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Epidemiological Survey of Bovine Trypanosomosis in Sayo District of Kellem Wollega Zone, Western Ethiopia

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Abstract: A cross-sectional study was conducted from November 2013 to April 2014 to assess the prevalence of bovine trypanosomosis and apparent density of tsetse flies in four kebeles of Sayo district of KelemWollega zone, Ethiopia. The overall apparent densities of tsetse flies in each study areas were determined by using monopyramidal, biconical and ingu traps. It indicated that, *Glossina tachnoides, Glossina morsitons sub morsitons* and *Glossina pallidipes* were the tsetse flies species caught along with other biting flies. Overall 16.9% prevalence of bovine trypanosomosis was recorded among 585 blood samples collected from randomly selected animals using buffy coat method. The result revealed that, *Trypanosoma congolense* was the dominant species (69.7%), while the lowest infection was co-infection of *Trypanosome congolense* and *Trypanosome vivax* (2.0%). The highest prevalence 33 (5.6%) of the disease was recorded in Ripa while the lowest 13 (2.2%) was recorded in Minko-lencha Kebeles. The mean packed cell volumes (PCV) were 24% and 26% (95%) in parasitaemic and in aparasitaemic animals respectively. There was statistically significant difference ($p<0.05$) in prevalence of the disease among the different kebeles, body conditions scores and PCV values. The presence of different species and high densities of vectors in the studied areas along with a relatively higher prevalence of the disease in the area warrants the initiation of appropriate prevention and control measures.

Key words: Bovine · Epidemiology · Ethiopia · Prevalence · Trypanosomosis · Tsetse Fly

INTRODUCTION

African animals Trypanosomosis (AAT) is a disease complex caused by *Trypanosome congolense*, *Trypanosome vivax* and *Trypanosome brucei* or mixed transmitted principally by tsetse flies [1]. Trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. Currently about 3 million livestock die every year due to tsetse fly transmitted trypanosomosis which covers one third of the continent estimated to be 10 million km^2. A recent study estimated the direct annual cost of trypanosomosis to be about 1.34 billion US$. African livestock producers are administering an estimated 35 million curative and prophylactic treatments annually which costs the producers and the government at least 35 million US$ [2]. The direct losses from trypanosomosis in livestock include mortality, morbidity, impaired fertility and the cost of implementing and maintaining tsetse fly and trypanosomosis control operations. Indirect losses stem from farmers responses to the perceived risk of the disease, including the reduction and in some cases, the exclusion of livestock from tsetse-infested grazing lands and reduced crop production due to insufficient animal draught power.

The tsetse flies (*Glossina*) occur in 37 sub-Saharan countries including Ethiopia. The vector fly occupied approximately 220,000 km^2 of areas of fertile land in Ethiopia and about 23.15 million livestock populations were at risk to contact the disease. Trypanosomosis is one of the most significant and costly disease in Ethiopia hindering the effort made for food self-sufficiency [3]. Livestock sector plays a significant role for the economy and has a great potential to assist the economic development by providing meat, milk, other food products, cultivation power, transport, security in times of crop failure and farm yard manure (fertility and energy) and also plays a major role in export commodity [4].

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Basically there are five species of trypanosomes in Ethiopia and the most important trypanosomes in terms of economic loss in domestic livestock are the tsetse transmitted species, *Trypanosome congolense, Trypanosome vivax* and *Trypanosome brucei* [5]. Tsetse flies in Ethiopia are confined to southwestern and northwestern regions. Tsetse transmitted animal trypanosomosis still remain as one of the largest causes of livestock production losses in Ethiopia. Five species of *Glossina (G. m. submorsitans, G. pallidipes, G. tachinoides, G.f. fuscipes*and *G. longipennis*) have been recorded in Ethiopia [6].

Three elements influence the epidemiology of the disease, namely the distribution of the vectors, the virulence of the parasite (trypanosome) and response of the host. All three species of *Glossina* transmit trypanosomes in various mammals and also biting flies may act as mechanical vectors, but their significant in Africa is still undefined [7].

Sayo district is potentially a productive place for agricultural activities and raise livestock. Unfortunately, the area is infested with medium to high tsetse transmitted trypanosomosis. As a result, the people suffer from low level of draught power and productivity that is manifested by low level of meat, milk and other animal products that compromise the socio-economic and nutritional status of inhabitants. There is no any surveillance conducted previously in this area. Therefore, the objective of the study was to determining the prevalence of bovine trypanosomosis and characterizes the apparent density, distribution and species of tsetse flies in four selected Kebeles.

