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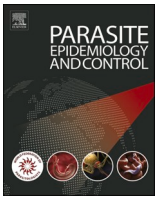

Epidemiological investigation of trypanosomosis in livestock and distribution of vector in Dabo Hana district, Southwest Oromia, Ethiopia

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ABSTRACT

The trypanosomosis remains unresolved due to its impact on various hosts, leading to production losses in Ethiopia. In the Southwest of Oromia, multiple livestock species share grazing land in tsetse-infested areas. Thus, a cross-sectional study was conducted from December 2020 to December 2021 to determine the prevalence and associated risk factors of trypanosomosis in bovines, small ruminants, and equines, as well as the distribution of the vector in the Dabo Hana district of Southwest Oromia, Ethiopia. A vector survey was carried out using 60 monoconical traps placed at intervals ranging from about 100 to 200 m. Out of the 1441 flies captured, 86.2 % were *Glossina*, 7.84 % were *Stomoxys*, and 5.96 % were *Tabanus*. The overall apparent density of flies was 12 flies per trap per day. Among the 1242 caught *Glossina* species, 85 % were identified as *G. tachinoides* and 15 % as *G. m. submorsitans*. The average age of male tsetse flies was 28 days, and the overall infection rate of trypanosomes in tsetse flies was 4.8 %. A total of 701 blood samples (190 from bovines, 384 from small ruminants, and 127 from equines) were analyzed using buffy coat and Giemsa techniques. The prevalence of trypanosomosis was found to be 10 % in bovines, 4.2 % in small ruminants, and 3.1 % in equines. A significant difference ($P < 0.05$) in trypanosome infection was observed among the three host species, as well as with respect to the age and body condition of the animals. The predominant cause of infection was *T. congolense*, accounting for 74.4 % of cases. The mean packed cell volume (PCV) values of infected bovines, small ruminants, and equines were significantly lower ($P < 0.05$) compared to those of non-infected animals. Trypanosomosis is a major livestock disease in the study area. The findings provide valuable insights into the prevalence and infection rates of trypanosomosis, identify the affected species, and highlight significant risk factors, such as age, body condition, and vector distribution. Implementing sustainable and integrated practices for trypanosomosis control is crucial, and conducting molecular techniques in different seasons is also recommended.

1. Introduction

Trypanosomosis, also known as African Animal Trypanosomosis (AAT) or nagana, is a significant disease affecting livestock in sub-Saharan Africa, posing a serious threat to the lives and livelihoods of communities (Nyimba et al., 2015). The distribution of AAT

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corresponds to the distribution of tsetse flies and covers about 10 million km^2 across 37 sub-Saharan African countries (Sachs, 2010). The disease has a substantial negative impact on food production and economic growth, particularly in sub-Saharan Africa (Cecchi et al., 2008). In these areas, around 55 million cattle and a significant number of other animals are at moderate to high risk of contracting the disease, resulting in the death of 3 million cattle annually (Cecchi et al., 2014). The economic burden of AAT in Africa is estimated to be around 4.5 billion USD per year (Yaro et al., 2016).

Several species of pathogenic trypanosomes, including *T. congolense*, *T. vivax*, *T. b. brucei*, *T. evansi*, and *T. equipardum*, affect animals in Africa, with mixed infections often occurring in areas where multiple species are present (OIE, 2013; Moti et al., 2015). While AAT can affect various domestic animals, its economic significance is particularly pronounced in cattle in sub-Saharan Africa (Namangala and Odongo, 2014). The disease also impacts a wide range of wildlife species, acting as reservoirs of infection for both humans and domestic animals (Auty et al., 2015; Robi and Diriba, 2021). However, certain African cattle breeds, including the N'Dama from West and Central Africa, the Shorthorn from West Africa, the Sheko in Ethiopia, and the Djallonke sheep and Dwarf goats from West Africa, have demonstrated a level of tolerance to infections (Naessens, 2006; Berthier et al., 2015; Mekonnen et al., 2019; Robi et al., 2021).

Trypanosomosis is primarily transmitted cyclically by tsetse flies, with *T. congolense*, *T. vivax*, *T. b. brucei*, and *T. simiae* being the major tsetse-transmitted trypanosomes (Namangala, 2011; Holmes, 2013). Mechanical transmission can occur through biting flies of the diptera genus, such as *Tabanus*, *Hematopota*, and *Stomoxys* (Kone, 2011; Baldacchino et al., 2013). Latrogenic transmission can also occur through the use of contaminated needles or surgical instruments (Desquesnes and Dia, 2003). Furthermore, *T. equiperdum* is primarily transmitted sexually (Namangala, 2012).

Ethiopia, with its large livestock population, is heavily affected by trypanosomosis, which remains a major cause of livestock production losses in the country. The disease hinders the optimal utilization of livestock resources, leading to significant economic losses (Bezabih et al., 2016; Leta et al., 2016; Efrem et al., 2022). One tsetse-infested area in Ethiopia is the Dabo Hana District, located in the Southwest Oromia region. The district is bordered by conserved areas for wildlife and river basins, making livestock grazing in and around these infested areas highly susceptible to trypanosomosis (Gebeyehu and Degneh, 2023).

Previous studies conducted in Dabo Hana District have focused on the impact of trypanosomosis on draught power and productivity of cattle, but did not thoroughly assess the prevalence of trypanosome infections in different livestock species such as sheep, goats, and equines (Iyob et al., 2018; Gebeyehu and Degneh, 2023). Preventing and controlling trypanosomosis hinges on minimizing contact

