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Tsetse fly density and trypanosoma infection rate in Bedele and Dabo Hana districts of Buno Bedele Zone, Southwest Ethiopia

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Abstract

Background Trypanosomiasis is an infectious disease caused by parasitic protozoa of the genus *Trypanosome* and primarily transmitted by tsetse flies. This study aimed to determine the density of tsetse flies and the rate of trypanosome infection in the Bedele and Dabo Hana districts of the Buno Bedele Zone in Ethiopia.

Results A cross-sectional study was conducted from January to February 2023 to catch tsetse flies, determine tsetse density, and estimate the trypanosome infection rate. We used 100 traps (40 NGU, 30 pyramidal, and 30 biconical) to catch the flies. The following standard procedures were followed to identify the specific trypanosome species in the collected tsetse flies: The flies were dissected, and the salivary glands were removed. We placed the salivary glands in a drop of saline solution on a microscope slide. A coverslip was placed over the salivary glands, the slide was examined under a microscope, and the trypanosomes were identified based on their morphology. A total of 3,740 tsetse flies were captured from 100 traps, resulting in an overall apparent density of 18.7 flies per trap per day. Within the study area, only one species of tsetse fly, *Glossina tachinoides*, was identified. Of the 1,320 dissected *Glossina tachinoides*, 1.82% were found to be infected with *trypanosome* parasites. Among these infections, 58.33% were attributed to *Trypanosoma congolense*, while the remaining 41.67% were caused by *Trypanosoma brucei*. The infection rate of *trypanosomes* was significantly higher in female tsetse flies (87.5%) as compared to male flies (12.5%). Furthermore, a significantly higher infection rate was observed in flies older than 20 days (83.33%) and in hunger stage 1 flies (58.33%) compared to hunger stages 2, 3, and 4.

Conclusions This study highlights the necessity of implementing control and suppression measures targeting the vector (tsetse flies) and the parasite (trypanosomes) to effectively manage and prevent pathogenic animal trypanosomiasis.

Keywords Tsetse fly, Fly density, Trypanosomiasis; infection rate, Ethiopia

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Introduction

Trypanosomiasis is a severe disease that poses a significant threat to both humans and animals. Caused by a protozoan parasite, this infectious disease is transmitted cyclically by tsetse flies and mechanically by various biting flies [1, 2]. The parasite targets the central nervous system, leading to a range of debilitating symptoms such as fever, anaemia, and weight loss. In severe cases, the disease can progress to coma and ultimately result in death [3, 4].

In Ethiopia, Trypanosomiasis affects an extensive area of approximately 220,000 km² of fertile land [5, 6]. The disease is transmitted by five species of tsetse flies that are prevalent in the country: *Glossina pallidipes*, *G.m. sub-morsitans*, *G. f. fuscipes*, *G. tachinoides*, and *G. longipennis*. Ethiopia is a home to five species of trypanosomes, namely *T. congolense*, *T. vivax*, *T. b. brucei*, *T. evansi*, and *T. equiperdium* [5, 6]. Among the African countries affected by trypanosomiasis, Ethiopia bears a significant burden, with an estimated 30% of its cattle population being affected [7, 8].

Despite the well-documented prevalence of trypanosomiasis in Ethiopia, there remains a dearth of data concerning tsetse fly distribution patterns and infection rate of *trypanosomes* in tsetse flies. Therefore, the objective of this study is to determine the density of tsetse fly and infection rate of *trypanosomes* in the Bedele and Dabo Hana Districts of the Buno Bedele zone in Ethiopia.

The study aims to contribute valuable insights into the dynamics of trypanosomiasis in Ethiopia and elsewhere, where trypanosome is highly prevalent. The finding provides information to design targeted control and prevention strategies, ultimately leading to more effective management of the disease. Furthermore, this study also provides a foundation for future investigations and interventions aimed at reducing the burden of trypanosomiasis on both human and animal populations in Ethiopia.

Methods

Study area

The study was conducted in the Bedele and Dabo Hana districts of the Buno Bedelle Zone, approximately 480 km southwest of Addis Ababa, Ethiopia. The Bedele district has a total area of 745.0 km² and is characterized by an altitude ranging from 1300 to 2200 m above sea level and a mean annual rainfall of 1200 to 1800 mm. The district's agro ecology is classified into three zones: midland (75%), lowland (20%), and highland (5%). The Dabo Hana district has an area of 747.3 km² and is located at an altitude of 1600 to 2200 m. It is subdivided into three agro-climatic zones: highland (10%), midland (70%), and lowland (20%). The area receives an annual rainfall ranging from 1500 to 1800 mm, with a mean annual temperature of 18 to 24 °C. This research was conducted at an altitude

range between 1300 and 1,800 m in both districts (Fig. 1) [9, 10].

