life and people [4]. The species of trypanosomes are known to exist in Ethiopia, which are pathogenic to cattle, are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*. They are distributed mainly in tsetse belt region of the Ethiopia. However, *T. vivax* is also found in areas outside of the tsetse belt, where it can possibly be transmitted by mechanical vectors of biting flies [5,6]. According to National Tsetse and Trypanosomosis Investigation and Control Center [1], tsetse transmitted animal trypanosomosis still remain as one of the largest cause of livestock production losses in Ethiopia. Trypanosomosis is characterisations are intermittent fever, anaemia, immunodepression, heart failure, oedemas and wasting. Methods used to reduce trypanosomiasis and its effects are use of drugs for curative and preventive and use of trypanotolerant cattle (Muturu and N'Dama). Economic impacts developed are direct losses (loss of production, mortality and abortion), cost of control (cost of drugs, transportation cost to field and operators salaries) and indirect losses (loss of potential production) [4,7].

There are 22 different species of tsetse flies inhabit Africa. These species are arranged into three groups based on their male genitalia construction. Groups are morsitans, palpalis and fusca. Studies interest for tsetse distribution are emanated from firstly tsetse belts continue from one country to the next, across the national boundary, secondly an expansion of a tsetse belt in one country may cause the belt to come up to and cross an international boundary and thirdly knowing the continental distribution of tsetse species gives a greater

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understanding of what stops the fly from spreading further, and whether a spread beyond the present limits in a particular country is likely. Distribution of morsitans group is limited by climatic (cold winter and hot dry) conditions and scarcity of game animals as well it is the most widespread group. However, palpalis group limited to very humid areas and fusca group limited to thickly forested areas. Tsetses are able to find their hosts by their sense of smell and by sight. Tsetse fly importance is its ability of spreading trypanosomosis among domestic animals and man. For this reason, very large areas of Africa are without cattle which are prevented economic development these areas. Tsetse infested areas lie in the lowlands and also in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe, and Omo in Ethiopia. Five species of *Glossina* (G. morsitans submorsitans, *G. pallidipes, G. tachinoides, G. fuscipes fuscipes* and *G. longipennis*) have been recorded in Ethiopia [1,4,5].

In Yayo district there is a paucity of information on the trypanosomosis and tsetse flies. Therefore, the aim of this study is to determine prevalence of bovine trypanosomosis and distribution of tsetse flies.

Methods and Materials

Study area, population and Sample size determination

The study was conducted from January to June, 2015 in Yayo district, Western Oromia, which is situated at 550km West of Addis Ababa in Ilu Aba Bora Zone. The mean annual rainfall in Yayo district ranges from 1000 to 1500 mm. The annual temperature ranges from 15 to 31°C. The altitude of the area ranges from 1,000 to 2060 meter above sea level (m.a.s.l). The Geba forest which is registered on the United Nations Educational, Scientific, and Cultural Organization (UNESCO) for its natural habitats is located in the study area. The area has a number of wild animals, such as African buffaloes, Bush pigs, warthog, bush buck, kudu, hippopotamus, crocodiles, hyena, antelopes and snakes which are claimed to serve as sources of food for the vector of trypanosomes. The cattle in the district are local breeds that are kept under traditional extensive husbandry systems with communal herding. Agriculture is the main livelihood of the society with mixed farming system and livestock play an integral role for agriculture. Currently, at the time of study the livestock populations of district were 60,000 cattle, 30,120 Sheep, 35,120 Goat, 5,232 Donkey, 4,693 Horse, 2,722 Mule and 80,000 Poultry. Sample size was determined using 95% confidence level, 50% expected prevalence and 0.05 desired absolute precision using the formula described by Thrusfield [8]. Therefore, a total of 408 cattle were randomly examined for bovine trypanosomosis to increase precision.

Study design and protocol

Yayo district was selected purposely based on the extent of the existing problems, the complaints of farmers and the level of medium to high tsetse challenge in the area from the report of the field veterinarian in the district. A cross-sectional study design was engaged and three peasant associations were selected based on the veterinary reports of the trypanosomosis and tsetse infestation in the district. Cattle body condition score was categorized as good, medium and poor and their age was categorized as young (< 3 years old), adult (3 to 9 years old) and old (> 9 years old) according to Nicholson and Butterworth [9].

