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Vector identification and prevalence of bovine trypanosomosis in Oda Buldigilu district of Benishangul Gumuze regional state, Western Ethiopia

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Abstract
Trypanosomosis is one of the major obstacles to livestock development and agricultural production in Ethiopia. The disease is transmitted by the bit of *Glossina* species and other biting flies. A cross-sectional study was conducted to identify the density and diversity of vectors and also the prevalence of bovine trypanosomosis from January, 2016 to May, 2016. The study was conducted in five kebeles of the Oda Buldigilu district of Benishangul Gumuze regional state, Western Ethiopia. Standard methods of sampling and identification were employed for both entomological and parasitological examination. One species of the genus *Glossina (Glossina morsitans submorsitans*) and three genera of biting flies (*Stomoxys*, *Tabanus* and *Haematopota*) were caught and identified. The overall apparent density of flies caught was 226(2.05) flies per trap per day), of which 67 (0.61 flies per trap per day) was *Glossina* species. Out of a total 395 cattle examined, 47 (11.89%; 95% CI: 8.88-15.51%) were found infected with trypanosomes. Three species of trypanosome were detected in the study area namely *T. congolense* 26(55.31%), *T. vivax* 18*(*38.29%), *T. brucei* 1(1.12%) and 2(4.28%) mixed infection. The prevalence of trypanosomosis was significantly affected by origin (PA'S), body condition score and PCV (P<0.05). Trypanosomosis significantly affected the status of anemia or packed cell volume (P<0.05). The mean PCV of parasitemic animals were significantly lower than that of aparasitemic ones (P<0.05). The relative abundance of *Glossina* species caught and trypanosome detected confirmed the continuous challenge of the disease in the areas. We recommend to reinforce community based vector and parasite control or prevention strategy in the study area.

Keywords: Biting flies, Buffy Coat, Tsetse fly, Packed Cell Volume, Trap, Trypanosomosis

1. Introduction
Trypanosomosis is a complex disease caused by the genus Trypanosoma which resides in the blood and other tissues of domestic and wild animals including human [1]. The disease can be expressed as chronic or acute which could cause sudden death in susceptible hosts if left untreated. Its distinguishing factors include intermittent fever, progressive anaemia, and emaciation [2].

Different trypanosomes of veterinary importance were reported in Ethiopia in various hosts. Langridge [3] reported trypanosomes in cattle, sheep and goats comprising of *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*, and *Trypanosoma evansi* in camels. The findings of Abebe [4] also showed *Trypanosoma equiperdum* in horses. Prior research works indicated that trypanosomosis is transmitted by tsetse flies and other biting flies such as stomoxys, Tabanus, Haematopota and Chrysops [5]. Trypanosomosis is a main limitation to agricultural production impeding the national economy in Ethiopia [6].

Swallow [7] showed that trypanosomosis increases calf mortality and calving intervals; however, it reduces milk production off-take and draught efficiency. In addition to its direct adverse effects on affected cattle, it can also preclude rearing of animals in endemic areas leaving large amount of arable lands uncultivated. For instance tsetse infests 10 million square kilometers between latitudes 14ºN and 29ºS in thirty eight African countries. In Ethiopia alone about 240,000km^2 of land is infested with tsetse flies and excludes farmers from rearing livestock [6, 8] Trypanosomes infects many domestic and wild animals such as cattle, water buffalo, sheep, goats, camels, horses, donkeys, pigs, dogs, cats, waterbuck, lion, greater kudu, bushbuck and other species. In Africa, cattle are the main preferred host by tsetse fly as result become the most affected species by Trypanosomosis [1].

The host preferences of each trypanosome species may differ, but *T. congolense*, *T. vivax* and *T. brucei* has a wide host range among domesticated animals. *T. godfreyi* and *T. suis* occur in pigs. *T. simiae* appears to be most important in pigs, but it has also been reported by PCR in camels, horses and cattle [9]. In Ethiopia, tsetse flies are confined to the South west and North western region between longitude 33^0w and 38 ^{0}E and latitude 5^0 S and 12^0 N covering an area of 220,000 km^2. According to NTTICC [10] tsetse infested area of Benishangul Gumuz Regional State is about 31,000 km^2. The presence of animal trypanosomosis in the area where more than 90% of Crop production are dependent on animal drought power mainly on ploughing oxen is a major constraint to utilize large land resource which worsens insuring food security [11].