Study design

Entomological study

Two kebeles (the lowest administrative division in Ethiopia) in Bedele and Dabo Hana districts were selected for this entomological study based on favourable conditions for tsetse flies and reports of tsetse-transmitted bovine Trypanosomiasis from animal owners. The Dedesa River is the main river in the study area, and the traps were deployed along it.

A total of 100 traps (40 NGU, 30 pyramidal, and 30 biconical traps) were deployed along the Dedesa River in Bedele and Dabo Hana Districts. 50 traps (20 NGU, 15 pyramidal, and 15 biconical) were deployed in Chefe Jalal kebele, Bedele district, and the remaining 50 traps (20 NGU, 15 pyramidal, and 15 biconical) were deployed in Didesa Sene kebele, Dabo Hana district.

The trap deployment activity was carried out with trained community members. The authors first gave appropriate training to the volunteer community and then worked with them jointly to deploy the traps.

To attract tsetse flies, traps were baited with a mixture of acetone, octenol (1-Octen-3-ol), and 3-week-old cow urine. They were deployed at 200-meter intervals, and their locations were recorded using GPS. The traps were left at each location for a maximum of 48 h before being collected. The trap deployment sites were selected to represent all habitat or vegetation types in the study area that could be associated with tsetse fly feeding, behavior, multiplication, and other related aspects. The traps were deployed along the Dedesa River, which is the main river in the study area. The Dedesa River is a river in Ethiopia. It is a tributary of the Awash River. The river is about 100 km long.

The catches from each trap were carefully counted and identified. The live flies were dissected immediately and preserved in formalin for further processing. The apparent density of tsetse flies was determined by dividing the total number of flies captured by number of catching days and by total number of traps (FTD). The sex differentiation was performed by observing the posterior end of the ventral aspect of the abdomen using a hand lens and stereomicroscope. Male flies were identified by the presence of an enlarged hypopygium in the posterior ventral part of the abdomen, which is absent in female flies. The age of a tsetse fly can be estimated by looking at the colour of its abdomen, the size of its wings, and the number of ommatidia in its eyes. Adult tsetse flies are typically black, but their abdomens will turn brown as they age. Adult tsetse flies have four wings, and the size of their wings will increase as they age. Adult tsetse flies have compound eyes, and the number of ommatidia

