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Study on Spatial Distribution of Tsetse Fly and Prevalence of Bovine Trypanosomosis and Other Risk Factors: Case Study in Bedele Woreda, Ilu Aba Bora Zone, South Western Ethiopia

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Abstract: A study was carried out from April to September 2014 in four peasant associations (PAs) in Bedele woreda, Oromia regional state to determine the prevalence of bovine trypanosomosis and its vectors, tsetse fly. A total of 391 cattle were randomly selected from the study population and examined first by Buffy coat and confirmed by Giemsa staining for positive samples. Out of the total cattle examined, 48(12.28%) cattle were positive for trypanosomes. The recorded trypanosomosis prevalence rate was varying from 8.16% to 17.17% indifferent sites of the study area. The identified trypanosome species were T. congolense, T.vivax and T.brucei; out of which 60.4% was T.congolense. This shows that, T.congolense was the most prevalence in all Pas; however, there was no statistical difference in distribution between the species of trypanosomes (p>0.05). Prevalence of disease in age was ($\leq 3=8.11\%$; ≥ 3 years=13.28%) and in sexes (male=13.02%; female=10.78%). However, the association between the infection rate within the age and sex groups was statistically insignificant. The mean PCV value of parastaemic animals was 20.6% in compared to aparastaemic animals which was 26.8% and analysis of the mean PCV values of parastaemic and aparastaemic animals showed statistically significant (p=0.000). Finally, entomological survey was performed by deploying of a total of 52 monoconical traps in the four PAs (13trap/site). The number of tsetse flies and other biting flies caught during the study period were 479, out of which 50 % of them were accounted by tsetse flies and the rest were biting flies. The overall apparent densities of flies caught were 2.31fly/trap/day for *Glossina*, 1.18fly/trap/day for *Stomoxys*, 0.94 fly/trap/day for Tabanus and 0.17 fly/trap/day for Hematopota. Identification to the species level of tsetse flies showed that: 47.1% Glossina morsitans submorsitans, 39.2% Glossina pallidipes and 13.8% Glossina tachinoid and other biting flies of genus Stomoxys, Tabanus and Hematopota. In conclusion, selective and rapid intervention on tsetse suppression and chemoprophylaxis actions should be done in Bakalcha Biftu PAs and farms involvement in the tsetse and trypanosomosis control strategy should be strengthened.

Key words: Tsetse Fly · Prevalence · Trypanosomosis · Cattle · Bedele Woreda

INTRODUCTION

Ethiopia has huge and diverse livestock population that plays an important role in the economy and livelihoods of farmers and pastoralists [1]. The country is estimated to have over 150 million livestock heads. According to the census conducted by Central Statistical Authority [2] there were approximately 53.4 million cattle, 48.28 million small ruminants, 8.58 million equines, 1 million camels and 49.3 million poultry. Despite the large animal population; production and productivity is very low in Ethiopia and even below the average for most countries in eastern and sub-Saharan African countries. This is due to poor nutrition, reproduction insufficiency, management constraints and prevailing animal diseases [1]. Trypanosomosis is one of the major disease impediments to livestock development and agricultural production, which negatively affect the overall development in agriculture in general and to the food self-reliance efforts of the nation in particular. In Ethiopia

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about 180,000 to 200,000km² of agriculturally suitable land in the west, southwest and north western low lands and the associated river basins of the country are infested by tsetse fly and trypanosomosis, making this land underutilized. In those areas, there are 14 millions heads of cattle, an equivalent number of small ruminants (equivalent to cattle), nearly 7 million equines and 1.8million camels are at risk of contracting trypanosomosis at any time [3-5]. Thus, if control of trypanosomosis could be achieved there would be the potential even at the current low rate of production to increase meat supply of continent by the approximately 16% and milk supply by 17% of the current production level [6]. To control the disease and its vector; tsetse fly, a base line data tsetse density and trypanosomosis concerning prevalence in the endemic areas like Bedele woreda is mandatory. Therefore, the objectives of this work were:

