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Prevalence of Bovine Trypanosomosis, Identification of the Vectors and Associated risk factors in Asossa District of Benishangul Gumuz Regional State, Western Ethiopia.

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Abstract: A cross-sectional study was carried out from December 2018 to June 2019 in Asossa district of Benishangul Gumuz Regional State, Western Ethiopia to determine the prevalence of bovine trypanosomosis, identification of circulating trypanosome species, vectors and associated risk factors. Blood samples were collected from a total of 250 cattle and examined using buffy coat technique. Overall 250 (4.8%) bovine trypanosomosis prevalence was recorded. The major species of Trypanosoma identified include; Trypanosoma congolense (58.33%), Trypanosoma vivax (25%), Trypanosoma brucei (8.33%) and mixed infection accounted for 8.33%. Mean packed cell volume (PCV) value of the infected animals was lower (22.33% ± 1.99) than uninfected animals (26.95% ± 2.9) and the variation was statistically significant (P< 0.05). Overall, anemia prevalence of 27.6% (69/250) was recorded and it was significantly higher (66.66%) in infected cattle than in non-infected (25.63%). Significant difference was not observed between sex groups and age categories (p>0.05) but there was significant difference in the prevalence of trypanosomosis among study sites and body conditions (P< 0.05). Glossina morsitans sub morsitans was the only tsetse fly caught and its mean apparent density measured as fly/trap/day was 0.39. In addition, mechanical vectors of trypanosomosis such as Tabanus (0.26f/t/d), Stomoxys (0.23 f/t/d),) and Haematopota (0.13 f/t/d) were identified. In conclusion, the result of the current study shows lower prevalence of Trypanosomosis, compared to the previous studies. Therefore, continuous and strategic control measures should be carried out to eliminate this economically important disease.

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Key words: Asossa, Trypanosomosis, Tsetse fly, prevalence, Risk factors

1. 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 (Azage and Alemu, 1997). Official figures give 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 (CSA, 2003).

Tsetse fly infest 10 million km^2 potentially productive land of Africa between 14 ^{0} N and 29 ^{0} S (Radiostits, 2006). 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 (Ford *et al*., 1976) based on 1500 masl, breeding limits in the south and southwestern valley of the country. Langridge (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 33^0 and 38^0 E and latitude 5^0 and 12^0 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 with Tsetse fly (NTTICC, 1996).

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 flies to spread to more populated areas and

the deforest land covered with savannah grass consequently newly invade by morsitans group (Jordan, 1986).

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 (Getachew A. and Yilma J., 1996). 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 (Radostits, 2006).

Mortality and the morbidity rate can be high and 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 (Enwezor *et al*., 2009). The disease distribution over 10 million km^2 of potentially productive land of Africa. The risk falls between 15^0 N and 29^0 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 (NTTICC, 2001) in Ethiopia. Therefore, the present study designed to assess the prevalence of bovine trypanosomosis, associated risk factores, to identify vectors of trypanosomosis and their density, factors and to address possible control and prevention measures.

1. Materials and Methods

1.1. Study area

Assosa district is located in Benishangul Gumz Regional State, west Ethiopia and it is 661 km away from Addis Ababa. The study was conducted from December 2018 to June 2019 in 5 PAs namely kushimengel, Abramo, Megelle39.Megelle32 and komoshiga 27 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 9^0 and 10^0 N, longitude 034^0 and 035^0 E and Altitude 1400 -1570 masl. With the lowest temperature 19^0c and the highest was being 34^0c. 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 & 34,710 Poultry (ADARDO, 2006). 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 (BG. BARD, 2004). 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 (Radostits, 2006).

1.2. 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: $n= (1.96)^2$ x Pexp (1-Pexp) $/d^2$ (Thrustfield, 2005).