The genus *Glossina* is blood sucking flies that belongs to the family *Muscidae*. Tsetse flies are found exclusively on the African continent and are closely related to the vegetation which protects them from solar radiation and wind. In Ethiopia, there are five species of Glossina and all are important vectors for African animal trypanosomosis (nagana). They are *G. pallidipes, G.morsitanssubmorsitans, G. fuscipes, G. tachinoides* and *G. longipennis* [4] Livestock in Oda Buldigilu district plays substantial role in the livelihood of the farmer as they are integral to the agricultural activities through provision of milk and meat.

The knowledge of the status of the disease prevalence, its health impact on animals affected, its vector distribution and the associated risks are very important for understanding the epidemiology of the disease and to devise suitable control measures. In Oda Buldigilu, trypanosomosis was found to be one of the factors that hampered livestock rearing in most peasant associations. Therefore, the objectives of the present study were to determine the trypanosomosis prevalence, status of anemia and the apparent density of tsetse and biting flies ascribed in the transmission of trypanosomosis.

Materials and Methods

Study Area

The study was conducted in the Oda Buldigilu district, which is located in Asossa zone of Benishangul Gumuz Regional States, South Western Ethiopia. It is located on the 648 kilometers West of Addis Ababa, and 82 kilometers from neighbouring Mendi district of Western Oromya. Geographically, Oda Buldigilu town falls between $10^0$00'58.1''N latitudes and $35^0$12'38.8''E longitudes. The five peasant associations namely, Oda, Godare, Dalati, Kambodushu and Tulli are settlement areas. The total land area covers 31,025 hectare with an altitudes ranges from 650 to 1140 meter above sea level. The annual mean temperature ranges from 25ºC to 38ºC and receives annual rainfall greater than 900-1400ml. The human population of the district is 71,948. The livestock populations of the district were estimated to be 12,573 cattle, 1,045 sheep, 22,571 goats, 18,473 poultry, 2,572 donkeys and 57 mules. The farming system of the area was mixed farming where 90% of the total population engaged in agriculture. Crop and livestock sales are an important source of income for all wealth groups according to Bureau of Agriculture and Rural development (BOARD).

Study Design

A cross-sectional study was used to estimate the current prevalence of bovine trypanosomosis and vectors in the study area from January, 2016 to May, 2016. The peasant associations (PA's) were selected based on their accessibility to transport and information from the district's administrative body. Multistage sampling was used to sample animals, where, herds were selected from each PA's by simple random sampling as primary sampling units. From the selected herds, individual animals to be sampled were selected by simple random sampling techniques as secondary sampling units.

Study Animals and Sample Size

The study was conducted on 395 local cattle breed selected from five peasant associations (PAs). The body condition score was classified as poor, medium and good by observing the body condition of the animals in the field [12]. The ages of animals were also estimated by the dentition method [13] and from owner information. The PA's were selected purposively based on their accessibility to transport and information from the district's administrative body. The number of animals required for the study was determined using the formula given by Thrusfield [14], 95% confidence level was used by 50% expected prevalence and 0.05 desired absolute precision. Thus, 384 cattle were needed for the study. However, to increase the precision the number sampled animals were increased to 395.

Entomological Survey

A total of 55 monoconical traps was deployed in five peasant association of Oda Buldigilu district (11 in Oda, 10 in Godare, 10 in Dalati, 10 in Kambodushu and 10 in Tulli PA's) at approximate interval of 200-250m. All traps were baited with acetone, and cow urine filled in separate bottles. Each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards. After 48 hours of deployment, tsetse flies in the cages were counted and identified based on their habitat and morphology to the genus and species level. Other biting flies were also identified according to their morphological structures such as size and proboscis at the genus level [15]. Tsetse flies were sexed just by observing the posterior end of the ventral aspect of the abdomen using a hand lens. Male flies were identified by their enlarged hypopygium in the posterior ventral end of the abdomen. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day [6].

Parasitological Survey

Blood samples were collected randomly from each cattle into a capillary tube after piercing the ear vein by using a lancet. One end of the capillary tube was sealed and centrifuged at 12,000rpm for 5 minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces as buffy coat. Then PCV was measured using haematocrit reader. The capillary tubes were broken just 1mm below buffy coat and expressed on microscopic slide, mixed and covered with 22x 22mm cover slip. Then it was examined under 40x objective of a microscope using dark ground buffy coat technique to detect the presence of the parasite [16]. Buffy coat positive samples were stained by Giemsa's in thin blood smears, fixed with methanol for 5 minute and examined under oil immersion using 100 x objectives to identify the species of trypanosomes.