- ► To determine the prevalence of bovine trypanosomosis in the study area,
- To determine the species and relative magnitude of the different species of trypanosomes of bovine in the study area,
- To determine PCV values in parasitaemic and aparastaemic animal,
- To identify the major vectors in the area

MATERIALS AND METHODS

Study Area: The present study was conducted in four peasant associations (PAs), namely: Qolosiri, Ambalta, Chafejalala and Bakalcha Biftu in Bedele woreda of Illu Abbaabora Zone in Oromia regional state South West Ethiopia. The woreda is located at about 480 km west of Addis Ababa at longitude 8.27 N and 36' 21 E and latitude 8.45° N and 36.35° E respectively. It is also located at an elevation ranging between altitude ranges from 1200-2200 meters above sea level. The climatology alternates with long summer rain fall (June- September), short rainy season (March-) and winter dry season (October-February). The study area receives a mean annual rainfall of 1200-1800mm with optimum temperature of 25°C.

Agriculture is the main stay of livelihood of people with a mixed farming system and livestock plays an integral role for agriculture. The major livestock kept in the study area are cattle, goats, sheep and equines. According to the information from Bedele woreda agricultural offices [7], there are about 17470 cattle, 9115 goats, 4214 sheep and 945 equines. Animals are left in communal grazing area far away from farm area or residential areas under close supervision by the owners.

Study Design: A cross-sectional study was implemented to assess the prevalence of bovine trypanosomosis and to investigate tsetse species composition in the area.

Study Animals: The target populations were local breeds of cattle of all age group and sex found in each site.

Sample Size Determination: The sample size was determined by using [8]. The expected prevalence of this disease in respective of the area was based on the previous study conducted by NTTICC [9] which was 19.6% and 95% confidence, 5% absolute precision was used.

$$N = \frac{1.96^{2}P(1-p)}{d^{2}}$$

$$N = \frac{1.962 \times 0.196 (1-p)}{d2}$$
Therefore $N = \frac{1.96 \times 1.96}{d2}$

Therefore, N=
$$\frac{1.96 \times 1.96 (0.5) (0.5)}{0.0025}$$

= 342 cattle

where N= the sample size d= the desired absolute precision p= the expected prevalence

However, to increase precision, the sample size was increased to 391 cattle.

Study Animals Selection: Simple random sampling techniques were followed to select the animals to be used for the study of the prevalence of bovine trypanosomosis in the study area.

Sample Collection and Laboratory Processing

Sample Collection, Parasitological and Hematological Examinations: Paired blood samples were collected from the auricular vein (Marginal ear vein) of each animal using two haematocrit capillary tubes. The tubes were filled ³/₄ of its height and sealed with crystal sealant. The capillary tube was also used to measure the PCV values for the determination of anemia and comparison of infected animals with non-infected animals. The capillary tube was cut 1mm below the Buffy coat to include the top layer of RBCs. The content of the capillary tube was expressed on to a clean microscopic slid, mixed and covered with cover slip. Then the slides were examined for trypanosomes based on the type of movement in the microscopic field.

Confirmation of trypanosome species by morphological characteristics were done after staining with Giemsa and examination with oil immersion microscopy under×100 power of magnification according to Murray *et al.* [10]. During sample collection; age, sex, PAs, altitude and body condition of each animal were recorded. The age of the animals was grouped as young (< 3 years) and adults (\geq 3 years) according to the classification used by Bitew [11]. On subjective basis body condition of examined animals were evaluated during sample collection. They were classified as poor, medum and good relative to the average body condition of local animals (zebu) [12].

Entomological Survey: To assess the apparent density of different tsetse fly species and other biting flies; entomological samples were collected and studied in selected sites of the study area. These entomological data were collected only at one time during the dry period season in February 2014. The flies were caught with monoconical traps baited with acetone and 3 days old cow urine according to Drans *et al.* [13]. The tarps were placed in selected sites (Places where animals stay for longer period i.e. watering and grazing areas) of the study area and 52 traps were deployed (13 traps/site) before sun rise in the morning and kept in position for 48 hours. During trapping, acetone was dispensed from open vials through an approximately 'o' sized hole and cow urine with open bottles was stationed.

