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Study on the Prevalence of Bovine Trypanosomosis, Vector Density and Associated Risk Fators in Assosa District of the Benishangulgumuz Region, West Ethiopia

Dinka Ayana and Mulugeta Zerihun

College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O. Box 34, Bishoftu, Ethiopia

Abstract: A cross-sectional study was carried out in Asossa district of Benishangul Gumuz Regional State, Western Ethiopia from November 2015 to May 2016 to determine the prevalence of trypanosomosis, identification of circulating trypanosome species, identification of the vectors and associated risk factors. Blood samples were collected from a total of 400 cattle and examined using buffy coat technique. Overall 85 (21.5%) trypanosomosis prevalence was recorded. The major species of identified Trypanosoma were *Trypanosomacongolese* (51.76%), *Trypanosomavivax* (28.23%), *Trypanosomabrucei* (11.76%) and mixed infection accounted for 8.23%. Mean packed cell volume (PCV) value of the infected animals was lower (21.6%±3.20) than uninfected animals (24.32%±2.22) and the variation was statistically significant ($P< 0.05$). Overall, anemia prevalence of 54% (216/400) was recorded and it was significantly higher (77.64%) in infected cattle than in non-infected (47.67%). Significant difference was not observed between sex groups and age categories ($p>0.050$) but there was significant difference in the prevalence of trypanosomosis among study sites and body conditions ($P< 0.05$). *Glossinamorsitans* sub *morsitans* was the only tsetse fly caught and its mean apparent density measured as fly/trap/day was 4.95. In addition, mechanical vectors of trypanosomosis such as *Stomoxys* (1.29 f/t/d), *Tabanus* (1.83f/t/d) and *Haematopota* (0.41 f/t/d) were identified. In conclusion, the result of the current study showed high prevalence of the economical important cattle disease, Trypanosomosis and we recommend urgent institution of control strategies in the study areas.

Key words: Asossa · Trypanosomosis · Tsetse Fly · Prevalence · Risk Factors

INTRODUCTION

The livelihoods of more than 85% of the people of Ethiopia depend on the agricultural sector. This sector mainly possesses crop production, livestock production and mixed farming. Since people are dependent on this sector, the presence of livestock is one of the necessities to this sector. This fact has made Ethiopia to be one of the richest countries in livestock production in Africa [1].

Official figures gives a National Ethiopia animal population of 40.9 million cattle, 25.5 million sheep, 23.4 million goats, 2.7 million horses, 0.63 million mules, 5.2 million donkeys and 1.07 million camels [2].

Tsetse fly infest 10 million km^2 potentially productive land of Africa between 14° N and 29° S [3]. There are 23 different species of tsetse fly and they exist in 37 countries of Africa. Five of them namely *G.m.submorsitans*, *G.pallidipes, G.tachinoides, G.fuscipes* and *G.longipennis* are reported in Ethiopia. Several reports made in Ethiopia revealed that tsetse fly occupy over 66,000 km^2 areas [4] based on 1500 masl, breeding limits in the south and southwestern valley of the country. NTTICC [5] (1976) has reported that some 98,000km^2 areas 1600 masl breeding limits in the southern and southern western of Ethiopia. The tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N which amounts to about 200,000 km^2. Out of this 31,000 km^2 or (62%) Regional land area of Benishangul-Gumuz is infested bytsetse fly [6].

Tsetse flies are hard to control and the tsetse fly infestation is becoming more and more serious in Africa. The clearing of large forest tracks some time cause the

Corresponding Author: Dinka Ayana, College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O. Box 34, Bishoftu, Ethiopia.

flies to spread to more populated areas and the deforest land covered with savannah grass consequently newly invade by morsitans group [7].

Tsetse flies are enormous health risks in part of Africa they can transmit a disease trypanosomosis. African trypanosomosis is heamoparasitic disease considered as the main obstacle to animal production development [8]. It is the wasting disease; affected animals are chronically unproductive in terms of milk, meat, manure, traction. The number of trypanocidal drugs available for treating and preventing infections in endemic areas is limited and about 6 million doses are administered yearly in Africa. The drugs have been in the market for over 30 years, their range of therapeutic safety is small. The disease in Africa costs livestock producers and consumers an estimated US $ 1340 million each year [3].

Mortality and the morbidity rate can be high. There is a direct association between increase prevalence and proximity herd pens watering points distance but no association of herd pens to grazing point distances which suggests that hydrological network played an important part in trypanosomosis. The disease distribution over 10 million km^2 of potentially productive land of Africa. The risk falls between 15° N and 29° S latitudes. As the result a total of 14.8 million cattle 6.12 million sheep and goats, 1 million camels and 1.23 million equines are at risk of contracting the disease in Ethiopia [6].