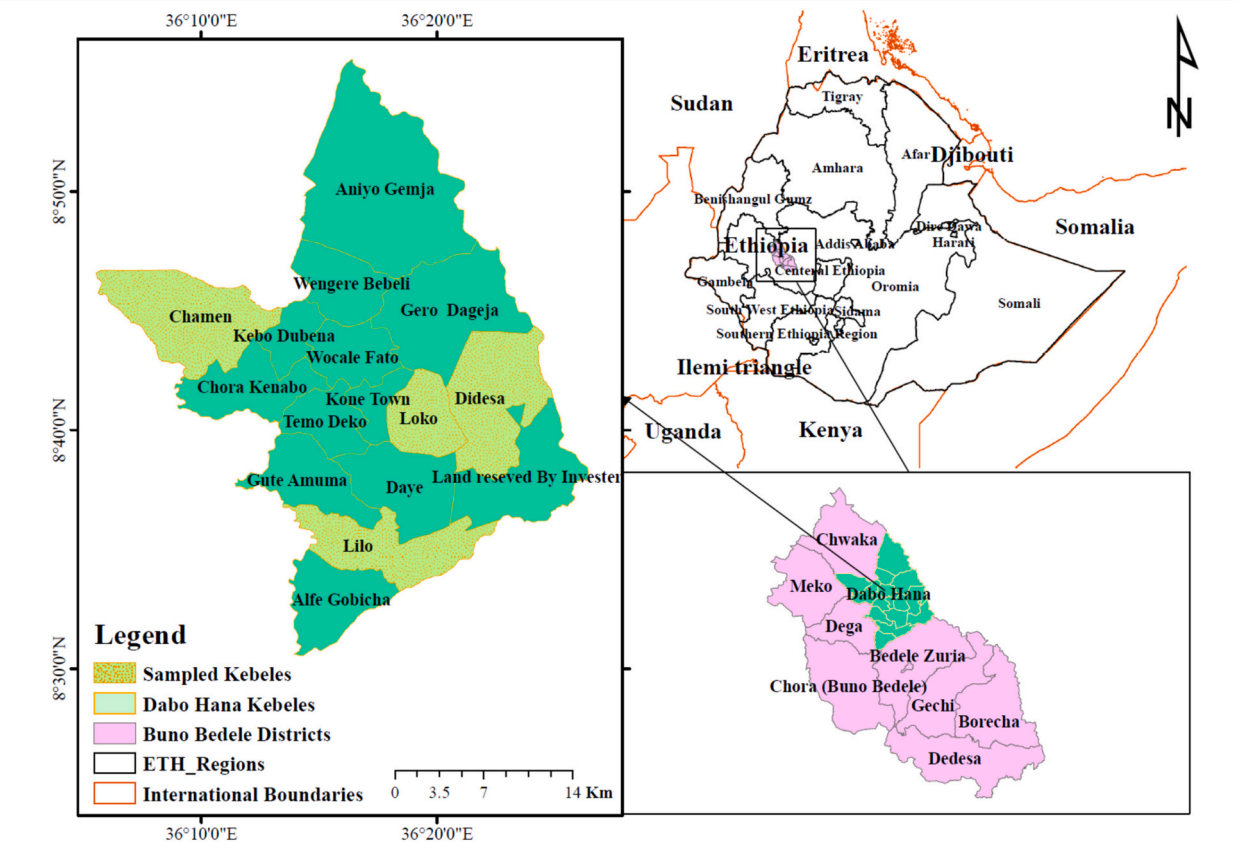

Fig. 1. Map showing Dabo Hana district.

between livestock like cattle, small ruminants, and equines, and the vectors that transmit the disease. Strategies for controlling trypanosomosis primarily involve reducing tsetse fly populations, administering trypanocidal drugs, and breeding animals that are tolerant to the disease (Achenef and Bekele, 2013; Bouyer et al., 2014; Robi et al., 2021). Understanding the epidemiology of the disease in these animals and the distribution of its vectors in specific areas is crucial for effective trypanosomosis management (Ebhodaghe et al., 2018). Therefore, this study aims to determine the prevalence and associated risk factors of trypanosomosis in bovines, small ruminants, and equines, as well as the distribution of the vector in the Dabo Hana district of Southwest Oromia, Ethiopia.

2. Materials and methods

2.1. Study area

The study was carried out in the Dabo Hana District, located in Southwest Oromia, Ethiopia. This district shares borders with Gechi to the south, Chora to the southwest, Diga to the west, the southern exclave of the Benishangul-Gumuz region to the north, and the Didessa River to the northeast. Situated around 480 km southwest of Addis Ababa (Fig. 1), the area's elevation ranges from 1600 to 2200 m above sea level. It categorized into three agro-climatic zones based on altitude: highland (10 %), midland (70 %), and lowland (20 %). The average annual temperature in the study district spans from 18 to 24 °C, accompanied by an annual rainfall of 1500–1800 mm. Mixed crop-livestock farming was the main source of livelihoods. The vegetation cover was dominated by savanna grassland, forest, bush lands, riverine and cultivated land. The major livestock species found in the study area were cattle, small ruminant, equine and poultry. Communal grazing with a minimum supplementary feeding was the major livestock husbandry system throughout the year. As most of the land was intensively cultivated during the long rainy season (April to October), livestock are moved to the vicinity of the forests with permanent grassland where wild games and reptiles are commonly found.

2.2. Study design and animals

A cross-sectional study into livestock trypanosomosis was carried out in Dabo Hana District from December 2020 to December 2021. The study focused on bovine, small ruminants, and equines aged one year or older, all kept in smallholder extensive husbandry systems. These animals exhibited variations in age, body condition, and sex, and were managed under an extensive management system in the Dabo Hana District. Various variables such as species, sex, age, hair color, and body condition scores were documented during sampling. The age of the animals was estimated using their dentition, following the method outlined by Pasquini et al. (2003), and categorized as young ($\leq$3 years) or adult (>3 years) (Radostits et al., 2007). Additionally, the body condition animals were assessed and categorized as good, medium, or poor based on the visibility of ribs and dorsal spines, in accordance with the criteria proposed by Nicholson and Butterworth (1986). During sample collection, the hair color of animals was classified into white, red, black, and mixed colors based on visual observation. The management system classification followed the criteria established by Richard (1993), with animals in the extensive management system being those kept outdoors during the daytime and allowed to graze on communal or private pasture lands.

2.3. Sampling methods and sample size determination

The prevalence of trypanosomosis in the Dabo Hana district seems to be influenced by various ecological and human factors. The reduced presence of biting flies, which transmit trypanosomes, may result from environmental conditions and management practices that limit their proliferation. Key risk factors for the spread of trypanosomosis in livestock include the proximity of grazing areas to forests, which serve as breeding grounds for vectors, and the migration patterns of herds that may expose them to infected flies. Human activities, such as farming, deforestation, and urban expansion, significantly affect vector distribution by altering habitats and disrupting ecosystems, creating conditions that can either support or reduce the population dynamics of these disease-carrying insects. The study shows that trypanosomosis mainly affects livestock in the district, with no evidence of zoonotic transmission to humans. However, inadequate veterinary services hinder timely diagnosis and treatment, potentially increasing morbidity and mortality in affected animals, which negatively impacts local livestock productivity and the livelihoods of farmers. Seasonal changes in the distribution of tsetse flies and other vectors are crucial for understanding the epidemiology of trypanosomosis. Vectors are more abundant in specific habitats, such as riverbanks and wetlands, during the rainy season, while habitat desiccation during the dry season may cause declines in their populations. Understanding these patterns is essential for developing effective control strategies. Consequently, the Dabo Hana district was purposefully selected due to its large livestock population, including bovines, small ruminants, and equines, and its perceived high prevalence of trypanosomosis. Four kebeles, named Chamen, Didesa, Lilo, and Loko, were selected using a simple random sampling method from a total of sixteen kebeles in the Dabo Hana district. Subsequently, twelve villages were selected from these kebeles based on their village count. The lottery method was used to select herds/flocks from these villages. A herd/flock refers to a group of animals that live in a village, sharing grazing and watering areas. The sampling frames for individual animals were obtained from *Abba Ulle* in each respective village. *Abba Ulle* served as an important contact person facilitating cooperation among animals' owners (Robi and Diriba, 2021). Study animals were then selected from herds/flocks using the simple sampling technique. Sample sizes from each herd varied based on numbers of the herds/flocks. The sample size calculation followed the method by Thrusfield and Christley (2018) with an expected prevalence of 14.45 % for bovine (Iyob et al., 2018), and 50 % expected prevalence for small ruminants and equines, at a 95 % confidence interval and 5 % desired absolute precision for all animals. The calculated

sample sizes were 190 for bovine, 384 for small ruminants, and 384 for equines with a total of 958 animals. However, due to the limited number of equines (only 127 donkeys were selected), the final sample size included only 701 animals.