MATERIALS AND METHODS

Description of Study Area: The study was conducted in Sayo district which is located in Kellem Wollega zone, Oromia Regional state, Western Ethiopia. Dembi-Dollo is a capital (town) of Kellem Wollega zone and situated about 652 km West of Addis Ababa. The study site has a latitude 8°32'N and longitude 34°48'E with an elevation between 1701 and 1827 meters above sea level. It has a tropical climate and remains mostly hot and humid throughout the year. The area receives an average annual rainfall of 700 to 1100 mm. The zone is bordered by West Wollega zone to the north, Gambella regional state to the south, Illubabor zone to the east and Benishangul Gumuz regional state to the west.

Study Population: A total of 585 cattle were selected from indigenous cattle breed kept under extensive management system. The livestock populations that are found in Sayo district include cattle (78,492), sheep (54,159), goat (13,888), horses (4,637), mule (1,750), donkey (10,822) and poultry (70,282). Among these animals, cattle are the dominant species raised in the area. The total human population of this area is 35,065 [8].

Study Design and Sampling Method: A cross-sectional study design was conducted from November 2013 to April 2014 to determine the prevalence of bovine trypanosomosis and identify the major vector population by selecting the district and kebelles purposively. The study animals were selected by using simple random sampling method by taking age, sex and body condition into account. The subjects were selected randomly from the population up to the household-cattle level whereby all the animals in the selected areas had equal chances to be selected for this study [9].

Sample Size Determination: The sample size was calculated at 50% expected prevalence with the precision at 5% and at 95% confidence interval, since there was no previous study conducted in the study area. Accordingly, the required minimum sample size was 384 animals [9]. However, a total of 585 animals were sampled to increase the precision.

$$n = \frac{a^2 \text{ pexp } (1\text{-\_pexp})}{d^2}$$

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

where

n=the required sample size
Pexp=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, kebelles, 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 Ingu traps baited with acetone, octenol and cow urine [10]. In the selected sites of the study area, about 62 traps were deployed in the morning 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 odours were placed on the ground about 30cm upwind of the trap. The coordinates of each trap position were recorded with a Global Positioning System (GPS) and found in the range between 1300 to 1600 meters above sea level. 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 [11].

Sexing were done just by observing the posterior end of the ventral aspect of the abdomen by microscopic lenses as a result male flies were easily identified by enlarged hypophgeum in the posterior ventral part of the abdomen. Tsetse fly apparent density was determined as the mean catches in traps deployed, expressed as the number of tsetse catch/trap/day [12].

Parasitological Survey: The blood samples were collected from the ear vein of 585 cattle to determine the prevalence of trypanosomosis and PCV after recording the age, sex and body condition of animals. The randomly collected samples were then examined by the capillary microhematocrit centrifugation method to estimate the packed cell volume (PCV) and buffy coat result. Phase contrast microscope was used for the detection of trypanosomes in the blood. Species identification was done based on movement in wet films.

Blood was collected from an ear vein using heparinized micro–haematocrit capillary tube and the tube was sealed. A heparinized capillary tube containing blood was centrifuged for 5 min at 12,000 rpm. After the centrifugation, the capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto slide, homogenized onto a clean glass-slide and covered with cover-slip. The slide was examined under 40× objectives and 10× eye pieces for the movement of parasite and trypanosome species diagnosed was recorded.

Data Analysis: For the management, analysis and interpretation of data collected based on the study methodology, descriptive statics and SPSS statistical software were employed. The prevalence of trypanosomosis across different variables (kebeles, sex, age, *trypanosome species* and body conditions) were compared by using Chi-square test. The mean PCV values of parasitaemic and aparasitaemic animals were analyzed. In all cases differences between parameters was tested for significance at probability levels of less than 0.05.

RESULTS

Entomological Survey: A total of 558 tsetse flies, 625 Stomoxys and 1020 other biting flies were caught from the four selected Kebelles during study period (Table 1).