Therefore, the objectives of the study are:

- To determine prevalence of bovine trypanosomosis
- To identify the species of trypanosomes in the study areas
- To determine the degree of anemia between infected and non infected cattle
- To identify vectors of trypanosomosis and determine their density
- To identify the associated risk factors

MATERIALS AND METHODS

Filed Data Collection

Study Area: Assosa district is located in BenishangulGumz Regional State, west Ethiopia and it is 661 km away from Addis Ababa. The study was conducted from November 2015 to May 2016 in 6 peasant associations namely Kushimengel, Alubo, Abramo, Komoshiga26, Komoshiga27 and Tsetse of Assosa district.

According to Assosa district Agricultural and Rural Development Office, the total size of the district is 2317 km^2. It divided in to 74 PAs with a total population of 92,144. The district is situated in the latitude 9° and 10° N, longitude 034^0 and 035°E and Altitude 1400 -1570 masl. With the lowest temperature 19°c and the highest being 34°c. It is characterized by uni-modal rain fall. The average rain fall ranges from 900 mm to 1200 mm, it extends from May to October with peak rainy periods from June to August. The vegetation that constituent available grass land predominantly native grass and bamboo forest.

Livestock population in the study area comprises: Cattle 26,124 Sheep 4,382, Goat 17,509, Equines 5,930 and 34,710 Poultry.They provide with vast range of products and services such as milk, meat, skin, hair, horns, bones and manure etc.

The commonly encountered animal diseases in the area were trypanosomosis, pasteurellosis, black leg, CBPP, PPR, LSD, external parasites and internal parasite. Basic clinical syndrome on cattle affected by trypanosomosis appear after an incubation periods of 8-20 days. There is fever which is likely to be intermittent and to last for long periods. Affected cattle are dull, anorexic watery ocular discharge and lose condition. Superficial lymph nodes become visibly swollen, mucus membranes are pale, diarrhea occasionally occurs. Estrus cycle become irregular, pregnant cow may abort. The animals become very emaciated and die within 2-4 months or longer. Thin rough hair coat, anemic, lethargic cattle with generalized lymph node enlargement are said to have a fly struck appearance [3].

Study Design and Sample Size Determination: A cross sectional epidemiological study design was employed for this study. The sample size for the study group was calculated using a formula given byThrust field [9].

$n= (1.96)^2 \times Pexp (1-Pexp)/ d^2$.

where

n = the required sample size for the district

Pexp = expected prevalence (22.05 % in this case)

d^2 = desired absolute precision (5% in this case)

Therefore; $1.96^2 \times 0.2205 (1-0.2205)/ (0.05)^2 = 264$

Study Population: A total of 400 blood samples were collected from cattle during the study period.

Table 1: Prevalence of trypanosomosis by peasant associations

Peasant association (PA)	Location			Date of sample collection	Sample size	No Negative	No positive	Prevalence %	X^2	p- value
	Latitude	Longitude	Altitude							
Kushimengel	101013.3	03429153	1363	16/02/08	54	34	20	37.03	11.101	0.049
Tsetse	100852.0	0343116.3	1471	23/02/08	53	42	11	20.75		
Alubo	101868.1	0344950.1	1288	01/03/08	70	59	11	15.7		
Komeshiga26	100044.5	0344122.8	1468	10/03/08	90	76	14	15.55		
Komeshiga 27	100310.6	0347086.1	1390	20/03/08	64	50	14	21.87		
Abrahamo	099940.3	0345059.7	1493	30/03/08	69	54	15	21.7		
	Total				400	315	85	21.25		

Questionnaire Survey: To assess the socio-economic impact of trypanosomosis animals owners of the study group were interviewed about the husbandry practices, the farming practices, treatment cost (Expenses against trypanosomosis control) and other trypanosomosis and its vector related questions.

Fly Survey: During the study one type of traps was deployed: 73 monoconical, traps. Every trap was odor baited with acetone, Octanol and cow urine. The underneath of each trap 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 trap deployment time was 48 hours. After the flies captured in the collecting cage they were then sorted by sex and species and recorded. The species of tsetse was identified based on the characteristic morphology. Other biting flies were also separated according to their morphological characteristics such as size, color, proboscis and wing venation structures at the genus level [10]. Sexing was done for tsetse fly just by observing the posterior end of the ventral aspect of abdomen by hand lens, as a result male flies easily identified by enlarged hypophageum.