2.4. Morbidity, mortality, treatment, and control of trypanosomosis in the study areas

Livestock owners commonly report several clinical signs associated with AAT, which can vary depending on the species affected and the severity of the infection. Key clinical signs include weight loss, poor body condition, anemia, weakness, swelling of the lymph nodes, recumbency, and death (Radostits et al., 2007). The morbidity associated with AAT significantly impacts livestock productivity, leading to poor growth rates, reduced milk yield, and infertility. Odeniran et al. (2020) noted that morbidity rates can vary depending on factors such as species, infection severity, and availability of veterinary care. If left untreated, mortality rates can reach alarming levels, with case fatality rates hitting 50 % in severely affected populations, particularly among young and immunocompromised animals. This loss imposes a substantial economic burden on farmers and disrupts local food security. Furthermore, the economic impacts extend beyond mortality, encompassing veterinary treatment costs, decreased productivity, and increased management expenses to control outbreaks. In the Dabo Hana district, current control strategies include trapping tsetse and biting flies with varying intensities (i.e., different numbers of traps), applying insecticide-treated cattle through methods such as pour-on treatments, general body spraying, and using insecticide-treated targets (Beshir et al., 2024). To address trypanosomes, approaches include administering trypanocides (such as diminazene aceturate (DA) or isometamidium chloride (IC)), implementing seasonal cattle movements to evade tsetse fly attacks, and providing access to better grazing areas during the dry season.

2.5. Parasitological study

Blood samples were acquired from the marginal ear vein through a sterile lancet, followed by blood retrieval using a heparinized capillary tube. Subsequently, the collected samples underwent centrifugation at 12,000 rpm for 5 min in a hematocrit centrifuge. After centrifugation, the tubes were transferred to a microhematocrit reader for determining the packed cell volume (PCV) of each sample. For bovine, a PCV below 24 % indicated anemia (Van den Bossche et al., 2000; OIE, 2008), while PCV levels below 26 % and 30 % were considered anemic for small ruminants and equines, respectively (Radostits et al., 2007; Douglas and Wardrop, 2010). The contents of the capillary tubes, specifically about 1 mm above and below the buffy coat, were examined using the buffy coat technique under a × 40 magnification light microscope to detect *Trypanosoma* parasites (Murray et al., 1977). *Trypanosoma*-positive samples underwent thin blood smear preparation and Giemsa staining for species identification under an oil immersion ×100 objective lens. Species differentiation relied on criteria such as size, kinetoplast position, undulating membrane presence, and free flagella length as described by Picozzi et al. (2002) and OIE (2008).

2.6. Vector collection and morphological identification

A total of 60 monoconical traps were strategically positioned at watering holes and grazing spots in selected kebeles of the Dabo Hana district (Malele et al., 2003). Three attractants for tsetse flies (acetone, octenol (1-oct-3-nel), and cow urine aged three days) were employed (Brightwell et al., 1997; Kassa, 2005). These attractants were individually placed in bottles on the ground about 30 cm upwind from the trap location. These traps were elevated on poles to maintain their entrances about 45 cm above ground level. To deter ants from climbing and potentially harming the trapped tsetse flies, the lower sections of the support poles were coated with grease. The sites selected for trap deployment were intended to encompass all vegetation types associated to aspects of the tsetse life cycle of fly, including reproduction, behavior, feeding habits, and other relevant factors. The traps were spaced at intervals ranging from 100 to 200 m and were left undisturbed for 48 h once deployed. After this period, the flies captured in the traps were gathered, tallied, and classified. Identification of biting flies was conducted at the genus level based on observable features such as size and wing structure, referencing the works of Waiswa et al. (2006) and Taioe et al. (2017). For tsetse fly identification, the guidelines outlined in the FAO (2008) manual for collecting entomological baseline data for *Glossina* species were followed, focusing on morphological traits analysis. Male tsetse flies were distinguished through observation of their hypopygium. The apparent density of flies captured per trap per day was determined using Leak (1999) formula.

Traps were deployed regularly to capture fresh tsetse flies. The age of male tsetse flies was assessed based on wing fray, which correlates with their mating behavior. In contrast, determining the age of female tsetse flies requires dissection of their ovaries to examine reproductive development. However, due to limitations in available facilities and resources, we were unable to perform this dissection. The average age of male tsetse flies was estimated by removing the wings from recently captured and identified males. These wings were then placed on slides, mounted, and examined under a low-power compound microscope. The wear on the trailing edge of the wings was recorded and compared with standard wing fray charts (Jackson, 1946). The infection rate of tsetse flies was determined by catching fresh flies and following the dissection method outlined in the FAO training manual for tsetse control personnel (FAO, 2000). During dissection, the wings and legs of the flies were removed. Subsequently, freshly killed tsetse flies were dissected under a dissecting microscope using 0.9 % normal saline. *Trypanosome* infections in the dissected body parts of the flies were observed using a compound microscope at a magnification of ×40. Parasites identified in various body regions were classified as: Trypanozoon (*T. brucei*) when located in the midgut, salivary gland, and proboscis; Nanomonas (*T. congolense*) when detected in the proboscis and midgut; and a cluster of Duttonella (*T. vivax*) if solely present in the proboscis (Rotureau and Van Den Abbeele, 2013). Finally, Giemsa-stained smears were examined under a compound microscope at ×100 magnification for species identification based on morphological appearance. The infection rate was calculated using the formula provided by FAO (1982): Infection rate = (number

of positive flies / total number of dissected flies) * 100.