Table 1: Total flies caught during the study period from different kebelles

		Types of flies				
			Sex			
Kebeles	Altitude	Species	M	F	Total	fly/trap/day
Walgahi- bubuka	1384m	*G. Pallidipes*	21	44	65	9.63
		G. m.submorsitans	31	64	95	
		G. tachnoides	44	85	129	
		Stomoxys	-	-	291	9.7
		Other biting flies	-	-	317	10.57
Ripa	1486m	*G. pallidipes,*	11	16		3.2
		G.m. submorsitans	9	16		
		G. tachniodes	21	29		
		Stomoxys	-	-	111	3.5
		Other biting flies	-	-	279	8.7
Karro- baha	1437m	*G. pallidipes,*	8	22	30	3.34
		G.m. submorsitans	8	24	32	
		G. tachniodes	15	30	45	
		Stomoxys	-	-	145	4.5
		Other biting flies	-	-	263	8.2
Minko- lencha	1551m	*G. pallidipes,*	9	11	20	1.83
		G.m. submorsitans	5	7	12	
		G. tachniodes	8	15	23	
		Stomoxys	-	-	78	2.6
		Other biting flies	-	-	161	5.37

G. = Glossina, G.m. = Glossinam morsitons, M= Male, F = Female

Table 2: The mean fly catches by three traps types during the study period through F/t/d

		Mean fly catches/trap/day		
No. of Traps Deployed		*Tsetse flies*	*Stomoxys*	Other biting flies
Monopryamidal	(30)	394/30/2 = 6.57	402/30/2 = 6.7	515/30/2 = 8.58
Biconical	(16)	101/16/2 = 3.16	106/16/2 = 3.31	285/16/2 = 8.91
Ingu	(16)	63/16/2 = 1.97	117/16/2 = 3.66	220/16/2 = 6.875
Total	62	558/62/2 = 4.5	625/62/2 = 5.04	1020/62/2 = 8.22

F/t/d=fly- time- day

Table 3: Apparent densities of tsetse fly species (F/t/d) in different vegetation types

	Mean fly catches/trap/day		
Vegetation types	*G. Tachnoides*	*G.m.submorsitans*	*G. Pallidipes*
Riverine	1.99	-	1.15
Savanna	-	1.32	-

F/t/d= fly- time- day

Table 4: Apparent density of tsetse flies according to their sex

	Mean catches/trap/day			
Sex	*G. Tachnoides*	*G. M.submorsitan*	*G. Pallidipes*	Total
Male	0.71	0.43	0.36	1.5
Female	1.28	0.89	0.90	3.08

Table 5: Prevalence of Trypanosome infection and its univariate association with the different kebeles, age, sex and body conditions scores of the animals

				95% C.I.		
Variables	No. of examined animals	No. of positive n (%)	Odd ratio	Lower	Upper	P- value
Kebeles						
Ripa	153	33 (5.6)				
Karro- baha	159	27 (4.6)	0.20	0.083	0.46	0.00
Walgahi- bubuka	139	26 (4.5)	0.43	0.185	1.01	0.05
Minko- lencha	134	13 (2.2)	0.34	0.143	0.81	0.02
Age (years)						
≤ 3	151	23 (3.9)				
> 3	434	76 (13.0)	1.50	0.777	2.89	0.23
Sex						
Female	329	46 (7.9)				
Male	256	53 (9.0)	1.74	1.002	3.01	0.05
Body condition						
Poor	95	36 (6.1)				
Medium	386	62 (10.6)	0.09	0.011	0.73	0.02
Good	104	1 (0.2)	0.11	0.014	0.83	0.03

Table 6: Mean of PCV values in Aparasitimic and Parasitimic Animals

PCV values Aparasitimic	Parasitimic	Total
PCV = 24 58 (9.9%)	74 (12.6%)	132 (22.6%)
PCV > 24 428 (73.2%)	25 (4.3%)	453 (77.4%)
Total 486 (83.1%)	99 (16.9%)	585 (100)

Table 7: Trypanosomes species distribution across sex, age and body condition scores of the animals

	Trypanosomes species and their percent n (%)				
Variables	*T.congolense*	*T. vivax*	*T. brucei*	*T.congolence* and *T. vivax*	Total (%)
Sex					
Female	32 (32.3)	9 (9.1)	4 (4.0)	1 (1.0)	46 (46.5)
Male	37 (32.3)	10 (10.1)	5 (5.1)	1 (1.0)	53 (53.5)
Age					
1-3yrs	15 (15.2)	5 (5.10)	3 (3.0)	0 (0.0)	23 (23.2)
> 3yrs	54 (54.5)	14 (14.1)	6 (6.1)	2 (2.0)	76 (76.8)
Body condition					
Poor	24 (24.2)	9 (9.1)	2 (2.0)	1 (1.0)	36 (36.4)
Medium	44 (44.44)	10 (10.1)	7 (7.1)	1 (1.0)	62 (62.60)
Good	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)

Table 8: Distribution of trypanosome species in different kebeles based on hematological finding