Data Analysis

Data obtained were stored in Microsoft Excel spreadsheet. The data were summarized and presented in tables and analyzed by using STATA version 12.0 for Windows (Stata Corp. College Station, TX).The density of fly population was

calculated by dividing the number of flies caught by the number of traps deployed and number of days of deployment and expressed as Fly/Trap/Day (F/T/D), [17]. The association between trypanosome infection and risk factors or variables (age, sex, body condition and origin/peasant association) were determined by univariable logistic regression. Those risk factors with *P*-value <0.25 by the univariable analysis were further analysed by multivariable logistic regression. Two sample student t-tests were used to compare mean PCV of study animals. A statistically significant difference between variables exists when $P<0.05$ at 95% confidence level (CI). The origin of the animals was categorized in to five peasant associations, body condition score was categorized in to three (poor, medium and good). The age of animals was categorized into two; less than or equal to two year are calf raised near the homestead and assumed to have less frequent contact with the vectors and greater than one year are adult animals raised far from home and went for grazing and watering in the grassland, bush and river side's which might get frequent contact with the vector. PCV value was analysed in two ways as categorical and continuous variables. PCV was categorized as anemic if it is less than 24% and normal if it is greater than or equal to 24%.

Results

Entomological Results

Only one species of *Glossina (G. morsitans submorsitans)* and three genera of biting flies (*Tabanus, Stomoxys and Heamatopota*) were identified during the entomological survey. The relative abundance of Glossina species and other biting flies were indicated in Table 1. The overall Glossina species caught per 48 hours with sex proportion in different peasant associations were shown in (Table 2).

Table 1: Relative abundance of Glossina and other biting flies in study areas PA's No of traps over all flies

Peasant associations	No of traps	Over all flies	Glossina species		Other biting flies			
			Gm	(F/T/D)	Stomoxy	Tabanus	Haematopota	(F/T/D)
Oda	11	57	26	1.18	1	27	3	1.4
Godare	10	33	15	0.75	0	16	2	0.9
Dalati	10	30	14	0.7	0	15	1	0.8
Kambo	10	68	5	0.25	3	51	8	3.1
Tulli	10	30	7	0.35	1	21	1	1.15
Total	55	226	67	0.61	5	130	15	1.36

GM= Glossina morsitans submorsitans, F/T/D= Fly/trap/day

Table 2: Proportion of male and female Glossina species on the study area

Peasant associations	Total number of Glossina	Male	Female
		Number (%)	Number (%)
Oda	26	8 (30.7%)	18 (69.2%)
Godare	15	4 (26.6%)	11 (73.3%)
Dalati	14	5 (35.7%)	9 (64.2%)
Kambo	5	2 (40%)	3 (60%)
Tulli	7	2 (28.5%)	5 (71.4%)
Total	67	21 (31.3%)	46 (68.7%)

Parasitological Results

Out of 395 cattle examined, the prevalence of Trypanosome infection in the area was 11.89% (95% CI=8.88-15.51%).The association between infection and study variables were shown in Table 3. Among the 47 cattle positive for trypanosomes, 26 (55.31%), 18 (38.29%), 1 (1.12%), and 2(4.28%) cases were caused by *T. congolense*, *T. vivax*, *T. brucei*, and mixed, respectively. High infection of trypanosome was recorded in Oda (30%), followed by Godare (14.04%) and the list was recorded in Dalati (6.33%). The infection was also significantly higher ($P<05$) in cattle with poor body condition (17.9%). In addition, infection with trypanosome was slightly greater with age greater than or equal to 2 years (12.6%) and in female animals (13.6%) although not significant ($P>0.05$).

Table 3: Univariable logistic regression model for origin (PA's), body condition, age and sex with buffy coat result

Risk factors	Risk category	Number Examined	Number Positive	Prevalence% (95% CI)	OR (95% CI)	*P*-value
Origin of animals (PA's)	Oda	60	18	30(18.27-41.73)	Reference	-
	Godare	57	8	14.04(4.91-23.16)	0.38(0.15-0.96)	0.042
	Dalati	126	8	6.35(2.06-10.64)	0.15 (0.06-0.39)	0.000
	Kambo	91	8	8.79(2.92-14.66)	0.22 (0.09-0.56)	0.001
	Tulli	61	5	8.20(1.23-15.16)	0.20(0.07-0.60)	0.004
Body condition	Good	117	8	6.84(2.23-11.44)	reference	-
	Medium	150	17	11.33(6.23-16.43)	1.74 (0.72-4.19)	0.22
	Poor	128	22	17.19 (10.61-23.77)	2.83 (1.21-6.63)	0.02
Age	<2year	71	6	8.45(1.92-14.99)	1.57 (0.64-3.85)	0.33
	>=2year	324	41	12.6 (9.02-16.29)		
Sex	Male	118	10	8.48(3.41-13.53)	1.67(0.80-3.47	0.17
	Female	277	37	13.36(9.33-17.38)		