The different fly catches in each trap were counted and the species of tsetse fly were identified based on the characteristic morphology [14] and for other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level [15]. Sexing was done just by observing the posterior end of the ventral aspect of the abdomen by hand lens and stereomicroscope hence male flies were identified by enlarged hypopygium in the posterior ventral part of the abdomen. Tsetse fly apparent density mean catches in traps deployed was expressed as the number of tsetse catch /trap/ day [14].

Data Analyses: Data collected from each study animal and laboratory analyses were coded in to appropriate variables and entered in Microsoft excel, 2007 spread sheet. All statically analyses were performed using STATA-7 soft ware. The prevalence was calculated for all data as the number of infected individuals divided by the number of individuals sampled times 100. Categorical data were analyzed by using chi-square (X^2) test of independence where as t-test was used to examine the

difference in mean PCV between the study variables. In all cases, 95% of confidence intervals were used and p value less than 0.05 were considered as significant.

RESULTS

Parasitological Findings: The research was conducted in 4 selected peasant associations (Pas) of Bedelle woreda. Out of the total of 391 local breeds of cattle examined, 48 animals were found positive for trypanosomosis. The overall trypanosomosis prevalence was found to be 12.3 %. The prevalence of trypanosomosis was 12.9%, 10.4%, 8.16 and 17.7% in Qolo siri, Ambelta, Chafejalala and Bakalcha Biftu respectively. Out of these, the highest prevalence was found in Bakalcha Biftu (17.71%) and the lowest prevalence was observed in Chafe Jalaal peasant associations (8.16%) (Table 1), however, there was no statistical significant differences in prevalence of trypanosomosis among the four PAs (p>0.05).

According to the present study, out of the total positive animals for trypanosomosis, 29 (60.4%) were infected with *T. congolense*; followed 13 (27.1%) by *T. vivax* but the least was found to be due to *T. brucei* (12.5%) (Table 2).

Age and sex based comparison in prevalence of the parasite was performed. Accordingly, the overall prevalence in male was found to be13.02% and in females 10.78%. However, there was no statistical significant difference between sexes of animals. Furthermore, the prevalence of the trypanosomosis in different age groups was found to be 8.11 % and 13.25% in cattle less than 3 years and greater than and/or equal to 3 respectively but there was no statistical significant difference (P>0.05) in the different age groups (Table 3).

To evaluate the debilitating effect of trypanosomosis in diseased cattle which were living under similar environment and management systems, showed the following prevalence levels in different body scores of animals: 9.91%, 10.27%, 16.42% in good, medium and poor body condition respectively. In this present study, even though relatively high prevalence was recorded in animals with poor body condition; followed by medium, however, the differences in prevalence of the parasite (s) was not statistically significant (p>0.05) among animals with different body scores.

Hematological Examination: PCV for all study animals was analyzed to estimate the degree of anemia. From the total 391 animals; 36.1% had PCV value less than 24% and

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	Test results								
Qolo siri	13	88	101	12.87%	4.5	0.21			
Ambalta	10	86	96	10.42%					
Chafe Jalaal	8	90	98	8.16%					
Bakalcha Biftu	17	79	96	17.71%					
Total	48	343	391	12.3%					

Table 1: Prevalence of trypanosomosis in four peasant association (PAS)

Table 2: Summary of the prevalence of trypanosomosis in different peasant associations and by different species of Trypanosomes

Site (PAs)	Examined animals	T. brucei	T .congolense	T. vivax	Total	Prevalence	X^2	P-value
Qolo siri	101	1	9	3	13	12.87%	4.5	0.21
Ambalta	96	1	6	3	10	10.42%		
Chafe Jalaal	98	2	4	2	8	8.16%		
Bakalcha Biftu	96	2	10	5	17	17.71%		
Total	391	6	29	13	48			
Prevalence (%)	12.5	60.4	27.1	12.3				