2.7. Data analysis

The data obtained from the vector survey and laboratory tests underwent coding to create relevant variables, which were then inputted into a Microsoft Excel spreadsheet. The apparent density of the vector was determined by dividing the number of flies captured by the number of traps deployed and the duration of deployment, expressed as flies per trap per day. The average age of male tsetse flies was assessed through wing fray category analysis. The infection rate among tsetse flies was calculated by dividing the number of positive flies by the total number dissected, multiplied by 100. *Trypanosome* prevalence was determined as the ratio of infected individuals to the total individuals sampled, also multiplied by 100. The prevalence of livestock trypanosome infections was compared across different categories such as species, sex, age, hair color, and body condition score using the chi-square ($\chi 2$) test. The *t*-test was utilized to compare mean PCV (packed cell volume) values between infected and non-infected animal groups. Statistical analyses were conducted using SPSS version 20, with significance set at $P < 0.05$ and a confidence level of 95 %.

3. Results

3.1. Parasitological findings

Among the 701 animals (Table 1), the prevalence of trypanosomosis was 10 % in bovines, 4.2 % in small ruminants, and 3.1 % in equines, with an overall prevalence of 5.6 %. Notably, the prevalence in bovine was significantly higher ($P = 0.02$) compared to small ruminants and equines. During the study period, it was found that *T. congolense* (74.4 %) was the most prevalent, followed by *T. vivax* (17.9 %), with mixed infections of *T. vivax* and *T. congolense* accounting for 5.1 %, as detailed in Table 2.

The prevalence of trypanosomosis was 8.4 % in Didesa kebele, 5.9 % in Loko, 4.4 % in Chamen bareda, and 2.8 % in Lilo. However, these differences were not statistically significant ($P = 0.8$) (Fig. 2).

The prevalence of trpanosomosis was slightly higher in female cattle (11.5 %) than in males (8.1 %), although this difference was not statistically significant ($P = 0.5$). Age was significantly associated with trpanosomosis ($P = 0.02$): adult cattle had a higher prevalence (13.7 %) compared to young cattle (3.0 %). This is reflected by a high positive standardized residual (2.10) for adults and a negative residual (−2.10) for young animals, suggesting greater vulnerability to trpanosomosis among adults. Hair color showed a strong and statistically significant association with trpanosomosis prevalence (P = 0.0). Black-haired cattle had the highest trpanosomosis prevalence (27.3 %), with a significant positive standardized residual (3.50), indicating a much higher likelihood of infection compared to other hair colors. Cattle with mixed and white hair colors showed no infections, as reflected by strongly negative residuals (−3.50 and − 2.50, respectively), suggesting these colors may be less susceptible or exposed. Body condition score (BCS) was also significantly associated with trpanosomosis ($P = 0.04$). Cattle with a poor body condition had a higher prevalence of infection (18.6 %) compared to those with medium (6.9 %) and good (3.3 %) body condition. The positive residual (1.60) for poor body condition and the negative residuals for medium (−0.60) and good (−1.40) body conditions indicate that cattle in poorer health are more prone to infection (Table 3).

The prevalence of trypanosomosis was higher in females (6.1 %) than in males (1.8 %), with the Chi-square test indicating a significant association ($P = 0.03$). The standardized residuals suggested a higher-than-expected infection rate in females (1.89) and a lower-than-expected rate in males (−1.89). Adult animals exhibited a significantly higher prevalence of infection (6.3 %) compared to young animals (2.1 %) (P = 0.04). The standardized residuals indicated that adult animals were more likely to be infected (1.67), while young animals showed a negative residual (−1.67). Infection rates varied significantly among animals with different hair colors ($P < 0.001$). Black-haired animals had the highest prevalence (12.2 %), with a standardized residual of 3.45, indicating a much higher-than-expected infection rate. In contrast, mixed and white-haired animals showed no infection (0.0 %), with standardized residuals of −2.90 and − 2.25, respectively, indicating significantly lower-than-expected infection rates. Trypanosome infection was more prevalent in animals with poor body condition (7.2 %) compared to those with medium (4.8 %) and good body condition (0.0 %), showing a significant association with infection rates ($P = 0.04$) (Table 4).

The analysis revealed no statistically significant association between sex and trypanosome infection ($P = 0.7$) in small ruminants. Among females, the prevalence was 1.9 %, while for males, it was 4.1 %. A significant association was observed between age and trypanosomosis (P = 0.02). Trypanosomosis was detected exclusively in adult equines, with a prevalence of 8.3 %. In contrast, no cases were found in younger equines, as indicated by the standardized residuals (−1.64 for young and 1.64 for adults), which suggest a significant deviation, with adults being more susceptible to trypanosomosis. No statistically significant association was found between

Table 1
Distribution of samples in Dabo Hana district in Southwest Oromia, Ethiopia.

Study animals	Kebeles				
	Chmen bareda	Didesa	Lilo	Loko	Total
Bovine	42	66	37	45	190
Small ruminant	86	110	102	86	384
Equine	30	38	34	25	127
Total	158	214	176	156	701

Table 2
Proportions of trypanosomes species identified in study animals.

Study animals	Total sampled	Species of trypanosomes				Total positive (%)
		T. congolense (%)	*T. vivax* (%)	*T. congolense* and *T. vivax* (%) mixed	*T. brucei* (%)	
Bovine	190	12 (30.8)	4 (10.3)	2 (5.1)	1 (2.6)	19 (10.0)
Small ruminant	384	13 (33.3)	3 (7.7)	0 (0.0)	0 (0.0)	16 (4.2)
Equine	127	4 (10.26)	0 (0.0)	0 (0.0)	0 (0.0)	4 (3.1)
Overall	701	29 (74.4)	7 (17.9)	2 (5.1)	1(2.6)	39 (5.6)

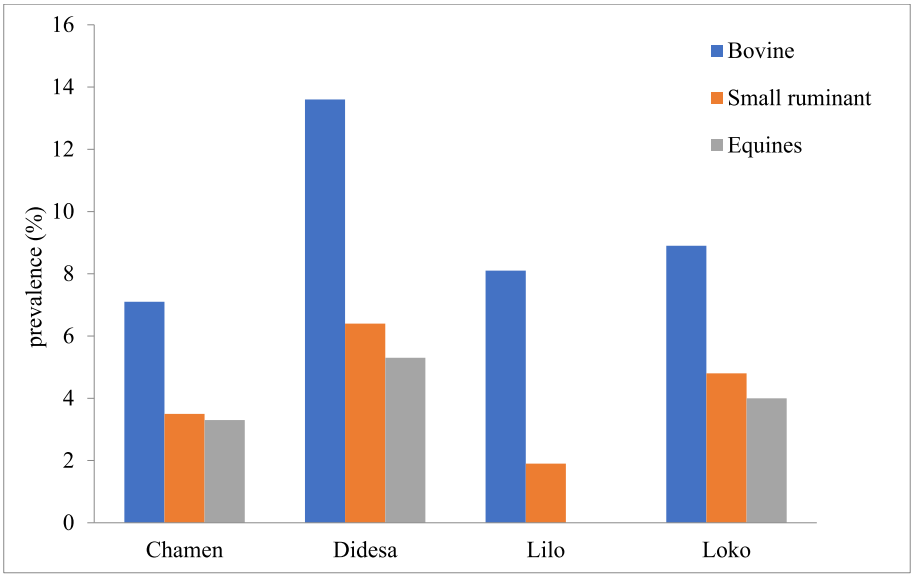

Fig. 2. Prevalence of livestock trypanosomes infections in study area.