CI=confidence interval, OR=odds ratio, PA's=Peasant associations

Multivariable logistic regression analysis showed the presence of significant difference between the different origins (in almost all kebele's except Godare) and also between PCV status. However, body condition and sex didn't show any significant difference in the multivariable analysis (Table 4).

Table 4: Multivariable logistic regression model for origin (PA's), body condition, sex and PCV with buffy coat result

Risk factors	category	Odds ratio (95% CI)	P-value
Origin (PA's)	Oda	Reference	-
	Godare	0.43(0.16-1.14)	0.09
	Dalati	0.21(0.08-0.53)	0.001
	Kambo	0.310.12-0.81)	0.02
	Tulli	0.20(0.07-0.59)	0.004
Body condition	Good	Reference	-
	Medium	1.59(0.63-4.04)	0.33
	Poor	2.26(0.90-5.69)	0.08
Sex	Male Female	1.60(0.74-3.46)	0.23
PCV	Anemic Normal	0.49(0.25-0.97)	0.04

CV=packed cell volume, CI= confidence interval, PA's= peasant associations

Hematological Findings

The mean PCV of anemic (19.06 + 0.28SE) and normal (27.59 ± 0.23SE) cattle showed significant difference (P<0.05). The proportion of anemic animals infected with the parasite were significant (P<0.05) as compared with non-anemic positive animals (Table 5).

Table 5: Univariable logistic regression analysis of anemic and normal cattle's in relation to buffy coat result

Category	Number examined	Number positive	Proportion (95% CI)	Mean PCV ± SE	OR (95% CI)	*P*-value
Anemic (<24)	165(41.77%)	29	17.56(11.73-23.42)	19.06 ± 0.28	0.40 (0.21-0.74)	0.004
Normal (≥24)	230 (58.23%)	18	7.83(4.34-11.32)	27.59 ± 0.23		
Total	395(100%)	47	11.90(8.88-15-51)	24.03(23.48-24.57)		

PCV= packed cell volume, CI= confidence interval, SEM=standard error of the mean

Packed cell volume of parasitemic animals fall in the range of 9-37%, while in aparasitemic cattle the PCV was in the range of 11-45%. The overall mean PCV in the examined animals was 24.03±0.28SE. The mean PCV of parasitemic animals were significantly lower than that of aparasitemic ones (P<0.05) table 6. Among 348 non parasitized animals, 39.08% had PCV<24 and from the 47 parasitized animals 61.70% had PCV<24. The prevalence of parasitemic and anemic animals (61.70%) were significantly higher as compared to the parasitemic and nonanemic 39.08% (P<0.05), table 6. Linear regression analysis indicated that a unit variation or increase of the buffy coat result leads to a reduction in the PCV by 2.35% (P<0.05) (Table 7).

Table 6: Mean PCV value of parasitemic and aparasitemic animals

Infection status	No examined	Mean PCV (%)	95% CI	SEM	T-test	P-value
Parasitemic	47	21.96	20.30-23.61	0.82	2.77	0.006
Aparasitemic	348	24.31	23.73-24.88	0.29		
Total average	395	24.03	23.48-24.57	0.28		

PCV= packed cell volume, CI= confidence interval, SEM=standard error of the mean

Table 7: Linear regression analysis of PCV and buffy coat result

PCV	Coefficient	Standard error	P-value	95% CI
Buffy coat result	-2.35	0.85	0.006	-4.02- (-0.68)
Constant	24.31	0.29	0.000	23.73-24.88

PCV= packed cell volume, CI= confidence interval

Discussion

In this study, the entomological survey revealed that one species of *Glossina* (*G. morsitans submorsitans*) and three genera of biting flies (*Tabanus, Stomoxys and Hematopota* species) were detected in the selected five PA's of Oda Buldigilu district. *Glossina morsitans submorsitans* have been reported in the Western and South Western parts of the country [18, 19, 20]. The overall apparent density of Glossina species was 0.61 flies per trap per day. The apparent densities of Glossina species were 1.18, 0.75, 0.7, 0.25, 0.35 flies per trap per day in Oda,Godare, Dalati, Kambo and Tulli, respectively.