Table 3: The prevalence of trypanosomosis based on different risk factors

		Test result	l est result						
Variables		Positive	Negative	Total	Prevalence	X ² - value	P- value		
Sex	Male	34	227	261	13.02	0.0002	0.989		
	Female	14	116	130	10.77				
Age	<3 years	6	68	74	8.11%	1.4725	0.225		
	\geq 3 years	42	275	317	13.25%				
Body condition	Good	11	100	111	9.91%	3.2551	0.196		
	Medium	15	131	146	10.27%				
	Poor	22	112	134	16.42%				

Table 4: Proportion of the mean PCV of parasitemic and aparasitemic cattle

No/of cattle	Parasitemic PCV<24(%)	Parasitemic PCV>24(%)	Aparasitemic PCV<24(%)	Aparasitemic PCV>24(%)
391	42(87.5)	6(12.5)	99(28.86)	244(71.14)

Table 5: Proportion of fly species collected and their density in different peasant associations

Elias con ano identificad	Oala airi	Ambalta	Chafa ialala	Deletake Diffe	Total flux accepted	0/	ETD
Files genera identified	Qolo siri	Ambaita	Chare Jalala	Bakalcha Bittu	I otal fly caught	%0	FID
Tabanus	23	21	19	25	98	20.5	0.94
Stomoxys	32	29	27	35	123	25.7	1.18
Haematopota	5	4	3	6	18	3.8	0.17
Glossina	62	52	52	74	240	50	2.3
G. m. submorsitans	31	27	25	30	113	47.08	
G. pallidipes	18	20	21	35	94	39.17	
G. tachnoides	13	5	6	9	33	13.8	

*FTD refers to fly per trap per day

from 343 trypanosomosis negative animals, 28.86% had less than 24% PCV values. From trypanosome positive 48 animals, 87.5% had PCV value less than 24%. Furthermore, 12.5% of the cattle have had a PCV value in the normal range (24-46%) while they were react positive to trypanosomosis infections. The mean PCV of the present finding of parasitemic (20.6%) was significantly lower than that of aparasetmic animals (26.8%).

Entomological Survey: A total of 479 tsetse and other biting flies were caught during the study period. Out of

these, *Glossina* accounts 50%, *Stomoxys* 25.7%, *Tabanus* 20.5% and *Haematopota* 3.8%. The apparent fly density was found to be 2.31flies/trap/day for *Glossina* species, 1.18flies/trap/day for *Stomoxys*, 0.94flies/trap/day for *Tabanus* and 0.17flies/trap/day for *Haematopota*. The composition of *Glossina* species identified in the present study were 47.1% (113) *G. morsitans sub morsitans*, 39.2 %(94) *Glossina pallidipes* and 13.8% (33) *Glossina tachnoides* and out of these, 180 of the 240 tsetse flies were females (75%) and 60 of them were males (25%).

DISCUSSION

Accordint to the present study, the overall prevalence of bovine trypanosomosis in the study area was 12.28%. This was in line with the findings of Solomon and Fitta [16] which was reported to be 12.41% in Metekel and Awi zones of northwest Ethiopia. However, this was relatively higher than the finding of Tafese *et al.* [17] which was reported to be 8.55% in the Diga and Sasiga, East Wollega Zone; 9.1% in Mada Talila kebeles of Hewa Gelan, Western Ethiopia [18]. It was also lower than the report of Tilahun *et al.* [19] which was recorded to be 23.0% in Daremello district of southwestern Ethiopia.

The present work reveled that T. congolence was the predominant species and found to be a major cause of cattle trypanosomosis in the study area followed T. vivax and T.brucei. Similar results were also reported by Shimelis et al. [20] and Fedasa et al. [21]. The result in this study area showed that from the total trypanosome positive animals; 60. 42% was affected by T.congolence, 27.1% by T. vivax and 12.5% were by T. brucei. Except for T. brucei, the current result was relatively lower than the previous study conducted in the same area by NTTICC [22] which was reported to be: 71% for T. congolense, 20% T. vivax and 9% T. brucei. The predominance of T. congolense infection in cattle may be due tothe development of better immune response to other trypanosome species by infected animals [14, 23]. The lower infection rate of domestic animals by T. brucei than by other trypanosome species may be due to the seasonal absence of the parasite in circulation (Parasitaemia) as indicated by Losos and Chovinard [24] and one might miss many latent infection which only become apparent after rat inoculation [3].