Sample collection

Buffy coat technique was used immediately as samples collected for the determination of bovine trypanosomosis prevalence. Blood sample collection was performed by piercing the marginal ear vein with a sterile lancet and blood was drawn by a heparinized capillary tube. Then one end (the heparinized end) of capillary tubes were sealed with crystal sealant and centrifuged at 12,000rpm for five minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces. Then the packed cell volume (PCV) was determined by packed cell volume reader and recorded. The PCV value ≥ 25 and < 25 were considered as non-aneamic and aneamic, respectively. The capillary tubes were then broken just below buffy coat using diamond pencil and expressed on microscopic slide and covered with a cover slip. It was examined under 40× objective of microscope to identify and detect the presence of the parasites [10]. Those positive were thin smeared to be stain by geimsa staining for further identification into species using their morphological characteristics under oil immersion microscope in laboratory.

Entomological survey

For the entomological survey a total of 45 monopyramidal baited traps were deployed in three PA's of altitude ranges from 1237m - 1500m a.s.l. to assess the apparent density, distributions and species of tsetse flies and other biting flies involved in transmission of trypanosomosis. All traps were baited with acetone, Octenol (1-3-Octane) and cow urine filled in separated bottles and labeled and deployed at an interval of 200 to 250 m. After 72h of trap deployment, the cages were collected and captured flies were identified and sexed according to morphological characteristics, and counted. The tsetse flies were identified as species level and the other biting flies as the genus level. The apparent density was determined based on the mean catches of flies in traps deployed and expressed as the number of fly catch/trap/day [11].

Data management and analysis

Raw data were entered into a Microsoft Excel spreadsheet and descriptive statistics were used to summarize the data. The prevalence was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between the prevalence of trypanosome infection and risk factors were assessed by chi-square, whereas the student's *t*-test was used to assess the difference in mean PCV between trypanosome positive, negative and overall examined animals. All statistical analyses were conducted using SPSS version 20.0 software. The test result was considered significant when the calculated p-value was less than 0.05. The apparent density of 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 (FTD).

Result

Trypanosomosis survey

In current study 408 cattle were randomly selected for the study of bovine trypanosomosis prevalence in the district. Blood sample collected from ear vein of cattle drawn into heparinized capillary tube then centrifuged and buffy coat techniques used to detect the trypanosomes and those positive were thin blood smeared to identify

Table 1: Prevalence of *Trypanosoma* species with regard to PA's.

PA's	Sample size	Positive	Prevalence[95%CI]	*Trypanosoma* Species			
				Mixed	*T. congolence*	*T. vivax*	*T. brucei*
Webo	144	8	5.6[2.1, 9.2]	2(1.4%)	4(2.8%)	2(1.4%)	0
Gere	120	8	6.7[2.5,12.5]	0	5(4.2%)	3(2.5%)	0
Witete	144	14	9.7[4.9,15.1]	0	11(7.6%)	3(2.1%)	0
Over all	408	30	7.4[5.1,10.0]	2(0.5%)	20(4.9%)	8(2%)	0

PA= Peasant Association, CI= Confidence Interval, Mixed= *T. congolence* and *T. vivax.*

Table 2: Proportions of trypanosomes species.

Trypanosomes species	Prevalence
T.congolense	20(66.7%)
Mixed infection	2(6.7%)
T. vivax	8(26.7%)

Mixed infection= *T.congolense* and *T. vivax.*

Table 3: Prevalence of trypanosomosis based on risk factor.