Where: n = the required sample size for the district
Pexp = expected prevalence. (20% in this case)
d^2 = desired absolute precision (5% in this case)

Therefore: $n= (1.96^2) \times 0.2(1-0.2)/0.5^2=245$

Accordingly total of 250 cattle were randomly sampled from five peasant associations to determine the prevalence of trypanosomosis.

1.3. Study animals

A total of 250 local breed cattle of different age groups and sex categories were randomly sampled from the district of the study sites to determine the prevalence of trypanosomosis parasite.

1.4. Fly survey

During the study 50 monoconical, traps was deployed and 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 and after the flies captured in the collecting cage they were sorted by sex and species and the data was 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 (Langridge, 1976; Fischer and Say, 1989). 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 hypopegeum.

1.5. Blood sample collection

The farmers were brings their cattle to the animal health clinic for examination 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 (Murray and Dexter, T.M. (1988).

1.6. Laboratory Analysis

2.6.1. Hematological examination

The blood samples were centrifuge at high speed (12,000 rpm) for 5 minutes. Finally, the packed cell volume (PCV) value was 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 (Uilenberg,1998).

2.6.2. Parasitological examination

More sensitive technique utilizes centrifugation in micro-heamatocrit then 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 was 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 (Murray, 1977).

2. Data Management and Analysis

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

4. Results

Out of total animals examined (n=250), 12/250 (4.8%) were infected with trypanosomes. The prevalence in terms of trypanosome species were 2.8 % *T. congolense*, 1.2 % *T.vivax*, 0.4% T. brucei and 0.4% mixed infection. The proportion of trypanosome species was 7/12(58.33 %) *T. congolense*, *3/12 (*25%) *T. vivax, 1/12(8.33%) T. brucei and 1/12(8.33%) mixed* (Table 2).

Table 1: Prevalence of trypanosomosis by peasant associations

Peasant association (PA)	Sample size	No positive	Prevalence %	X^2	p- value
Kushimengel	25	1	4	11.101	0.049
Megelle 39	65	4	6.15		
Megelle 32	60	2	3.33		
Komeshiga 27	55	4	7.27		
Abrahamo	45	1	2.22		
Total	250	12	4.8		

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

Peasant association (PA)	Sample size	Parasites identified				Total	X^2	p- value
		T.congolense	T.vivax	T.brucei	mixed			
Kushimengel	25	1	0	0	0	1	254.04	0.000
Megelle 39	65	2	2	0	0	4		
Megelle 32	60	1	0	0	1	2		
Komeshiga 27	55	2	1	1	0	4		
Abrahamo	45	1	0	0	0	1		
Total	250	7	3	1	1	12		
Prevence	%	2.8	1.2	0.4	0.4	4.8		

The mean PCV value for whole examined animals was 26.73 ± 2.8 SE. However, the mean PCV value for uninfected animals was 26.95 ± 2.9 SE and mean PCV value of the infected animals was 22.33± 1.99 SE. The mean PCV values of cattle were significantly (P = 0.000) influenced by trypanosome infection as 22.33 % and 26.95 % PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 4). The overall anemia prevalence in the studied district was 27.6 % (69/250). The anemia prevalence was significantly higher in trypanosome infected cattle (66.66%) than in non-infected cattle (25.63%) (P <0.000). Out of 27.6 % anemia prevalence, 11.59% (8/250) was trypanosome infected animals. However, large number of animals 24.4% (61/250) had anemia (PCV < 24) without having trypanosome infection. Some animals 1.6% (4/250) were infected by trypanosome but their PCV was found normal (Table 4).

Table 3: Mean PCV value between infected and uninfected Bovine of the study site.