During the study period, the prevalence of bovine trypanosomosis was assessed between sexes of animals and among 48 trypanosome positive animals; out of the total male animals sampled in this study (261), only 34(13.03%) of them were positive for one or more species of trypanosomes and similarly; out of 130 female animals sampled, only 14 female animals were positive for the parasite (10.77%), i.e. relatively higher infection rates in male than female animals. This was in agreement with the findings of Teka *et al.* [25], Daya and Abebe [26], Terzu [27], Nigatu [28] and Tewelde *et al.*[29]. This may be attributed to stress factors related to working male animals which are commonly used for drought power in Ethiopia and they walk long distance even in areas with high risk of tsetse challenges.

In this study, animals with age \geq 3 years (old) were relatively more prone to high infection rate (13.25%) that animals with age <3 years (young) (8.11 %). Similar results were reported by Addisalem [30], Daud and Molalegne [31] and Molalegne et al. [32]. This could be associated to the fact that older animals travel long distance for grazing and draught as well as harvesting crops in tsetse challenging areas. Rowlands et al. [33] 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 [34]. This could be the reason for lower prevalence of trypanosomosis that was observed in calves. Moreover, tsetse flies are attracted significantly more by odor of large animals and animals that showed less defensive behavior according to Torr et al. [35] and Torr and Mangwiro [36].

In the current study, the highest prevalence was recorded in animals with poor body condition and the least was in animals with good body conditions. This was in agreement with Dawud and Molalegne [31] and Molalegne *et al.* [32]. Obviously, the disease itself results in progressive emaciation of the infected animals. In addition to that; animals with good body condition might have well developed immunity to this particular disease than animals with poor body condition. Furthermore, animals with poor body condition may have compromised immunity presence of concurrent diseases or malnutrition [37].

According to Van den Bossche and Rowlands [38], cattle with $PCV \le 24\%$ are considered anemic and anemia is a principal sign of trypanosomosis in livestock [39]. From the total cattle populations sampled during this study, 36.1% of them had a PCV \leq 24%. In the other side, despite of 28.86% of total animals sampled had a PCV ≤24%, they react negatively for trypanosomosis infection and this may have occurred due to less sensitivity and specificity of the tests used [10] or delayed recovery of anemic situations after recent treatment with trypanocidal drugs or may be due to the combined effect of poor nutrition and hematophagus helminth infection such as haemonchosis and bunostomiasis [40]. These suggest that even anaemia is characteristic of trypanosomosis, other factors can also contribute to the reduced PCV value. In the present study, out of 48 trypanosome positive animals, 87.5% had PCV $\leq 24\%$, i.e. they were both infected and anemic This clearly showed that, PCV values can be affected by many factors other than trypanosomosis, but these factors are likely to affect both trypanosomosis negative and positive animals [38]. However, the difference in mean PCV between parasitaemic and aparasitaemic animals clearly indicates trypanosomosis reduces the PCV values in infected animals.

The present study also revealed that almost 12.5% of the cattle have a PCV value in the normal range (24-46%) while they were react positive to trypanosomosis infections. This may have occurred due to recent infection with trypanosomosis [41]. This result also agrees with the previous result of Teka *et al.* [25] and Garoma [42] who concluded that cattle's having PCV value of normal range were shown to be infected with trypanosome parasite. Some trypanosome infected animals can also keep their PCV within the normal range for a certain period of time. So, diagnosing of trypanosomosis on the basis of PCV is not accurate but can be an indicator.