Risk factor		Sample size	Positive	Prevalence[95%CI]	P-value
Sex	Male	149	9	6[2.0,10.1]	0.643
	Female	259	21	8[5.0,11.6]	
Age	Young	26	0	0	0.162
	Adult	374	28	7.5[5.1,10.2]	
	Old	8	2	25[0.0,62.5]	
BCS	Good	88	7	8[3.4,14.5]	0.436
	Medium	212	13	6.1[2.8,9.9]	
	Poor	108	10	9.3[3.7,14.8]	
PA's	Gere	120	8	6.7[2.5,12.5]	0.246
	Webo	144	8	5.6[2.1,9.2]	
	Witete	144	14	9.7[4.9,15.1]	

BCS=Body Condition Score; PA= Peasant Association; CI= Confidence Interval.

morphologically species of trypanosomes. This study revealed that overall prevalence 7.4%, 95% CI: 5.1 - 10.0% with the highest rate of infection of *T. congolonse* (66.7%) than *T.vivax* 8(26.7%) and mixed infection 2(6.7%) and none of *T. brucei* were recorded and trypanosomosis highly infested Witete peasant association (9.7%) than the rest (Table 1, Table 2). Prevalence of trypanosomosis higher in female cattle 8%, 95% CI: 5.0 – 11.6% than male cattle 6%, 95% CI: 2.0 – 10.1%; old cattle 25%, 95% CI: 0.0 – 62.5% than adult cattle 7.5%, 95% CI: 5.1 – 10.2%; poor body condition score cattle 9.3%, 95% CI: 3.7 – 14.8% than good body condition score cattle 8%, 95% CI: 3.4 – 14.5% and medium body condition score 6.1%, 95% CI: 2.8 – 9.9%; Witete peasant association 9.7%, 95% CI: 4.9 – 15.1% than Gere peasant association 6.7%, 95% CI: 2.5 – 12.5% and Webo peasant association 5.6%, 95% CI: 2.1 – 9.2% and all of them were insignificantly associated with the bovine trypanosomosis prevalence (Table 3).

Bovine trypanosomosis prevalence was higher in aneamic 26(7.54%) than non-aneamic 4(6.3%) cattle; cattle of poor body condition score 10(9.26%) was harbored more infection of trypanosomosis than good 7(7.95%) and medium 13(6.13%) body condition scores cattle; female cattle 21(8.11%) was more exposed to trypanosomosis than male cattle 9(6.04%), Witete peasant association 14(9.72%) was with highest infection of trypanosomosis than Webo 8(5.56%) and Gere 8(6.67%) peasant association; old cattle 28(7.49%) ported more infection than adult 28(7.49%) and all the risk factors are insignificantly associated with trypanosomosis prevalence (Table 5).

Heamatological survey

The mean PCV of the infected cattle 17.767%, 95% CI: 15.96 – 19.58% lower than non-infected cattle 20.542%, 95% CI: 20.14 – 20.95%; male cattle 20.274%, 95% CI: 19.78 – 20.77% slightly lower than female 20.450%, 95% CI:19.76 – 21.14%; medium body condition score cattle 20.09%, 95% CI: 19.53 – 20.65% lower than good and poor body condition score cattle 20.51%, 95% CI: 19.59 – 21.43% and 20.68%, 95% CI: 19.96 – 21.41%, respectively (Table 4).

Entomological survey

This study incorporated 45 monopyramidal traps to assess apparent density of tsetse flies and other biting flies in the district. Species of Tsetse flies caught in the study areas were *G. pallidipes* and *G. f. fuscipes* with overall apparent density of 142.41 FTD. Other biting flies Tabanus, Stomoxys and Heamatopota were trapped during the study period. All monopyramidal traps were trapped within the range of 1237m 1500m altitude above sea level. Webo peasant association with the range of altitude 1237m 1257m were mostly infested with tsetse flies than other two peasant associations (Table 6).

Discussion

The current study reveals that overall prevalence 7.4% in the study area. Similar findings ranges from 6.7% to 8.7% were reported in Dale Wabera and Dale sedi districts of Kellem Wollega zone, in southwestern Ethiopia and Guto Gida district of East Wollega zone [12-14]. However, prevalence ranges from 13.6% to 16.9% higher than the present finding were reported in Humbo district of southern Ethiopia, Chora district of Ilu Aba Bora zone and Sayonole district of western Oromia [15-17]. Even though, from Dale Wabera district of Kellem Wollega zone reported lower prevalence (2.86%) of trypanosomosis [18]. These differences were due to ecological differences and seasonal variations of study areas [19,20].