Sample Collection

Blood Sample Collection: The farmers were informed to bring their cattle to the animal health clinic /post or convenient shady place of examination sites a day before commencement of sampling. The cattle were chosen randomly and blood samples were collected by ear vein puncture using sterile lancet in to a pair of heparin-zed capillary tubes filled ¾th of its length (75x1.2mm) from each of randomly selected cattle. Each tube was sealed with crystal seal on one end [11].

Method of Transportation and Submission: Each sample was identified with code number, owner name, animal species, sex and age, date of collection, breed and address on the paper attached to the samples. The identified capillary tubes containing the samples still on the sealant in their code number were submitted to the laboratory for Buffy coat preparation and the thin smears were dried and stored at slide storing boxes and submitted to the laboratory for Giemsa stain preparation for further analysis.

Data Management and Analysis: All data recorded in this study was entered in to Microsoft excel and subsequently analyzed using STATA version 7 soft ware. Chi-square test was used to determine the variation in Trypanosomes between sex, age, body condition, PCV and species.

Laboratory Analysis

Sample Storage and Processing: The sampling informed formats were documented at the laboratory for future information. The capillary tubes were centrifuge immediately after arrival of the laboratory to prepare Buffy coat to detect the parasites based on their movements, to provided screening the case and PCV evaluation. Before staining blood films need to be fixed with acetone free methyl alcohol (Methanol) for some minutes in order to prevent hemolysis while staining them with aqueous (Water –based) stain such as Giemsa. The slides were stored at slide storing boxes until staining and examination.

Analysis of Specific Samples

Hematological Examination: The blood samples were centrifuge at high speed (12,000 rpm) for 5 minutes. Finally the packed cell volume (PCV) value were read by micro-hematocrit reader which could be adjusted individually for the length of the blood column in each tube to get value indication presence, absence and degree of anemia [12].

Parasitological Examination: More sensitive technique utilizes centrifugation in micro-heamatocrit the followed by microscopic examination of the interface between the Buffy coat and plasma. Capillary tubes containing blood after centrifugation were cut with diamond pointed pen

1mm below the Buffy coat to include the upper most layer of red blood cell and 3mm above to include the plasma so that the contents were gently expressed onto a slide, mixed and covered with cover slip (22mm x22mm).The preparation were then examined using a 10x eye piece in combination with 40x objective to get optimum views allowing large visual field and sufficient magnification for easy identification of trypanosomes based on their movement [13].

RESULTS

Trypanosomes Infection Prevalence: Out of total 400examined animals 85(21.25%) were infected with trypanosomes. The prevalence in terms of trypanosome species was 11, 6, 2.5 and 1.75 % for *T.congolense, T.vivax*, *T. brucei* and mixed infection, respectively. The proportion of trypanosome species was 44/85 (51.76 %) *T. congolense*,*24/85 (*28.23%) *T. vivax, 10/85 (11.76%) T.brucei* and *7/85(8.23%)* mixed (Table 2).

Cattle Pcv Distribution and Anemia in Studied Area: The mean PCV value for whole examined animals was 23.75±3.97. However, the mean PCV value for uninfected animals was 24.32±2.22 and mean PCV value of the infected animals was 21.6±3.20. The mean PCV values of trypanosome positive and trypanosome negative in cattle 21.6 % and 24.32 %, respectively with significant difference between of them (*P<0.001*) (Table 4). The overall anemia prevalence in the studied district was 54 % (216/400). The anemia prevalence was significantly higher in trypanosome infected cattle (77.64%) than in non-infected cattle (47.62) (??<0.001). Of 54 % anemia prevalence, 16.5% (66/400) was trypanosome infected animals. However, large number of animals 37.5% (150/400) had anemia (PCV <24) without having trypanosome infection. Some animals 4.75% (19/400) were infected by trypanosome but their PCV was found normal (Table 5).

Prevalence of Trypanosomosis by Age, Sex, Study Sites and Body Condition: The highest trypanosomosis prevalence (21.63%) was recorded in 2-7 years old animals whilst the lowest prevalence (19.56%) were >7years old. Slightly higher prevalence was registered in males 23.5% than in females 19.6 %, which was statistically non-significant.Trypanosomosis was recorded across the study sites with the highest (37.03 %) prevalence in Kushemengel PA and the lowest 15.55 % inKomeshiga-26 Peasant association. Trypanosomosis prevalence was statistically significant among study sites. There was a significant difference (P < 0.005) in the prevalence of