Table 3
Chi-square (χ2) analysis of risk factors associated with bovine trypanosomosis in the study area.

Variable	Category	Number examined	Number positive	Prevalence (%) (95 %CI)	Standardized Residuals	X^2-test	*P*-value
Sex	Female	104	12	11.5 (5.40–17.68)	0.72	0.6	0.5
	Male	86	7	8.1 (2.36–13.92)	−0.72		
Age	Young	66	2	3.0 (1.11–7.17)	−2.10	5.5	0.02
	Adult	124	17	13.7 (7.66–19.76)	2.10		
Hair color	Black	44	12	27.3 (14.11–40.43)	3.50		
	Mixed	21	0	0.0 (0.00–0.00)	−3.50	20.8	0.0
	Red	98	7	7.1 (2.04–12.24)	−1.20		
	White	27	0	0.0 (0.00–0.00)	−2.50		
BCS	Good	30	1	3.3 (3.09–9.76)	−1.40		
	Medium	101	7	6.9 (1.98–11.88)	−0.60	7.4	0.04
	Poor	59	11	18.6 (8.71–28.88)	1.60		

hair color and trypanosomosis ($P = 0.24$). Equines with black hair color had a higher prevalence of 8.7 % compared to gray-haired equines, who had a prevalence of 1.9 %. There was a significant association between body condition and trypanosomosis ($P = 0.04$). Equines with poor body condition had the highest prevalence at 11.1 %, followed by those with medium condition at 1.6 %, while no trypanosomosis cases were observed in animals with good body condition. The standardized residuals (−1.38 for good, −0.71 for medium, and 1.55 for poor) indicate that poor body condition is significantly associated with a higher likelihood of trypanosomosis (Table 5).

The packed cell volume (PCV) ranges between 14 % and 45 % for bovine, 15 % and 44 % for small ruminants, and 18 % and 47 % for equines. The average PCV values in parasitized animals were notably lower ($P < 0.05$) compared to non-parasitized ones. Furthermore, trypanosome infection rates were higher among anemic animals across all species than in non-anemic animals (Table 6).

Table 4
Chi-square ($\chi 2$) analysis of risk factors associated with trypanosomosis in small ruminants in the study area.

Variable	Category	Number examined	Number positive	Prevalence (%) (95 %CI)	Standardized Residuals	X^2-test	P-value
Sex	Female	214	13	6.1 (2.87–9.28)	1.89	4.4	0.03
	Male	170	3	1.8 (0.21–3.74)	−1.89		
Age	Young	193	4	2.1 (0.04–4.08)	−1.67	4.3	0.04
	Adult	191	12	6.3 (2.84–9.72)	1.67		
Hair color	Black	49	6	12.2 (3.07–21.42)	3.45		
	Mixed	56	0	0.0 (0.00–0.00)	−2.90	17.3	0.00
	Red	161	10	6.2 (2.48–9.94)	0.22		
	White	118	0	0.0 (0.00–0.00)	−2.25		
BCS	Good	92	0	0.0 (0.00–0.00)	−1.83		
	Medium	209	10	4.8 (1.89–7.68)	−0.12	6.1	0.04
	Poor	83	6	7.2 (1.66–12.80)	1.99		

Table 5
Chi-square ($\chi 2$) analysis of risk factors associated with trypanosomosis in equines in the study area.

Variable	Category	Number examined	Number positive	Prevalence (%) (95 %CI)	Standardized Residuals	X^2-test	P-value
Sex	Female	54	1	1.9 (1.74–5.45)	−0.44	0.5	0.7
	Male	73	3	4.1 (0.44–8.66)	0.44		
Age	Young	79	0	0.0 (0.00–0.00)	−1.64	6.8	0.02
	Adult	48	4	8.3 (0.51–16.15)	1.64		
Hair color	Black	23	2	8.7 (2.82–20.21)	0.87	3.1	0.24
	Gray	104	2	1.9 (0.72–4.56)	−0.87		
BCS	Good	37	0	0.0 (0.00–0.00)	−1.38		
	Medium	63	1	1.6 (1.50–4.67)	−0.71	7.3	0.04
	Poor	27	3	11.1 (0.74–22.97)	1.55		

3.2. Entomological findings

During the study, a total of 1441 flies were captured, with *Glossina* accounting for 86.2 %, *Stomoxys* for 7.84 %, and *Tabanus* for 5.96 %. The overall fly density was 12 flies per trap per day. Specifically, the densities of *Glossina*, *Stomoxys*, and *Tabanus* were 10.4, 0.9, and 0.7 flies per trap per day, respectively. Among the 1242 *Glossina* caught, 85 % were *G. tachynoides*, and the remaining 15 % were *G. m. sub-morsitans*. Of all the *Glossina* captured, 65 % were females and 35 % were males, resulting in a sex ratio of 1.9 females to 1 male. Notably, higher numbers of *Glossina* were observed in Didesa compared to Lilo kebele (Table 7). The statistical analysis revealed significant differences among the study areas, with Chmen Bareda compared to Didesa showing a P-value of 0.001, Chmen Bareda compared to Lilo having a *P*-value of 0.034, and Didesa compared to Lilo yielding a P-value of 0.002.

Throughout the study period, 92 freshly captured male *G. tachnoides* were examined to determine the average age of the male population (Table 8). This was done using the formula MWFV = Sum of products/Total flies = 330.4/92 = 3.6. By comparing the wear on the wing's trailing edge with established wing fray charts (Jackson, 1946), it was found that this MWFV of 3.6 corresponds to an estimated average age of 28 days for the entire male population.

Out of the 248 *G. tachnoides* that were captured and dissected, a total infection rate of 4.8 % was recorded among the flies. Notably, female flies exhibited a higher prevalence of trypanosome infection compared to males, as shown in Table 9. The infection rate of tsetse flies was determined by capturing and dissecting fresh flies. During this study, only *G. tachnoides* were captured and dissected, while *G. m. submorsitans* was not observed within the 2–4-h time frame, and its infection rate could not be determined. Furthermore, *T. brucei* was not identified in any of the examined samples during the dissection, which was conducted within 2–4 h of capture.

Table 6
Association between mean PCV of parasitamic and aparasitamic study animas in study area.