Tsetse fly distribution is strongly associated with the presence or absence of diversity of wild life in a given ecosystem. The distribution and abundance of G. morsitans and G. pallidipes are closely related to the number and habits of certain wild animals. The densities of these tsetse fly species are directly correlated with thedensities of wild animals and low human population in a given area [14]. According the present study, about 479 tsetse and other biting flies were caught during the study period. These findings were almost 4 times lower than the previous findings of Mezene et al. [43] in Bure District of western Ethiopia. Out of 479 flies collected, 50% (240) were Glossina species and the rest 50% were biting flies. The major Glossina species identified were: 47.1% (113) G. morsitans sub morsitans; followed by 39.2% (94) Glossina pallidipes and 13.8% (33) Glossina tachnoides. These findings were in agreement with the findings of Leak [14] who concluded that the distribution and abundance of some species of tsetse flies such as G. morsitans and G. Pallidipes to be closely associated with the number and habits of certain wild animals.

The risk of trypanosomosis is also influenced by apparent density and types of vectors in the area. The apparent fly density in the present work was found to be 2.31flies/ trap/ day for *Glossina* species, 1.18flies/ trap/ day for *Stomoxys*, 0.94 flies/trap/day for *Tabanus* and 0.17 flies/trap/day for *Haematopota*. This result was relatively lower for *Glossina* species but higher for biting flies when compared to the findings of Solomon and Fitta [16] at Metekel Awi zones of Northwest Ethiopia, who reported 6.49 f/t/d and 0.65 f/t/d for tsetse and biting flies, respectively. It is also lower for both tsetse species and biting flies' findings of NTTICC [22], at Bure Iluababor zone of Western Ethiopia which was reported to be 7.23 f/t/d, 3.13 f/t/d and 0.06 f/t/d for tsetse, Stomoxys and Tabanus, respectively. It is also fur lower than the report of Teka et al. [25] at Arbaminch, Ethiopia, who reported 14.97 f/t/d for tsetse and Fentahun et al. [18] at Hewa Gelan, Oromia region Ethiopia, who reported 11.9 f/t/d and 10.2 f/t/d for tsetse and Stomoxys respectively, however, it was in line with Fentahun et al. [18] for Tabanus which was reported to be 0.9 f/t/d. The relatively low level of tsetse population in present study may be due to the use of insecticide impregnated targets and insecticide-treated livestock undertaken in the area by NTTICC and the expansion of settlements and farmlands in the area in the expense of deforestation limits the tsetse and other flies habitats. It may also be related to the level of dryness, which resulted in the migration of game animals from the study area.

In the current study, tsetse flies sex identification was performed and greater numbers of female (75%) were recorded. This was in agreement with the finding of workers [44] in southern Ethiopia and Leak [14] who had reported in un-biased sample; female to comprise 70-80% of the mean populations. So, in the present study, traps may have been deployed in un-biased manner. Furthermore, the higher population of female may be attributed to the fact that females live longer than males (mean female fly life span being 8 weeks than male about 4 weeks), so that more female could be caught.

CONCLUSION AND RECOMMENDATIONS

The current study revealed that despite frequent interventions were applied in the study area against tsetse and trypanosomosis, significant numbers of flies and 12.3 % overall prevalence of trypanosomosis was reported. Out of the four peasant associations involved in this study; relatively highest trypanosomosis prevalence and high flies density was recorded in Bakalcha Biftu (17.7%) PA and the lowest in Chafejalala (8.16). In this study, T. congolense (7.4%) was found to be the most prevalent Trypanosoma species in the area; followed by T. vivax (3.3%) but the least was found to be due to T. brucei (1.5%). Lastly, similar to other previous studies conducted somewhere in the country, trypanosomosis was confirmed to bring anemia in cattle. Generally, the cost of treatment and/or prevention and death of animals due to this disease is supposed to be significant. Therefore, base on above conclusions the following recommendations are forwarded:

- Selective and rapid intervention on tsetse suppression and chemoprophylaxis actions should be done in Bakalcha Biftu PAs
- The intervention implemented by NTTICC in the study area should be evaluated for its efficacy by external experts
- Farms involvement in the tsetse and trypanosomosis control strategy through extension should be strengthened
- Further study should be conducted on overall efficiency of the control strategy implemented in the area.

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