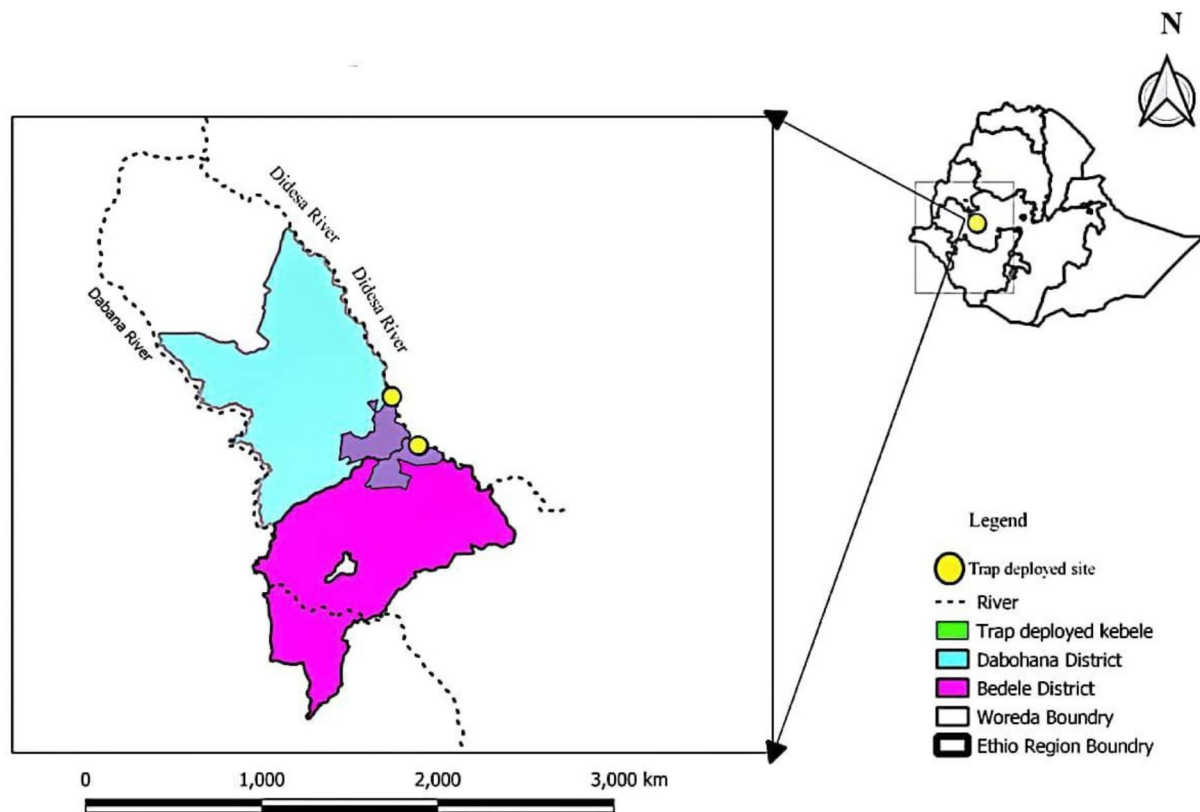


Fig. 1 Map of the study area

Table 1 Tsetse fly distribution in Bedele district, Buno Bedele Zone, Ethiopia

District	Kebele	Type of trap deployed	No of trap deployed	Species of fly	No of fly caught		Total	FTD
					Male	Female		
Bedele	Chefe Jalal	NGU	20	<i>G. tachinoides</i>	224	844	1068	26.7
		pyramidal	15	<i>G. tachinoides</i>	318	549	867	28.9
		Biconical	15	<i>G. tachinoides</i>	57	88	145	4.8
Total			50		599	1481	2,080	20.8

(individual units that make up the compound eye) will increase as they age. The hunger stage of a tsetse fly can be determined by looking at the fly’s behavior. A fly that is not hungry will be less active and will not fly as much. A fly that is starting to get hungry will be more active and will fly more. A fly that is very hungry will be very active and will fly around erratically. A hungry fly became very aggressive and bites anything moving otherwise it will die soon if it does not feed on a blood meal [11].

Fly dissection

To dissect the tsetse flies, we followed the guidelines outlined in the FAO Training Manual for tsetse control [12]. We randomly selected 50% of the live and healthy flies from each trap and dissected them. Initially, the wings and legs of the collected tsetse flies

were removed to prevent them from flying and damaging their internal organs. Freshly killed tsetse flies were then dissected under a dissecting microscope using 0.9% normal saline as a medium. The dissected body parts, including the midgut, salivary gland, and mouthpart (proboscis), were observed under a compound microscope at a magnification of $\times 40$ to detect trypanosome infections. The trypanosomiasis infections observed in the midgut, salivary glands, and mouthparts were classified as *Trypanozoon (T. brucei)*. Infections detected in the mouthparts and midguts were categorized as *Nanomonas (T. congolense)*. Immature infections were identified when they were solely found in the midgut [13]. For species identification of trypanosomes, Giemsa-stained smears on slides were examined under an oil immersion compound microscope at a magnification of 100x. The

Table 2 Tsetse fly distribution in Dabo Hana district, Buno Bedele Zone, Ethiopia

District	Kebele	Type of trap deployed	No of trap deployed	Species of fly caught	No of fly caught		Total	FTD
Dabo Hana	Didesa Sene	NGU	20		Male Female		903	22.6
		pyramidal	15	<i>G. tachinoides</i>	303	600		
		biconical	15	<i>G. tachinoides</i>	240	414	654	21.8
			15	<i>G. tachinoides</i>	41	62	103	3.4
Total			50		584	1076	1,660	16.6

Table 3 Trypanosome infection rate in *G. tachinoides* and identified trypanosome species

District	Species of fly caught	No. of fly Dissected	Trypanosome species found			Infection rate (%)	
			<i>T. congolense</i>	<i>T. brucei</i>	Total infected		
Bedele	<i>G. tachinoides</i>	M	178	1	1	2	1.1
		F	482	7	5	12	2.5
Dabo Hana	<i>G. tachinoides</i>	M	179	1	0	1	0.6
		F	481	5	4	9	1.9
Total			1320	14	10	24	1.82

morphological appearances of the trypanosomes were used for identification.