Study animals	Infection status	Number of animals	Anemic (%)	Mean PCV (%)	95 % CI	*P*-value
Bovine	Parasitamic	19	68.4	22.8	21.0–24.5	
	Aparasitamic	171	40.4	25.5	24.7–26.2	0.00
	Overall	190	43.2	25.2	24.5–26	
Small ruminants	Parasitamic	16	62.5	25.5	22.9–28.0	
	Aparasitamic	368	36.1	30.2	29.5–31.0	0.00
	Overall	384	37.2	30.0	29.3–31	
Equine	Parasitamic	4	75.0	30.0	23.5–36.5	
	Aparasitamic	123	19.5	34.7	33.7–35.7	0.001
	Overall	127	21.3	34.5	33.6–35.5	

Table 7
Distribution of *Glossina*, *Stomoxys* and *Tabanus* in study area.

Genus of fly	Kebeles				Total	%	FTD
	Chmen bareda	Didesa	Lilo	Loko			
Glossina	177	601	112	352	1242	86.2	10.4
Stomoxys	36	37	19	21	113	7.84	0.9
Tabanus	25	36	12	13	86	5.96	0.7
Overall	238	674	143	386	1441	100	12

FTD: Fly/trap/day.

Table 8
Wing fray categories (1–6) of male *G. tachnoides* in study area.

Glossina species	Wing fray category	Number of flies in each category	Factor	Product
G. tachinoides	1	7	1	7
G. tachinoides	2	15	2	30
G. tachinoides	3	30	3	90
G. tachinoides	4	24	4.4	105.6
G. tachinoides	5	9	5.5	49.5
G. tachinoides	6	7	6.9	48.3
Overall		92		330.4

Table 9
Infection rate of trypanosome in *G. tachnoides* in study area.

Risk factors	Number of flies dissected		Trypanosome species			Infection rate (%)
			T. congolense (%)	*T. vivax* (%)	Total (%)	
Sex	Male	92	2 (16.7)	2 (16.7)	4 (33.3)	4.3
	Female	156	3 (25.0)	5 (41.7)	8 (66.7)	5.1
	Total	248	5 (41.7)	7 (58.3)	12 (100)	4.8
Hunger stage	Engorged	32	0 (0.0)	0 (0.0)	0 (0.0)	0.0
	Fed	123	1 (8.3)	2 (16.7)	3 (25.0)	2.4
	Starved	93	4 (33.3)	5 (41.7)	9 (75.0)	9.7
Overall		248	5(41.7)	7(58.3)	12 (100)	4.8

4. Discussion

This study helps in the implementation of an effective approach for controlling and preventing trypanosomosis and its vectors in the Dabo Hana district, Southwest Oromia, Ethiopia. The findings of this study indicate that *T. vivax*, *T. congolense*, and *T. brucei* are responsible for a prevalence of 10 % in bovines, 4.2 % in small ruminants, and 3.1 % in equines. Moreover, the study confirms that *Glossina* species, specifically *G. tachinoides* and *G. m. submorsitans*, as well as other biting flies, have the potential to transmit *Trypanosoma* infection. The overall infection rate among the flies was recorded at 4.8 %, specifically observed in *G. tachinoides*. However, it is important to note that *G. m. submorsitans* was not captured and dissected during the study, despite representing 15 % of the fly population. The infection rate of tsetse flies was assessed using only freshly captured *G. tachinoides*, as no *G. m. submorsitans* were caught within the critical 2–4-h time frame when dissection was feasible. Therefore, the infection rate for *G. m. submorsitans* could not be determined, which limits the ability to fully assess the role of this species in *Trypanosoma* transmission in the study area. Furthermore, the occurrence of trypanosomosis is associated with factors such as the age and body condition of bovines, small ruminants and equines. Furthermore, the hair color of bovines and small ruminants was found to be associated with the occurrence of trypanosomosis. Sex has been found to be associated with the prevalence of trypanosomosis in small ruminants.

The prevalence of bovine trypanosomosis (10 %) observed in this study aligns with the prevalence reported by previous studies: 9.1 % in Hawa Gelan district by Fentahun and Tekeba (2013), 11.2 % in Sayo district by Degneh et al. (2018), and 9.46 % in Jawi district by Dagnachew et al. (2020), all in Ethiopia. These similarities may be attributed to comparable environmental conditions, livestock management practices, and vector densities in the regions studied. Conversely, a lower prevalence than the present one was reported by Olani and Bekele (2016) and Bekele et al. (2018), who found 7.7 % in Lalo-Kile district and 5.47 % in Didessa district, respectively, also in Ethiopia. These discrepancies may arise from differences in ecological conditions, agricultural practices, and vector control efforts between these districts and the study area. However, a higher prevalence of 21.5 % was found by Yalew and Fantahun (2017) in the Bambasi district of Western Ethiopia. This stark difference may be attributed to higher tsetse fly populations, variations in livestock density, or inadequate control measures in that area.

The prevalence of trypanosomosis (4.2 %) recorded in small ruminants aligns with the prevalences of 3.24 % and 5.1 % reported by Kumela et al. (2017) and Dinka (2003), respectively. This similarity suggests a stable epidemiological status for trypanosomosis in small ruminants in the areas covered by these studies. Conversely, the higher prevalence of 8 % reported by Kebede et al. (2016) in the

Mareka district may be attributed to various factors, including differences in geographic locations, ecological conditions, vector populations, and husbandry practices that may influence trypanosome transmission. The prevalence of trypanosomosis (3.2 %) observed in equine in this study is in agreement with the findings of Hailegebrael and Shimelis (2012) and Dagnachew et al. (2020). However, higher prevalences of 6 %, 10.7 %, and 18.8 % were reported by Assefa et al. (2015), Solomon et al. (2010), and Zelalem et al. (2019), respectively. The variations in the prevalence of trypanosomosis observed in equine in different studies can be attributed to factors such as differences in geographic location, vector distribution, and seasonal fluctuations in tsetse fly populations.

Bovines show the highest prevalence of trypanosomosis at 10 %, which can be attributed to several factors. Bovines are a primary host for tsetse flies, the vectors of trypanosomosis, which prefer larger hosts due to their size and blood availability (Courtin et al., 2005; Radostits et al., 2007). Furthermore, their behavior of grazing over large distances increases their exposure to fly-infested areas. This, coupled with their group dynamics, makes them an attractive target for tsetse flies (Vale and Torr, 2004). In contrast, small ruminants exhibit a lower prevalence of trypanosomosis at 4.2 %. Their smaller size, individual foraging behavior, and more agile movements may reduce their attractiveness to flies. Small ruminants are also known for defensive behaviors against biting flies, which may further lower their risk (Ravel et al., 2015). Equines show the lowest prevalence of trypanosomosis at 3.1 %. Their role in trypanosomosis transmission dynamics is less well-documented compared to bovines and small ruminants. However, like small ruminants, their smaller size compared to bovines and relatively lower exposure to fly-infested environments may contribute to the reduced prevalence (Dagnachew et al., 2020).