	Trypanosome species n (%)				
Kebeles	*T.congolense*	*T. vivax*	*T. brucei*	*T. congolense* and *T. vivax*	Total n (%)
Ripa	24 (24.2)	7 (7.1)	2 (2.0)	0 (0.0)	33 (33.3)
K. baha	20 (20.2)	4 (4.0)	3 (3.0)	0 (0.0)	27 (27.3)
W. bubuka	15 (15.2)	6 (6.1)	3 (3.0)	2 (2.0)	26 (26.3)
M. lencha	10 (10.1)	2 (2.0)	1 (1.0)	0 (0.0)	13 (13.1)
Total	69 (69.7)	19 (19.2)	9 (9.1)	2 (2.0)	99 (100)

K. baha = karro-baha, W. bubuka = Walgahi-bubuka, M. lencha = Minko-lencha, n = number

From the three glossina species collected, relatively higher number of *G. tachnoides* was caught in riverine than savanna vegetation types (Table 3).

From the total tsetse flies collected, majority (3.08) were females. Among these flies high number is *glossina tachnoides* (Table 4).

Parasitological Survey: From a total of 585 cattle examined in the four Kebeles, 99 (16.9%) were positive and the individuals prevalence of the disease in each Kebeles of Sayo district is indicated in Table (5). There was statistically significant difference ($P<0.05$) in prevalence of trypanosomosis among kebeles.

The mean PCV value for the aparasitemic and parasitemic cattle was 26.14% and 24.12% respectively. Parasitic cattle having PCV = 24% (anemic) was 12.6% whilst in the parasitic cattle having PCV > 24% (non-anemic) was 4.3% (Table 6).

DISCUSSION

The overall apparent density of tsetse flies in the study areas was found to be 4.5 fly/trap/day, 5.04 fly/trap/day and 8.22 fly/trap/day for *tsetse*, *Stomoxys* and other biting flies respectively. This result was higher than the study of [13] at southern rift valley of Ethiopia who reported 1.4 flies/trap/day and Fura 0.3125 flies/trap/day but less than 29.625 flies/trap/day at Eligo. A dramatic reduction of mean apparent density of the tsetse flies at Fura village is because of the presence of considerable suppression of flies' population by the use of insecticide impregnated targets and insecticide-treated livestock undertaken in the area [13].

Entomological survey conducted indicated that, *G. tachnoides, G. m. sub morsitons* and *G. pallidipes* were the tsetse flies species caught in the study area along with other biting flies. This result was in agreement with [14] who reported that, *G. pallidipes, G. m. sub-morsitans, G. fuscipes* and *G. tachinoide* species were found in Gawo Dale District of Kelleme Wollega zone.

Apparent tsetse flies density of 9.63 in Walgahi-bubuka, 3.2 in Ripa, 3.34 in Karro- baha and 1.83 in Minko-lencha, were recorded in four villages. These flies were found in the Birbire, Adama and Meti basin areas (rivers) of selected kebelles of sayo district. *Stomoxys* 5.04 fly/trap/day and 8.22 fly/trap/day other biting flies were also caught along with tsetse flies. These finding indicate that tsetse and stomoxys flies were highly prevalent in kebeles adjust to river basin.

The geographical distributions of the tsetse flies were concentrated in the low land area as climatic conditions are more favorable. Typical habitat pattern found in the study area is favorable for the savannah species *G. m. submorsitans* and is favorable for riverine species *G. tachnoides*and *G. palliidipes*. Most of the tsetses were caught in the low land areas and the apparent density decreases as altitude increases. This finding is supported by earlier works [15-17] who indicated that climate, which is largely dependent (influenced) by altitude, has an impact on tsetse population.

Glossina tachinoides was the major species of tsetse fly caught in the study area. Similar findings were reported previously by various researchers that the dominant species of *Glossina* in the upper Anger and Didessa river valley was *G. tachinoides* [17].

Regarding the sex composition of the flies, female flies constitute 65% and this was in agreement with [18] who reported female flies to comprise 70-80% of the mean population.

The overall prevalence of trypanosomes (16.9%) observed in this study can be considered high due to high vector density. This high prevalence of pathogenic trypanosomes in Sayo District was similar with the result of different works in Ethiopia, [19] who stated that, the occurrence of the disease is also consistent with the general knowledge of the occurrence of the vectors, 17.2% in Metekel [20] and 17.5% in the Upper Didessa of tsetse infested regions [21] but less than 25% prevalence done in Gawo Dale district [6] and 29% prevalence done along the escarpment of the Upper Didessa Valley [22]. On other hand, the current study result was higher than (10.1%) prevalence done at Gimbi- district by during the dry season [23].