Species of trypanosomes predominant in the study area was *T. congolonse* (66.7%) followed by *T. vivax* 8(26.7%). This study agrees to previous report of Takile et al [14], Begna et al [15], Tola et al [16], Biyazen [18], Kassaye [17] and Kassaye and Tsegaye [12] that their prevalence ranges from 53.3% to 88.40%. Unlike to this finding *T. vivax* predominant in Cameroon and Nigeria [21,22]. This difference occurred because of geographical factors and *G. pallidipes* and *G. m. sub-morsitans* are efficiently transmits *T. congolense* than *T. vivax* in Eastern Africa. In addition, the predominance of *T. congolense* infection in cattle may be due to the high number of serodams of

Table 4: Association of PCV with the factors.

Risk factors	Sample size	Mean PCV	95%CI	Std.dev.	df	P-value	*t*-value
Trypanosomes infection							
Infected	30	17.767	[15.96,19.58]	4.847	29	0.00	20.076
Non-infected	378	20.542	[20.14,20.95]	3.982	377		100.299
Sex							
Male	259	20.274	[19.78,20.77]	4.012	258	0.00	81.319
Female	149	20.450	[19.76,21.14]	4.285	148		58.261
Body Condition scores							
Poor	108	20.685	[19.96,21.41]	3.825	107	0.00	56.204
Medium	212	20.090	[19.53,20.65]	4.156	211		70.374
Good	88	20.511	[19.59,21.43]	4.334	87		44.399

PCV = Packed Cell Volume; CI = Confidence Interval; Std.dev. = Standard deviation; df = Degree of freedom.

Table 5: Association of trypanosomosis prevalence with risk factors.

Risk factors		Sample size	Trypanosomosis prevalence			Total	X^2	P-value
			T. congolense	Mixed(*T. congolense* and *T. vivax*)	*T. vivax*			
PCV	Aneamic	345	16(4.6%)	2(0.6%)	8(2.3%)	26(7.54%)	2.153[a]	0.541
	Non-aneamic	63	4(6.3%)	0(0.0%)	0(0.0%)	4(6.3%)		
BCS	Good	88	4(4.5%)	1(1.1%)	2(2.3%)	7(7.95%)	5.889[a]	0.436
	Medium	212	7(3.3%)	1(0.5%)	5(2.4%)	13(6.13%)		
	Poor	108	9(8.3%)	0(0.0%)	1(0.9%)	10(9.26%)		
Sex	Female	259	13(5.0%)	2(0.8%)	6(2.3%)	21(8.11%)	1.672[a]	0.643
	Male	149	7(4.7%)	0(0.0%)	2(1.3%)	9(6.04%)		
PA	Gere	120	5(4.2%)	0(0.0%)	3(2.5%)	8(6.67%)	7.898[a]	0.246
	Webo	144	4(2.8%)	2(1.4%)	2(1.4%)	8(5.56%)		
	Witete	144	11(7.6%)	0(0.0%)	3(2.1%)	14(9.72%)		
Age	Adult	374	18(4.8%)	2(0.5%)	8(2.1%)	28(7.49%)	9.203[a]	0.162
	Old	8	2(2.5%)	0(0.0%)	0(0.0%)	2(25%)		
	Young	26	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)		

PCV = Packed Cell Volume; X^2= Chi-square; BCS= Body Condition Score; PA= Peasant Association; Aneamic = PCV < 25; Non-aneamic= PCV ≥ 25.

Table 6: Apparent densities of tsetse flies and other biting flies caught in three peasant associations.

PA's	Altitude	No. of trap	No. of days	*G. pallidipes*	*G. f. fuscipes*	Total	FTD	Tabanus	Stomoxys	Heamatopota
Webo	1237-1257m	15	3	56,533	96	56,629	1258.42	3	216	95
Gere	1372-1500m	15	3	100	92	192	4.26667	1	114	20
Witete	1300-1498m	15	3	566	289	855	19	2	204	119
District		45	9	57,199	477	57,676	142.41	6	534	234

PA= Peasant Association; FTD= Number of tsetse flies caught/no. of trap deployed/no. days.

cattle as compared to *T. vivax* and development of better immune response to *T. vivax* by the infected animal [11].