The Infection Rate (IR) was calculated for the collected data by dividing the number of infected individuals by the total number of individuals sampled and multiplied by 100 [14].

$$\text{Infection Rate (IR)} = \frac{\text{Number of tsetse flies infected}}{\text{Total number of dissected flies}} \times 100$$

Data analysis

Entomological data collected from each deployed traps were coded and recorded in a Microsoft Excel 2010 spreadsheet. Statistical analysis was performed using STATA version 14 software at a 95% confidence interval [15]. The infection rate (IR) of trypanosomes in *G. tachinoides* was calculated as the number of microscopically positive flies divided by the total number of dissected flies and multiplied by 100. The apparent density of tsetse flies was expressed as the number of each type of fly per trap per day (FTD). Trypanosome infection in *G. tachinoides* and its association with potential risk factors were computed using univariable logistic regression analysis. Categorical data were analyzed using the Chi-square (χ^2) test of independence. In all cases, 95% confidence interval was used, and a *p*-value of less than 0.05 was considered significant [16].

Results

A total of 3,740 tsetse flies were captured from 100 traps (40 NGU, 30 pyramidal and 30 biconical), resulting in an overall apparent density of 18.7 flies per trap per day (FTD). Of these, 1,971 flies were captured in NGU traps, 1,521 flies were captured in pyramidal traps, and 248 flies were captured in biconical traps. There is only one

species of tsetse fly, *G. tachinoides*, was found in both districts of the study areas. Specifically, in Bedele district, 2,080 flies were trapped, yielding an apparent density of 20.8 FTD. Of these, 1,068 flies were captured in NGU traps, 867 flies were captured in pyramidal traps, and 145 flies were captured in biconical traps (Table 1).

In Dabo Hana district, 1,660 flies were trapped with an apparent density of 16.6 FTD. Of these, 903 flies were captured in NGU traps, 654 flies were captured in pyramidal traps, and 103 flies were captured in biconical traps (Table 2).

From a total of 1320 dissected *G. tachinoides*, 24 flies were infected with trypanosomes resulting in an overall infection rate of 1.82% in the study area. Infection of *G. tachinoides* due *T. vivax* and mixed type of infection was not found in the study area during study period High-trypanosome infections were observed in the Bedele district 14(2.1%). *T. congolense* 8(1.21%) was the predominant species and found to be a major cause of tsetse fly infection in the study area followed by *T. brucei* 6 (0.91%). Less-trypanosome infection were observed in Dabo Hana district as compare to Bedele district 10(15.5%). *T. congolense* 6(0.91%) was the predominant species and found to be a major cause of tsetse fly infection in the study area followed by *T. brucei* 4 (0.61%) (Table 3).

Among the infected flies, 58.33% were found to be infected with *T. congolense*, while the remaining 41.67% were infected with *T. brucei*. The infection rate of trypanosomes was significantly higher in female tsetse flies (87.5%) as compared to male flies (12.5%). Furthermore, there was a high difference in the trypanosome infection rate between flies older than 20 days (83.33%) and those younger than 20 days (16.67%), as well as between flies in hunger stage 1 (58.33%) and hunger stages 2, 3, and 4 (Table 4).

Table 4 The infection rate of trypanosomes in *Glossina tachinoides* between sex, age, and hunger stage in Bedele and Dabo Hana district

Risk factors	No of fly dissected	Trypanosome species found			Infection rate (%)	chi-squire value	P-value
		<i>T. congolense</i>	<i>T. brucei</i>	Total infected			
Sex							0.262
Male	357	2	1	3	0.84	2.675	
Female	963	12	9	21	2.9		
Age							0.008
Young(≤ 20 days)	608	1	3	4	0.66	9.727	
Old(> 20 days)	712	13	7	20	2.81		
Hunger stage							0.002
Stage4 (Teneral)	263	0	1	1	0.38	21.252	
Stage3 (Intermediate)	331	1	1	2	0.60		
Stage 2 (replete)	410	3	4	7	1.71		
Stage 1 (Gorged)	316	10	4	14	4.43		

P-value < 0.05 considered as significant

Discussion

The survey conducted in Bedele and Dabo Hana Districts of Buno Bedele zone, Ethiopia, revealed the presence of *G. tachinoides*, a single species of tsetse fly that serves as the main vector of trypanosomes. A total of 3,740 tsetse flies were captured from 100 traps, resulting in an overall mean density of 18.7 flies per trap day (FTD). This density aligns with a study conducted in Daramallo district (19.14 FTD) [17], but is higher compared to that of Sokoru District (4.36 FTD) [18], Botor tolay district (10.9 FTD) [19], and Arbaminch Zuria district (15.03 FTD) [20]. However, other studies reported significantly higher densities of tsetse flies in certain southern regions of Amaro district (23 FTD) [21] and in Bambasi district, Western Ethiopia (29.62 FTD) [22]. These variations may be attributed to differences in environmental conditions, agro-ecological factors, and seasonal variations during sampling.