Table 2: The species of Trypanosoma identified from the study sites

	Location		Date of sample			Parasites identified						
PA	Latitude	Longitude	Altitude	collection	Sample size	*T.congolense*	*T.vivax*	*T.brucei*	mixed	Total	X^2	p- value
Kushimengel	101013.3	03429153	1363	16/02/08	54	12	5	1	2	20	182.75	0.0001
Tsetse	100852.0	0343116.3	1471	23/02/08	53	5	3	2	1	11		
Alubo	101868.1	0344950.1	1288	01/03/08	70	6	4	1	0	11		
Komeshiga26	100044.5	0344122.8	1468	10/03/08	90	6	4	3	1	14		
Komeshiga 27	100310.6	0347086.1	1390	20/03/08	64	7	4	1	2	14		
Abrahamo	099940.3	0345059.7	1493	30/03/08	69	8	4	2	1	15		
Total	400	44	24	10	7	85						
%	11	6	2.5	1.75	21.25							

Table 3.: Vectors of trypanosomosis identified from the study sites

									Tsetse fly			Biting fly					
									Glossinam. submoristance			*Stomoxys*		*Tabanus*		*Hematopota*	
Kebele	Latiteude	longitude	altitude	Site/river	Trap code	Trap type	Deployed day	Collecting day	M	F	ftd	T	ftd	T	ftd	T	ftd
Kushmengel	101013.3	0342915.3	1363	sheslu	01-10	monoconical	15/02/08	17/02/08	35	46	4.05	31	1.55	39	1.95	12	0.6
Tsetse	100852.0	0343116.3	1471	Amerti	01-09	monoconical	22/02/08	24/02/08	23	40	3.5	19	1.05	`32	1.77	9	0.5
Alubo	101868.1	0344950.1	1288	Awshend	01-10	monoconical	30/02/08	02/03/08	32	71	5.15	15	0.75	28	1.4	11	0.55
Komeshiga 26	100044.5	0344122.8	1468	Qatana3	01-15	monoconical	09/03/08	11/03/08	49	62	3.7	23	0.76	48	1.6	14	0.46
Komeshiga27	100310.6	0347086.1	1390	selga	01-14	monoconical	19/03/08	22/03/08	66	84	5.35	55	1.96	53	1.89	8	0.28
abrahamo	099940.3	0345059.7	1493	Fomeshio	01-15	monoconical	29/03/08	01/04/08	80	135	7.16	46	1.53	67	2.23	6	0.2
Total					73		46	285	438	4.95	189	0.056	267	0.079	60	0.0178	

Table 4: Mean PCV value between infected and uninfected Bovine of Asossa district

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X^2	p-value
Infected	85	21.6	3.20	1836	25.37	0.000
Uninfected	315	24.32	2.22	7662		
Total	400	23.75	3.97	9498		

Table 5: Proportion of anemia in infected and uninfected Bovine population of Asossa district

Status	anemia	Frequency	Percent/%	Percent share per strata
Infected	Anemic	66	16.5	77.6
	Non-anemic	19	4.75	22.35
Non-infected	Anemic	150	37.5	47.6
	Non-anemic	165	41.25	52.38

Table 6: Prevalence of bovine trypanosomosis against the various risk factors in Asosa district

Risk factors	No. examined	No. positive	Prevalence (%)	x^2	p-value
Sex					
Male	166	39	23.5	0.85	0.35
Female	234	46	19.6		
Total	400	85	21.25		
Age group (years)					
<2	72	15	20.8	0.110	0.946
2 – 7	282	61	21.6		
> 7	46	9	19.56		
Total	400	85	21.25		
Body conditions					
Good	108	10	9.25	32.75	0.000
Medium	195	35	17.94		
Poor	94	40	41.2		
Total	400	85	21.25		

trypanosomosis between good and poor body conditioned animals with highest prevalence in poor body condition category.

Entomological Findings: A total of 1239 tsetse and biting flies were caught during the study period from different sites. Out of the total, 723 (58.35 %) were belonging to tsetse of the genus *Glossina*, followed by 189 (15.25%) *Stomoxys* and 267(21.54 %) *Tabanid* and 60 (4.84%) *Haematopota*.Only *Glossina* sub.*morsitans*were identified in the survey site with the overall apparent density of 4.95 F/T/D (Fly/trap/day). The highest fly density were observed in Abrhamo peasant association 334 (11.13 F/T/D) and the lowest recorded in Komishega-26 196 (6.53 F/T/D) (Table 3).

DISCUSSION

The present study revealed an overall prevalence of 85/400 (21.25%) in the study area. This finding was in agreement with earlier works ofMekuria and Gadissa [14] who reported 20.74% from Metekel zone who studied survey on bovine trypanosomosis and its vector in Metekel and Awi zones of North west Ethiopia. This result also concords with the reported bovine trypanosomosis prevalence of 24.7% from neighboring Mao- Komo special district [15].