The prevalence of trypanosomosis was higher in the female cattle 8%, 95% CI: 5.0 – 11.6%, old 25%, 95% CI: 0.0 – 62.5%, cattle of poor body condition score 9.3%, 95% CI: 3.70 – 14.8% and Witete peasant association 9.7%, 95% CI: 4.9 – 15.1% than rest of factors and insignificantly associated (P > 0.05) to the prevalence of trypanosomosis. Similar with earlier report of Takile et al [14] in Guto Gida district female (8.37%), Poor (19.67%) and old (9.21%), Tola et al [16] in Chora district female (9.2%) and poor (5.2%), Kassaye [17] in Sayonole district poor (19.7%) and Kassaye and Tsegaye [12] in Dale sedi and Wabera Poor (15%). In contrary to this, Biyazen [18] and Kassaye and Tsegaye [12] determined male cattle harbor more infection than female cattle in Dale Wabera district male cattle (3.64%) and Dale sedi and Wabera district male cattle (10.7%), respectively. This could be due to female cattle were kept for purpose of rearing or giving offspring and milking for a long period of time and in numbers than male cattle used for ploughing purpose that lead in the continuous exposure of tsetse flies infestation. Old cattle stayed in the areas for long time that increases their exposure to the tsetse flies, but poor body condition score cattle deprived immunity to fight against infection of trypanosomosis.

Mean of packed cell volume (PCV) varies among risk factors. Trypanosomes infected cattle mean PCV 17.767%, 95% CI: 15.96 – 19.58% lower than non-infected cattle mean PCV 20.542%, 95% CI: 20.14 – 20. 95%. This could be due to aneamic nature of trypanosomosis and malnutrition. Mean PCV of male 20.274%, 95% CI: 19.78 – 20.77% lower than female cattle mean PCV 20.450%, 95% CI: 19.76 – 21.14%; medium body condition score PCV 20.090%, 95% CI: 19.53 – 20.65% lower than poor and good body condition score cattle 20.685%, 95% CI; 19.96 – 21.41% and 20.511%, 95% CI: 19.59 – 21.43%, respectively and all risk factors statistically associated ($P < 0.05$) with PCV. Analogous to the previous report of Takile et al [14] in Guto Gida (20.23%) district, Begna et al [15] in Humbo (20.2%) district, Tola et al [16] in Chora (19.02%) district, Kassaye [17] in Sayonole (20.86%) district, Kassaye and Tsegaye [12] in Dale Wabera (20.9%) and Dale sedi (20.3%) districts and [23] in western Oromia (22.88%). Difference in aneamia status in trypanosome infected cattle was probably due to discrepancy in ecology [20], nutritional status [24], animal breed [25], season of the study [19], tsetse control and trypanocidal drug utilization level [26] and/or diagnostic method employed [27,28].

Monopyramidal type of traps 45 in number was deployed in three peasant associations of Yayo district. These traps were trapped *G. pallidipes* and *G. f. fuscipes* with their overall apparent density of 142.41 flies/ traps/ days and other biting flies Tabanus, Stomoxys and Heamatopota within the range of 1237m - 1500m altitude above sea levels [29]. Hence, *G. pallidipes* was predominantly with the apparent density 141.23 flies/ traps/ days in the district. Other study reported apparent density in Dale Wabera and Dale sedi (4.8 flies/traps/days) districts [12], Sayonole (13.01 flies/trap/days) district [17] and Chora (2.63 flies/ traps/days) district [16] which is lower than the current study. Hence, in Abaya district apparent density of *G. pallidipes* 0.5 flies/traps/days was the predominant in the district, but this apparent density is lower than the current study *G. pallidipes* apparent density. Generally, this indicates that higher rate of tsetse flies infestation in the district which is more responsible for the trypanosomosis transmission.

Conclusion and Recommendation

Current study revealed that highest apparent density of tsetse flies that transmit trypanosomosis infection to cattle which severely harm production and productivity of livestock in the district. In the study area *T. congolense* and *G. pallidipes* were predominantly occurred in the study area. Hence, the apparent density of tsetse flies in the district was abundant than other areas with similar geography. Female, old and poor cattle were incredible infected by trypanosomes species and those infected, medium body condition score and male cattle possessed a lower mean packed cell volume. Based on this the following recommendations are forwarded:

- Tsetse flies control techniques should be implemented.
- Chemotherapeutics and prophylactics techniques should be undertaken.

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