

Exploring the factors driving the recurrence of human African trypanosomiasis in Ethiopia after three decades

Abate Waldetensai^{*}, Ebise Abose¹, Abebe Getachew¹, Henok Tadesse¹, Behabilom Meharenet², Moges Hidoto², Araya Eukubay¹, Solomon Kinde¹, Alemayehu Kassahun³, Geremew Gonfa¹, Kokeb Kore², Netsanet Asfaw², Daniel Tsega¹, Adugna Abera¹, Belachew Bokicho⁴, Geremew Tasew¹ and Abraham Ali¹

^{*}Corresponding to Authors: abate@ephi.gov.et, abyw.res31@gmail.com

¹Ethiopian Public Health Institute, Addis Ababa, Ethiopia

²Animal Health Institute, Sebeta, Oromia, Ethiopia

³Neglected Tropical Disease Control, Arba Minch University, Arba Minch

⁴Neglected Tropical Disease Control, Southern Nation Nationalities and Peoples of Regional Health Bureau, Hawassa

Abstract

Background: Human African trypanosomiasis (HAT), or sleeping sickness, results from a parasitic protozoan transmitted by infected tsetse flies. The re-emergence of HAT in Ethiopia, reported since 1967, has recently seen cases re-emerged in disease-free areas, prompting investigation into contributing factors.

Objective: The aim of this study is to identify the possible drivers that caused the recurrence of HAT in Ethiopia.

Methods: A cross-sectional study design with descriptive data analysis was used. NGU and biconical traps were used to capture tsetse flies and dissected under a stereo binocular microscope to identify the parasite. Blood samples were taken from the animals and the parasites in the serum were identified using the buffy coat method.

Results: A total of 329 tsetse flies were identified: *G. pallidipes* 259 (60.4%) and *G. fuscipes* 70 (16.3%). Dembagofa accounted for 188 (51.1%) of the captures, with an apparent density of 94. 11.8% of flies had recently fed on hosts with high female density. Highest apparent densities were in forests (93), mostly *G. pallidipes* (76.5%). Forests (93) and shrublands (36.5) had the highest densities. Among 301 tested cattle, 9 were positive (3% prevalence): *T. congolense* 6 (2%), *T. vivax* 1 (0.3%), and 2 (0.7%) suspected *Brucei*. Parasite prevalence was higher in poorly conditioned cattle (4.6%).

Conclusion: The high density of tsetse flies and the prevalence of *Trypanosoma* parasites in cattle create an ideal environment for the transmission of this deadly disease. Addressing the resurgence of Human African Trypanosomiasis (HAT) in Ethiopia requires a collaborative effort from researchers, policymakers, and communities. The complex interplay of environmental, agricultural, social, and health system factors underscores the need for a comprehensive and multisectoral approach to control and eliminate HAT. This involves not only addressing health system deficiencies but also broader socioeconomic and environmental issues. Urgent measures include strengthening HAT surveillance and response capacity in Ethiopia, along with increasing public awareness of the disease and its prevention.

Key words: Trypanosomiasis, Tsetse fly, *Trypanosoma brucei rhodesiense*, Human, Animal, Ethiopia

Background

Sleeping sickness or Human African Trypanosomiasis (HAT) is a parasitic disease caused by the genus *Trypanosoma* (T.) and transmitted by infected tsetse flies. The disease poses a major threat to millions of people living in remote rural areas of 36 sub-Saharan African countries where access to health services is limited (Crump et al. 2021). The subspecies *T. b. rhodesiense*, which occurs in eastern and southern Africa, can cause acute and rapidly progressive HAT with epidemic potential (Alderton et al. 2018). HAT, without distinguishing between *T. b. gambiense* and *T. b. rhodesiense* was included in the first global assessments of the burden of disease and is estimated to result in a loss of 1.78 million DALYs across Africa and is the good reason for many parameters related to HAT used in the global assessments (e.g. disability weighting and incidence estimates) is not transparent and represents a major problem (Nagagi and Kweka 2017).

Animal trypanosomiasis (AAT), called Nagana, is caused by the same *Trypanosoma* species and affects both wild and domestic animals. Animal production in the affected regions is severely affected by the disease, resulting in reduced productivity (Geremew et al. 2018). Tsetse flies are the main vectors for HAT and AAT, highlighting their central role in the transmission of trypanosomes to vertebrate hosts. Most trypanosomal infections in wildlife do not appear to cause harm to their hosts, but some wildlife species are at extreme risk for trypanosomal infections (Nagagi and Kweka 2017). *T.b. rhodesiense* occurs in both domestic and wild animals and infects with *T. b. gambiense* and most likely serve as a reservoir. However, it is still unclear what the exact contribution of the animal reservoir is to the epidemiology of Gambiense-type disease. *T. brucei*, *T.*

congolense, *T. simiae* and *T. uniforme* are transmitted within the tsetse belt of Africa. *T. vivax* and *T. evansi* can be transmitted mechanically within and outside tsetse-infested zones (Vincent et al. 2015).