Status	Frequency	Mean PCV (%)	SE	Overall PCV	X^2	p-value
Infected	12	22.33	1.99	268		
Uninfected	238	26.95	2.9	6415	25.37	0.0001
Total	250	26.73	2.8	6683		

Table 4: Proportion of anemia in infected and uninfected Bovine population of the study site

Status	anemia	Frequency	Percent/%	Percent share per strata
Infected	Anemic	8	3.2	66.66
	Non-anemic	4	1.6	33.33
Non-infected	Anemic	61	24.4	25.63
	Non-anemic	177	70.8	74.36

The highest trypanosomosis prevalence (5%) was recorded in 2-7 years old and >7years older animals whilst the lowest prevalence (4.29%) was in<2years old. Slightly higher prevalence was registered in females 4.83% than in males 4.76 %, which was statistically non-significant. Trypanosomosis was recorded across the study sites with the highest (7.27 %) prevalence in Komeshiga 27 peasant association and the lowest 2.22 % in Abramo peasant association. Trypanosomosis prevalence was statistically significant among study sites. There was a significant difference (P < 0.005) in the prevalence of trypanosomosis between good and poor body conditioned animals with highest prevalence in poor body condition category.

A total of 101 Tsetse and biting flies were caught during the study period from different sites. Out of the total, 39 (38.61%) were belonging to tsetse genus Glossina, followed by 26 (25.74%) Tabanid and 23(22.77%) stomoxys and 13(12.87%) Haematopota. Only Glossina m.sub.morsitans was identified in the survey site with the overall apparent density of 0.39F/T/D (fly/trap/day). The highest fly density was observed in Megelle 39 peasant association 11 (0.55 F/T/D) and the lowest recorded in Megelle 32, 5(0.25F/T/D) (Table 6).

Table 5: prevalence of bovine trypanosomosis with associated risk factors

Risk factors	No. examined	No. positive	Prevalence (%)	χ^2	p-value
Sex					
Male	105	5	4.76		
Female	145	7	4.83	0.52	4.21
Total	**250**	**12**	**4.8**		
Age group (years)					
< 2	70	3	4.29		
2 – 7	140	7	5.00	0.53	0.4
> 7	40	2	5;00		
Total	**250**	**12**	**4.8**		
Body conditions					
Good	105	2	1.9		
Medium	115	5	4.34	32.75	0.000
Poor	30	5	16.66		
Total	**250**	**12**	**4.8**		

Table 6: Vectors of trypanosomosis identified from the study sites

village	Tsetse fly			Biting fly					
	Glossina m.submorsitance			stomoxys		Tabanus		Hematopota	
	M	F	ftd	T	ftd	T	ftd	T	ftd
Kushimengel	2	6	0.4	5	0.25	5	0.25	3	0.15
Megelle 39	3	8	0.55	7	0.35	`8	0.4	2	0.1
Megelle 32	1	4	0.25	2	0.1	3	0.15	2	0.1
Komeshiga 27	4	5	0.45	6	0.3	6	0.3	2	0.1
Abrahamo	1	5	0.3	3	0.15	4	0.2	4	0.2
Total	11	28	0.39	23	0.23	26	0.26	13	0.13

5. Discussion

The present study revealed an overall prevalence of 12/250 (4.8%) in the study area. This finding was in agreement with earlier works of (Dawit, T., et al., 2017) who reported 5.44% from Assosa District while studying Survey on prevalence of bovine trypanosomosis in Assosa district of the Benishangul Gumuz regional state, western Ethiopia. This result also concords with the reported bovine trypanosomosis prevalence of 6.25% from neighboring Bambasi district (Mubarik, K. et al., 2017).