The prevalence of *T. congolense* (74.4 %) noted in this study aligns with findings reported by Biyazen et al. (2014), Kassaye and Tsegaye (2016), and Kitila et al. (2017) in bovines, by Kebede et al. (2016) in small ruminants, and by Abebe and Wolde (2010) in equines. This similarity may be attributed to the increased resistance to drugs observed in *T. congolense* compared to the high susceptibility of *T. vivax* to treatment. Moreover, *T. vivax* and *T. brucei* have been reported to invade tissues, as mentioned by Biyazen et al. (2014) and Demelza (2018). Similarly, Kebede et al. (2009) and Zelalem et al. (2019) showed that *T. vivax* was the predominant species in small ruminants and equines, highlighting the significance of mechanical transmission. Prevalence of trypanosomosis did not show significance ($P > 0.05$) across kebeles (Chamen Bareda, Didesa, Lilo, and Loko) in the study district. A similar pattern was observed in studies conducted by Tafese et al. (2012) and Tulu et al. (2018) in bovines from East Wollega Zone and Jimma Horro district, and by Zelalem et al. (2019) in equines from the West Wollega Zone, Ethiopia. The lack of significant variation in trypanosomosis prevalence across the sampled area could be attributed to uniform tsetse fly control measures implemented by Bedele NTTICC (National Tsetse and Trypanosomosis Investigation and Control Center), including consistent fly belt coverage and treatment strategies. This uniformity may be influenced by differences in agro-ecological conditions, vector density, the choice of trypanocidal drugs, and the efficacy of fly control operations, all of which impact parasite occurrence (Majekodunmi et al., 2013; Geiger et al., 2015).

In this study, there was a significant difference ($P < 0.05$) in trypanosomosis occurrence between female and male small ruminants, with females showing a higher prevalence. This agrees with findings of Samdi et al. (2008). Similarly, a higher prevalence was observed in females in bovines compared to males; however, these differences were not statistically significant ($P > 0.05$). In agreement with this result, Teka et al. (2012) and Frehiwot and Samson (2010) found that there was no significant difference in trypanosomosis prevalence between sexes in bovines and equines. This lack of difference could be because both sexes are equally exposed to biting flies. The increased prevalence in female bovines and small ruminants may be due to physiological differences or the larger sample sizes used (Torr et al., 2006). Conversely, the higher trypanosomosis prevalence in male equines might be explained by their more frequent use for draught purposes, leading them to travel longer distances and encounter areas with more tsetse fly challenges.

The current study demonstrated that among bovine and small ruminants categorized by their hair colors (black, mixed, red, and white), a notably higher prevalence of trypanosomosis was found in black animals, a result that aligns with earlier findings reported by Gona et al. (2016) in bovine species. Equines were similarly categorized into black and gray hair color groups, with no significant difference in prevalence ($P > 0.05$), although a higher prevalence was noted in black animals compared to gray ones. This variance could be attributed to the observed preference of flies for black color, as mentioned by Teka et al. (2012). Furthermore, studies by Tulu et al. (2018) and Zelalem et al. (2019) from Jimma Horro district and East Wollega zone in Ethiopia revealed no statistically significant differences in the prevalence of bovine and equine trypanosomosis based on skin color, respectively.

In the present study, a significant prevalence of trypanosomosis ($P < 0.05$) was noted among different age groups of bovines, small ruminants, and equines. These findings corroborate previous studies conducted by Addisalem et al. (2012), Kebede et al. (2016), and Abebe and Wolde (2010) in bovines, small ruminants, and equines, respectively, indicating a higher prevalence in adult animals compared to younger ones. This disparity is attributed to older animals grazing in tsetse fly-infested areas, while younger animals tend to graze closer to human settlements, resulting in reduced exposure to tsetse bites (Alemayehu et al., 2012); Torr et al. (2006) reported that tsetse flies are significantly more attracted to the odor of older animals. Furthermore, studies have shown that *T. congolense* infection, being a chronic disease, escalates with the age of animals, leading to higher infection in adults compared to juveniles (McDermott et al., 2003). A significant association ($P < 0.05$) was also found between livestock and body condition scores, consistent with prior research conducted in Ethiopia (Taylor et al., 2007; Solomon and Fitta, 2010; Degneh et al., 2017). This correlation could be attributed to weakened resistance in animals with poor body condition or associated to gradual weight loss due to trypanosomosis (Radostits et al., 2007). However, Dawit et al. (2015) and Kitila et al. (2017) reported no significant association between bovine body condition and trypanosomosis prevalence. This could be due to poor body condition, which may be associated with malnourishment, internal parasites, and other diseases causing weight loss (OIE, 2009).

The PCV stands as a crucial quantitative measure for evaluating the anemic condition in tested animals. This study revealed that parasitemic animals exhibit notably lower mean PCV values compared to aparasitemic ones. Similar observations were made by previous studies in cattle (Bayisa et al., 2015; Mamoudou et al., 2015), in small ruminants (Samdi et al., 2008), and in horses (Abebe

and Wolde, 2010). Similarly, Mbewe et al. (2015) observed a notable decrease in the average PCV among trypanosome-infected animals compared to non-infected ones. This decline in PCV can be attributed to the destructive effect of trypanosomes on erythrocytes in infected animals. However, it is important to note that not all anemic animals are necessarily parasitemic but can also be caused by poor nutrition, helminthosis, and tick-borne diseases (Radostits et al., 2007; Moti et al., 2013). Conversely, not all parasitemic animals may exhibit anemia, as some hosts can effectively manage the pathological effects of the disease or delayed recovery from anemia post-treatment with trypanocidal drugs (Mekuriaw and Kebede, 2015; Simukoko et al., 2007). Furthermore, the presence of infected livestock with normal PCV values could be attributed to recent infections (Vanden Bossche and Rowlands, 2001).

The study identified two *Glossina* species, *G. tachinoides* and *G. m. submorsitans*, indicating tsetse fly species diversity in the area. *G. tachinoides* thrives near water bodies, particularly in districts with large rivers like Didessa, creating ideal breeding conditions (Meharenet and Alemu, 2020). In contrast, *G. m. submorsitans* occupies a wider range of habitats, including bushy and forested areas, but is sensitive to environmental degradation (Duguma et al., 2015). Similar findings were reported in Western and Southwestern Ethiopia by Duguma et al. (2015) and Lelisa et al. (2015). Furthermore, Malele et al. (2007) from Tanzania and Pagabeleguem et al. (2012) from Burkina Faso also reported the coexistence of two to three tsetse species. The overall fly density (12 flies/trap/day) recorded in this study aligns with the findings of Lelisa et al. (2015), who reported 11.9 flies/trap/day in western Ethiopia. The observed apparent density of *Glossina* (10.4 flies/trap/day) in this study is lower than the densities reported by Teka et al. (2012) and Fentahun and Tekeba (2013) in Western Ethiopia. However, it is higher than the apparent density reported by Dagnachew et al. (2005) and Getachew et al. (2020). These variations may stem from control interventions implemented in the study district, changes in land use due to agricultural expansion, seasonal factors, vegetation cover, and the movement of reservoir game animals (Leak, 1999; Vanden Bossche et al., 2010; Malele, 2011). The higher apparent density of *G. tachinoides* (85 %) than *G. m. submorsitans* (15 %) recorded in this study agrees with the report by Shaw et al. (2013). The lower numbers of *G. m. submorsitans* could be attributed to their avoidance of stationary traps or migratory behavior (Shaw et al., 2013).