In the Western part of the country, various reports indicated that the apparent density of Glossina species ranges from 0.3 to 24.4 flies per trap per day [20, 21, 22]. This wider range might be due to differences in the density of vegetation cover, presence or absence of rivers and favourable climatic conditions. According to Leak [5], vegetation is vital for providing suitable conditions; the savanna, forest and riverine tsetse flies concentrate in the wooden savanna, in the bush vegetation and near the edge of the river, where the vegetation is dense, respectively [23]. A higher number of female tsetse species 46(68.7%) were caught than male 21(31.3%), which is in line with various reports from the country [18, 19, 20, 22]. This could be attributed to the longer lifespan of female compared to male Glossina [5].

The prevalence of bovine trypanosomosis in Oda Buldigilu was 11.89% (95% CI=8.88-15.51%). The current finding was significantly lower than the reports (27.5%) from Southern Ethiopia, [24]. This is because of the ongoing tsetse and trypanosome prevention and control actions in Oda Buldigilu districts using different techniques such as the use of

prophylactic and curative drugs, vector control using traps and insecticide impregnated targets through community participation. The current finding is similar with the findings from different part of Ethiopia for instance 14.2% prevalence from Humbo District [25] and 10.8% prevalence from Hawa-Gelan district [22]. However, the finding was significantly higher than the reports from Didesa District of Oromia Regional State with prevalence of 4.86% [26], Arbaminch District, southern Ethiopia with prevalence of 4.43% [27], Yayo district, Illubabor Zone, western Ethiopia with a prevalence of 3.39% [20] and Metekel zone of Benishangul Gumuz regional state with a prevalence of 5.43% [19].

The current study confirmed that the occurrence of trypanosome infections in cattle is clearly linked to the presence of potential fly vectors (Glossina species and other biting flies). In this study three species of trypanosomes were identified, namely *T. congolense* (55.31%), *T. vivax* (38.29%), *T. brucei* (1.12%) and mixed infection (4.28%). These species were widespread in most parts of Western and South Western Ethiopia [4, 20, 22, 28, 29, 30]. Trypanosoma congolense was found to be the most prevalent compared to other species. This was in line with various reports from the Western and Southern parts of the country [20, 24, 27, 29, 30, 31].

Among the risk factors considered in the univariable analysis, origin and body condition score has shown a significant association with the occurrence of trypanosomosis (Table 3). This is because the difference in the agroecology Oda Kebele has a higher number of river, forest and grassland or bush cover than all the others according to our field observation. Moreover, poor body condition animals were 2.83 times more affected by trypanosomosis than medium and good body conditions (P<0.05). Sex and age did not show any significant difference (P>0.05).

In the multivariable analysis, origin (PA's) and PCV had shown a significant association with trypanosome infection (table 4). Infection with trypanosomosis was significantly associated with anemia or reduced PCV (Table 5). Among anemic cattle 17.56% were diseased with trypanosomosis. The current finding is higher than the reports by different researchers from different part of the country [20, 32, 33]. Body condition and sex didn't show any significant difference (P>0.05) in trypanosome infection. Similar findings were reported by different researchers from different areas of the country [20, 24, 30, 33].

The mean PCV of parasitemic animals were significantly lower than that of aparasitemic ones (P<0.05) table 6. The prevalence of parasitemic and anemic animals (61.70%) were significantly higher as compared to the parasitemic and nonanemic 39.08% (P<0.05), table 6. Similar findings were reported by [20, 24, 30]. Packed cell volume has been demonstrated to be a good indicator of trypanosomal infection [34]. The aparasitemic cattle with PCV<24% in the current study could be either due to the low sensitivity of buffy coat techniques in chronic cases of trypanosomosis or it might be due to other factors like poor nutrition and other diseases particularly parasitic diseases which cause anemia [35]. Moreover, linear regression analysis of PCV and parasitemic cattle also confirmed a unit variation/increase in the buffy coat result caused a reduction of the PCV value by 2.35%, (P<0.05) (Table 7). Similar findings were reported in a study done in the Southwestern part of the country [20].

The results of the current study identified one species of *Glossina* and 3 biting flies that serve as potential vectors for the transmission of trypanosomes. Similarly, three species of trypanosomes were detected in the area. The prevalence of trypanosomosis investigated in the area and the associated vectors confirmed the continuous challenge of trypanosomosis in the area. Considering the above facts, the ongoing tsetse and trypanosomosis prevention and control strategy should be strengthened in the area.

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