This study shows that, *T. congolense* was the dominant species with a proportion of (69.7%) and followed by *T. vivax* (19.2%), *T. brucei* (9.1%) and *T. vivax* and *T. congolense* mixed infection (2.0%). This result was in agreement with the previous work [20] that stated the predominance of *T. congolense* infection in cattle *as compared to T. vivax* and may be due to the development of better immune response to *T. vivax* by the infected animal. In addition it was reported that the dominant trypanosomes species in upper Didessa of tsetse infested regions was *T. congolense* [21]. Moreover, the most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax* [19].

In this study, there was a statistically significant difference in trypanosome infection rate between Kebeles. The probability of trypanosome infection in animals in Ripakebele is five (OR= 5), at least two (OR= 2.5) and three (OR=3) times more likely than karro-baha, walgahi-bubuka and minko-lencha respectively. This difference could be due to the variation in the ecosystems of the study locations that supported proliferation of both the tsetse and biting flies and regular application of prophylactic treatment in some study kebelles.

During the study period, the prevalence of bovine trypanosomosis was assessed between sexes of animals and among 99 trypanosome positive animals; 46 (7.9%) of them were female animals and 53 (9.0%) were males. The trypanosome infections in female animals were similar with male animals; this shows that both male and female cattle were equally susceptible to trypanosomosis. This result coincides with the results of previous studies that reported absence of significant difference in susceptibility between the two sexes [24-29]. Prevalence of trypanosome infection in male and female animals did not show significant difference (P>0.05) in prevalence.

In this study, the occurrence of disease in three different body condition (poor, medium and good) of animals, shows that statistical significant variation (P=0.00). The Odds of occurrence of the diseases in poor body condition animals were eleven (OR=11) and nine (OR= 9) than medium and good body condition animals respectively. This agreement [30] stated that, there is a significant difference (p<0.05) in trypanosome infection rate among body condition of animals. On other hands, disagreement with a study [31] who stated that, the prevalence of the disease is high in good body conditioned animals.

Trypanosome infection and mean PCV obtained between parasitaemic and aparasitaemic animals had statistically significant variation (P=0.00). This result was in agreement with the previous work done in Ghibe, Southwest Ethiopia where treatment was given for animals with PCV value of less than 26% and for positive animals where the authors indicated that an increase in PCV value, the proportion of positivity decreases and hence, the mean PCV was a good indicator for the health status of the herd in an endemic area [32].

The difference in mean PCV between parasitaemic and aparasitaemic animals indicates 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 pathogenicity, the low PCV in parasitaemic animals could have contributed in reducing the mean PCV for cattle [33-34]. Moreover, the result in consist with [35] state that, parasitaemic animals had generally lower mean PCV value could be attributed to the fact that animals in low altitude than aparasitaemic ones.

In the current study, the prevalence of 13.3% recorded in animals (>3 years old) was significantly higher than the prevalence of 3.6% recorded in young animals (≤ 3 years old). This could be associated to the fact that older animals travel long distance for feed and to serve for draught power as well as for harvesting crops and this may pose them to high tsetse fly challenge. In addition, young animals are also naturally protected to some extent by maternal antibodies. There was no statistically significant difference between age groups (P=0.23). This result was in agreement with the previous research report [36]. Furthermore agree with the finding of [37] reported from nearly some ecological location with our study areas, there was no significance difference observed in age group in the study period, but relatively higher rates in older age.

CONCLUSION AND RECCOMENDATIONS

The results of bovine trypanosomosis and apparent tsetse density survey in four villages of Sayo Woreda indicated that an overall 16.9% prevalence of the disease and the presence of high density of tsetse flies with an overall apparent density of 4.5% flies/trap/day. During entomological survey, three species of tsetse flies such as *G. pallidipes, G. morsitons submorsitons* and *G. tachnoides* were identified along with other biting flies. The study showed that, *T. congolense* was the dominant species of trypanosome with a proportion of (69.7%) and followed by *T. vivax* (19.2%), *T. brucei* (9.1%). There was ($P<0.05$) a statistical significant difference in prevalence of bovine trypanosomosis between Kebeles, Age and body conditions scores. The result of this study shows that trypanosomosis is very important disease of livestock in Sayo district of Kellem Wollega zone. The current situation may get worse as the prevention and control of trypanosomosis is facing a challenge due to limitation of vector control activities and chemotherapy. Therefore designing and implementation of control strategies of trypanosomosis focusing integrated approach (vector control and chemotherapy) should be undertaken in the studied areas.

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