Research shows that female tsetse flies are more active than males, primarily due to their nutritional requirements for viable larval development (Attardo et al., 2012). In this study, female tsetse flies comprised 65 % of the captured specimens, while males made up only 35 %. This finding supports the results of Fentahun and Tekeba (2013), which indicate a higher prevalence of females in various habitats. Contributing factors include the longer lifespan of females compared to males, as highlighted by Lehane (2005). Furthermore, the WHO (2013) notes that the reproductive demands of female tsetse flies drive their increased feeding activity, leading to their greater abundance in natural populations. Therefore, it is essential to consider these gender-specific behavioral and biological traits when evaluating tsetse fly populations and their implications for the transmission dynamics of trypanosomosis (Aksoy et al., 2014; Longbottom et al., 2020).

The identification of *Stomoxys* and *Tabanus* species in this study, alongside *Glossina*, underscores the role of mechanical vectors in the transmission dynamics of trypanosomes. Previous research (Sinshaw et al., 2006; Kone, 2011; Baldacchino et al., 2014) supports this finding, highlighting the significance of these vectors, particularly *Stomoxys* (stable flies) and *Tabanus* (horseflies). Unlike *Glossina*, which are biological vectors, *Stomoxys* and *Tabanus* facilitate mechanical transmission by transferring infected blood between hosts during feeding (Desquesnes et al., 2009; Baldacchino et al., 2013). This mode of transmission can sustain the trypanosomosis infection cycle, especially in regions where *Glossina* populations are controlled (Geiger et al., 2018; Odeniran et al., 2020). The presence of these mechanical vectors could contribute to the persistence of trypanosome infections among livestock, particularly during seasons when *Glossina* numbers decline (Dagnachew et al., 2020; Konan et al., 2023), due to their broader distribution beyond the tsetse belt.

The average age (28 days) of male *G. tachinoides* recorded in the current study agrees with the finding of Getachew (1983), who reported that flies younger than 14 days old could potentially act as vectors for trypanosome infection. This finding is consistent with the suggestion by Leak and Rowlands (1997) that the prevalence of trypanosome infections in natural tsetse populations rises with the age of the flies. In the present study, the proportion of young males with 1–3 wing-fray categories exceeded that of older males with 4–6 categories, which is similar to the findings of Abebe et al. (2005) on *G. pallidipes*. This could be attributed to young males being more suited to the climatic conditions of area, resulting in higher mortality among older age groups (Leak and Rowlands, 1997).

The infection rate (4.8 %) observed in *G. tachnoides* is similar to the findings of Desta et al. (2013), who reported an infection rate of 5.98 %. However, it is higher than the infection rate (1.76 %) reported by Meharenet and Alemu (2020). These differences may stem from seasonal variations, the involvement of different species of tsetse flies, or variations in fly-animal contact (Bourn et al., 2005). Higher rates of trypanosome infections were noted in female tsetse flies (5.1 %) compared to males (4.3 %), consistent with the observations of Meharenet and Alemu (2020). This could be attributed to differences in feeding habits and longer life expectancy in female tsetse flies (WHO, 2013). Among the trypanosome species infecting tsetse flies, *T. vivax* (58.3 %) was more prevalent than *T. congolense* (41.7 %), as similarly found in a study by Adams et al. (2010) in East Africa. This disparity may be due to the simpler life cycle of *T. vivax* compared to *T. congolense* and *T. brucei* (FAO, 2008). Notably, starved flies exhibited a higher infection rate (9.7 %) compared to fed (2.4 %) and engorged (0 %) flies. This could be attributed to age, as older flies have a higher likelihood of infection and more time for the infection to mature (FAO, 2000).

The time frame between capturing and dissecting *Glossina* flies is crucial for accurately assessing their infection status. Delays significantly reduce the number of infected flies, as parasites may degrade or be expelled from the host. Studies indicate that the viability of *T. brucei* diminishes quickly once the vector is removed from the host, leading to underreporting of infection rates (Mattioli et al., 2018). To minimize variability, our study mandated dissection within 2–4 h of capture, aligning with best practices to enhance the accuracy of our findings on trypanosomosis prevalence.

The study did not incorporate molecular techniques because of facility and resource constraints. Consequently, there is a need for further research that includes molecular techniques to explore trypanosomosis and its vectors. Furthermore, this study did not permit the collection of blood samples from cattle across all seasons to assess the seasonal impact on disease prevalence. It is also crucial to

conduct future studies using a longitudinal study design over longer durations. Despite these limitations, the study provides valuable insights into trypanosomosis epidemiology in cattle, small ruminants, and equines, aiding interventions to reduce the impact disease on livestock productivity and livelihoods of farmers.

5. Conclusion

The current study has highlighted the significance of trypanosomosis as a major disease affecting bovines, small ruminants, and equines. It has also provided baseline information that can inform the development and execution of a disease control program in the study area. The presence of the disease was correlated with two *Glossina* species (*G. tachinoides* and *G. m. sub-morsitans*) as well as two genera of biting flies (*Stomoxys* and *Tabanus*). The study revealed that *T. congolense* was the predominant trypanosome species among livestock, while *T. congolense* and *T. vivax* were prevalent in tsetse flies. The prevalence of trypanosome infections in the area was significantly influenced by factors such as animal species, age, and body condition. To effectively combat the spread of tsetse and trypanosomosis, an immediate and comprehensive integrated control strategy should be implemented, encompassing measures to minimize the distribution of the disease. Advanced techniques like PCR should be conducted to assess trypanosomosis prevalence in vectors and animal hosts during different seasons.

Ethical statement

The procedures were conducted in accordance with the experimental protocols and standards approved by the animal welfare and research ethics committee at the National Institute for the Control and Eradication of Tsetse Fly and Trypanosomosis in Bedele, adhering to international guidelines for animal welfare. Additionally, verbal consent was obtained from cattle owners, ensuring their full cooperation and voluntary participation while assuring them of the confidentiality of their involvement.

CRediT authorship contribution statement

Surra Gebeyehu: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Dereje Tulu Robi:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors have not disclosed any conflicts of interest.

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