Of the 1,320 dissected *Glossina tachinoides* flies, 1.82% were found to be infected with trypanosomes (*T. congolense* and *T. brucei*). The study found that a significant proportion of *Glossina tachinoides* flies in the study area were infected with trypanosomes, a serious disease in animals. It is important to take steps to control the spread of trypanosomiasis, such as using insecticides to kill tsetse flies and educating people about the risks of the disease. This finding is comparable to a study conducted in Limu Kossa district in Jimma zone, where 1.76% of flies were infected [23]. The infection rate in the current study is higher than that reported in the Adamawa region of Cameroon (0.9%) [24]. However, it is lower than the infection rates found in Amaro Special District (6.93%) [19] and Arebaminch Zuria district (17.67%) [20]. This difference may be due to the low fly-animal contact in the study area.

The study also showed a higher rate of trypanosome infections in female tsetse flies (2.9%) compared to males

(0.84%). This difference can be attributed to the longer life expectancy of females and the fact that pregnant female flies require more blood meals during their pregnancy, which increases their exposure to infection [25]. The lower infection rate in male flies can be explained by their younger average age at the time of trapping. Furthermore, based on microscopic examination of the uterus contents and wing fray analysis, a higher trypanosome infection rate was observed in *Glossina tachinoides* flies older than 20 days (20.83%) compared to those below 20 days of age (9.52%). This finding is in agreement with a previous study reported by Meharenet and Alemu [23]. The risk of infection increased with age, and older flies had more time for the infection to develop. Flies need more blood meals at their reproduction age where they get maturity because they need the nutrients in the blood to produce eggs. The blood meal also provides the flies with energy, which they need to fly and mate [26–28]. The study found that the infection rate of trypanosomes in stage 1 (gorged) 58.33% and stage 2 (replete) 29.17% tsetse flies was significantly higher than the infection rate in stage 3 (Intermediate) and stage 4 (unfed) flies. The findings of this study suggest that stage 1 (gorged) and stage 2 (replete) tsetse flies are more likely to transmit trypanosomes to animals. This is consistent with the findings of a previous study in the Limu Kosa district of Southwest Ethiopia [23].

Conclusion

The results of this study suggest that the density of tsetse flies and the rate of trypanosome infection are high in the Bedele and Dabo Hana districts of the Buno Bedele Zone in Ethiopia. This is a significant animal health concern, as trypanosomiasis is a debilitating disease that can be fatal. The findings of this study highlight the necessity of implementing control and suppression measures targeting the vector, *Glossina tachinoides*. Based on the results

of this study, integrated vector control strategies, including the use of insecticide-treated traps, targets, and sterile insect techniques, are recommended to manage the spread of trypanosomiasis. Further research is needed to determine the most effective way to control and suppress tsetse flies in the Bedele and Dabo Hana districts.

Limitation

One of the limitations of this study was lack of molecular tests using PCR (Polymerase Chain Reaction) to confirm the presence of trypanosome parasites in the tsetse flies. The absence of molecular tests in this study might have underestimated of the true infection rate of trypanosome parasites in the tsetse fly populations. In future studies, incorporating molecular tests alongside dissection methods would enhance the validity and reliability of the findings.

Abbreviations

FTD	Flies/Trap/Day
IR	Infection rate
G. tachinoides	Glossina tachinoides
T. congolense	Trypanosoma congolense
T. brucei.	Trypanosoma brucei

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Author contributions

Ahimedini Beshir and Samson Takele contributed equally to this research. They both contributed to the design of the study, the collection and analysis of data, and the writing of the manuscript. Mohammed kedir proposed the research idea and supervised the project. Senbeta Tasew and Temesgen Tareke contributed to data collection. Delenasaw Yewhalaw proposed the research idea and reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets developed and/or analysed during the current study are available from the first author or from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The Bedele Animal Health Research Centre's Ethical Review Board (ERB) approved the research study, and community participants willingly participated in the entomological survey.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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