The study showed that the infection was predominantly caused by *T. congolense 7/12 (*58.33%), *T. vivax* 3/12(25%), T. *brucei* 1/12(8.33%) and mixed 1/12(8.33%). This result agrees with previous report of (Mubarik, K., et al., 2017) who recorded proportional prevalence of T. *congolense of 56%* while studying prevalence of bovine trypanosomosis in neighbouring Bambasi district of Benishangul Gumuz regional state. This result was in agreement with prior reports of (Mekuria,S., et al., 2011) who studied prevalence of major trypanosomes affecting cattle in the Assosa district of Benishangul Gumuz Regional State, Western Ethiopia and found *T. congolense* proportional prevalence of 66.7%; (Abraham Z. et al.,2012) worked on the prevalence of bovine trypanosomosis in selected district of Arba Minch, Sothern Ethiopia and reported *T. congolense* proportional prevalence of 61.4%; (Biyazen, H. et al., 2014) reported *T. congolense* proportional prevalence 63.64% during his work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, Western Ethiopia; Bayisa, K et al.,2015 demonstrated *T. congolense* proportional prevalence of 85% during his research on cattle trypanosomosis prevalence in Asossa district, Benishangul Gumuz 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 (Leak, S., *et al*., 1993). In addition, 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 (Langridge WP.,1976; Leak, S *et al*.,1999). Different studies (Leak, S *et al.,* 1993; G. J. Rowlands *et al.,* 1995) 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 (L. E. Stephen, 1986).

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 (Mihreteab, B *et al*., 2011; 29-31, Ayele, T., et al., 2012; Lelisa, K *et al*., 2015). The overall anemia prevalence in the studied district was 27.6% (69/250). The anemia prevalence was significantly higher in trypanosome infected cattle (66.66%) than in non-infected cattle (25.63%) ($p <0.05$). This is in concordance with previous results from different researchers (Mihret et al., 2007; Bekele, M. et al.,2011, Biyazen, H et al., 2014). Out of 27.6% anemia prevalence 3.2% (8/250) was trypanosome infected animals. Nonetheless, 24.4% (61/250) 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 (P. van den Bossche et al., 2001).

This study revealed that 1.6% (4/250) of the cattle was infected by trypanosome even though 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 (Murray, M *et al*., 1988), other anemia causing diseases (P. van den Bossche et al., 2001), 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 (P. van den Bossche et al., 2001).

The overall mean PCV value for examined animals was 26.73 ± 2.8 SE. The mean PCV value of the infected animals was significantly lower (22.33 ± 1.99 SE) than that of uninfected animals (26.95 ± 2.9 SE). This result is in alignment with previous works (Ali, D. Eta., 2011, Mulaw, S *et al*., 2011, Bayisa, K., et al., 2015). *Glossina morsitans sub morsitans* was the only tsetse fly caught and its mean apparent density measured as f/t/d was 0.39. It accounts for 39 (38.61%) out of the total flies caught and in addition other mechanical transmitters of trypanosomosis such as Tabanid 26(25.74%), stomoxys 23 (22.77%), and Haematopota 13(12.87%) were recorded. The current findings were not in consistent with previous works of (Solomon, M et al., 2010) 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 was also lower than findings of (NTTICC, (2004) 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 fly, *Stomoxys* and *Tabanus*, respectively. This might be due to the establishment of Assosa tsetse fly and trypanosomosis surveillance and control center under national institute for control and eradication of tsetse fly and trypanosomosis which carried out control measures like pour on the back of animals by deltamethrin 1%, trap and target technology and ground spray.

The present study was also consistent with the previous findings of (NTTICC, 2012-2014) at neighboring Mandura 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 agreed with the previous findings of (NTTICC, 2012& 2014) at neighboring Dangur 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.

6. Conclusion

The most common trypanosomes species identified in the study sites was *T. congolense* followed by *T. vivax*. The animal parameters such as sex and age were not found to be a risk factor; however, study site and body conditions were identified as risk factors. The mean PCV value of infected animals was significantly lower than that of uninfected animals indicating the adverse effect of trypanosomosis on the PCV profile of cattle. Trypanosomes were not detected in some anemic cattle indicating the occurrence of other causes of anemia in the area. *G. moristans sub morsitans* was the only tsetse fly species discovered in this study. Other mechanical transmitters of trypanosomosis such as stomoxys, tabanus and haematopota were recorded in the area. In general trypanosomosis is an economically important disease threatening the health and productivity of cattle in Assosa district. Therefore, appropriate control strategies should have to be designed and implemented to minimize its effect on livestock production in the studied district.

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