The study showed that the infection was predominantly caused by *T. congolense 44/85 (*51.76%), *T.vivax*24/85 (28.23%), *T.brucei* 10/85 (11.76%) and mixed 7/85 (8.23%). This result was in agreement with prior reports of Abraham Zechariasand Zeryehun [16] who studied prevalence of major trypanosomes affecting cattle in the neighboring Asosa district of BenishangulGumuz Regional State, Western Ethiopia and found *T. congolense* proportionalprevalence of 66. 7%, Abraham Zecharias and Zeryehun [16] reported 61.4% prevalence of Trypanosomosis in selected districts of Arbaminch, Southern Ethiopia while [17] reported *T. congolense* proportionalprevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia; Bayisa *et al*. [18] recorded *T. congolense* proportional prevalence of 85% during his research on cattle trypanosomosis prevalence in Asossa district, BenishangulGumuz Regional State, Western Ethiopia.

The high proportion infection rate of *T. congolense*in cattle might be attributable to the high number of serodems of *T. congolense* relative to *T. vivax.* It could also be due to the possible development of better immune response to *T. vivax* by the infected animals as demonstrated by Jordan [7]. Further, it might be attributed to the efficient transmission of *T.congolense* by cyclical vectors than *T.vivax*in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T.vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia, respectively [19]. Different studies by Leak *et al.* [20] and Rowlands *et al.* [21] have indicated that *T. vivax*is highly susceptible

to treatment while the problems of drug resistance are higher in *T. congolense* and *T. congolense*is mainly confirmed in the blood, while *T. vivax*and *T. brucei*also invade the tissues [22].

There was a significant difference ($p<0.05$) in the prevalence of trypanosomosis among the study sites and body condition. This result is in agreement with previous reports Leak *et al*. [20], Mihreteaband Mubarek [23] and Bekele and Nasir [25].

The overall anemia prevalence in the studied district was 54% (216/400). The anemia prevalence was significantly higher in trypanosome infected cattle (77.64%) than in non-infected cattle (47.67%) ($P <0.05$). This is in concordance with previous results from different researchers [5,2425, 26]. Out of 54% anemia prevalence, 16.5% (66/400) was trypanosome infected animals. Nonetheless, 37.5% (150/400) of non-infected animals were found to be anemic (PCV <24). This indicates the fact that other factors such as gastrointestinal parasitism, nutritional deficiencies, fasciolosis and vector-borne diseases could affect the PCV value of cattle [27].

This study revealed that 4.75% (19/400) of the cattle were infected by trypanosome; however, their PCV was laid in the normal range. This might be attributed to the capability of infected cattle to maintain their PCV within the normal range for a certain period of time. It could also be possibly due to inadequacy of the detection method used [11] other anemia causing diseases [27] or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of the animals [27].

The overall mean PCV value for examined animals was 23.75±3.97. The mean PCV value of the infected animals was significantly lower (21.6±3.20) than that of uninfected animals (24.32±2.22). This result is in alignment with previous works [15, 18].

*Glossinamorsitans*sub*morsitans*was the only tsetse fly caught and its mean apparent density measured as f/t/d was 4.95. It accounts for 723 (58.35%) out of the total flies caught. In addition, other mechanical transmitters of trypanosomosis such as *Stomoxys* 189 (15.25%), *Tabanid* 267 (21.51%) and *Haematopota* 60(4.84%) were recorded. The current findings were in consistent with previous works ofSolomon and Fitta[28] at MetekelAwi zones of Northwest Ethiopia, who reported 6.49 f/t/d and 0.65 f/t/d for tsetse and biting flies, respectively. It was also in agreement with findings of NTTICC[6] at BureIluababor 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 fly, *Stomoxys*and *Tabanus*, respectively.

This result was also consistent with the previous findings of NTTICC [6] at neigbouringMandura districts of western Ethiopia which was reported to be 3.59 & 1.16 f/t/d; 0.15, 0.20 & 4.5 f/t/d; 0.02, 0.05 & 0.33 f/t/d ; 0.014, 1.38 & 4.5 f/t/d) for tsetse fly, *Stomoxys, Tabanus* and *Haematopota*, respectively. Similarly, It was also in consistent with the previous findings of NTTICC [6] at neigbouringDangur districts of western Ethiopia which was reported to be 1.14 f/t/d; 4.04 & 0.09 f/t/d ; 3.84 & 0.04 f/t/d, 0.4& 0.6 f/t/d) for tsetse fly, *stomoxys, tabanus* and *haematopota*, respectively.

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