In Ethiopia, the distribution of endemic human trypanosomiasis and its tsetse vectors appears to be limited to the south and southwest of Ethiopia (Oromia region (Illubabor and Wollega), Gambella and SNNPR) (Vincent et al. 2015). No major outbreak of the disease was recorded until the epidemics of 1969–1970, and then, after 21 years, in 1991, a case of HAT was reported in Gambella. Ethiopia recently reported five HAT cases from Gamo Zone, SNNP Region. Factors such as lack of surveillance, the presence of a favorable ecology for vector spread and disease transmission, and agricultural activities near vector habitats contribute to the re-emergence of HAT. Improved access to health services, better methods of controlling tsetse flies, and advances in diagnostics and treatment are critical steps in the fight against the spread of trypanosomiasis. Despite these challenges, there are efforts to combat the spread of HAT and Nagana. It is important to address the challenges posed by HAT and Nagana to reduce their impact on these remote rural communities. Tsetse fly eradication measures such as ground and aerial sprays, blue-black-blue targeting and artificial barriers are recommended (Longbottom et al. 2021).

Collaboration between national governments, international organizations and local communities is key to sustainable progress (Reid et al. 2012; Boundenga et al. 2022). It is the shared responsibility of all stakeholders to prioritize access to healthcare, develop effective prevention and control strategies, and support those most affected by these debilitating diseases (Boundenga et al. 2022). By working together, we can build healthier communities and achieve a better quality of life for everyone. A confirmed case of HAT was first reported in Ethiopia in 1967. The epidemiology of HAT in Ethiopia needs to be studied in detail to develop more effective and accessible diagnostic, treatment and control programs. Innovative research and community-based approaches can provide important interventions to halt the progression of these diseases (Duguma et al. 2015). The aim of this research is to determine the root cause of the recurrence of HAT in Ethiopia after 31 years. Data analysis of HAT cases by site of infection, detection of pathogenic trypanosomes in animals, and identification of tsetse flies and their distribution status within various environmental variables were performed. Such information can enable the use of appropriate control methods at the intersection of human vector activities and lead to improved disease control measures and policy making. This research provides insightful insights into the resurgence of

African trypanosomiasis (HAT) in humans in the country. The study highlights the need for ongoing surveillance and control efforts to prevent recurrence of the disease, as well as the importance of monitoring the spread of tsetse flies and pathogenic trypanosomes in animals. The findings have significant policy implications, with a focus on developing targeted control methods that address the specific environmental factors contributing to the re-emergence of HAT in Ethiopia. In addition, the study may increase public awareness and understanding of HAT, which may further encourage stakeholders to develop targeted interventions aimed at reducing the overlap between human and tsetse fly activities and preventing HAT transmission. Ultimately, this work has the potential to improve the lives of millions of people in HAT-endemic areas. Therefore, the aim of this research was to identify the entomological and epidemiological drivers of the recurrence of HAT in Ethiopia after three decades and to determine the implications for disease control and prevention strategies.

Material and Methods

Study periods and Setting: A cross-sectional study design was used that considered all historical and current information on HAT case report, tsetse transmission history, environmental variables, and animal case surveillance variables. The survey was conducted from August to November 2022 following the reported outbreak of the disease in two districts of the SNNPR in Ethiopia, namely Kucha alpha (Gamo zone) and Dembagofa (Gofa zone). SNNPR is known for its diverse population, which includes people of different languages, cultures and ethnic backgrounds (Zekarias et al. 2020). Kucha district, the first study area, is located 172 kilometers from Arba Minch town in the GamoGofa zone. The district is 440 kilometers from Addis Ababa, the capital of Ethiopia. The town of Selamber serves as the district capital (Senbeta et al. 2016). The second study area, DembaGofa District, is located 510 kilometers south of Addis Ababa and is divided into 38 rural kebeles. The town of Sawla serves as the capital of the woreda. The study area can be divided into three agro-climatic zones, namely highland altitude/Dega/ (7.8%), medium altitude/WoinaDega/(15.8%) and lowland altitude/Kola/(76.4%) (Figure 1).

Tsetse Fly sampling: Tsetse flies were captured using biconical traps, small adhesive targets, and NGU traps in and around the HAT case report areas. Depending

on the vegetation type, drainage system, accessibility and human activities, 30 traps were installed in each grid at approximately 4/km² for two days. All traps were baited with 100 mg/h of cow urine from plastic bottles and acetone and 500 mg/h from glass bottles and greased to prevent ants from damaging the catch (Vale et al. 2015). After 12 hours, flies were removed from the trap, identified using a *Glossina* species identification key, and preserved in ethanol for further molecular detection. The collected tsetse flies were then counted by species and sex, and those that were

either stunned or just dead were dissected to look for possible trypanosomes. The dissection was performed under a binocular stereomicroscope with saline solution (Boundenga et al. 2022). All dissected organs were collected individually in microtubes containing sterile distilled water and stored at 20 °C until DNA extraction. Unprepared tsetse flies were also preserved for further parasite analysis using PCR (Ngomtcho et al. 2017). All bottles containing captured flies were labeled with location, trap number, date and time, and GPS coordinates were recorded using GARMIN (72H).

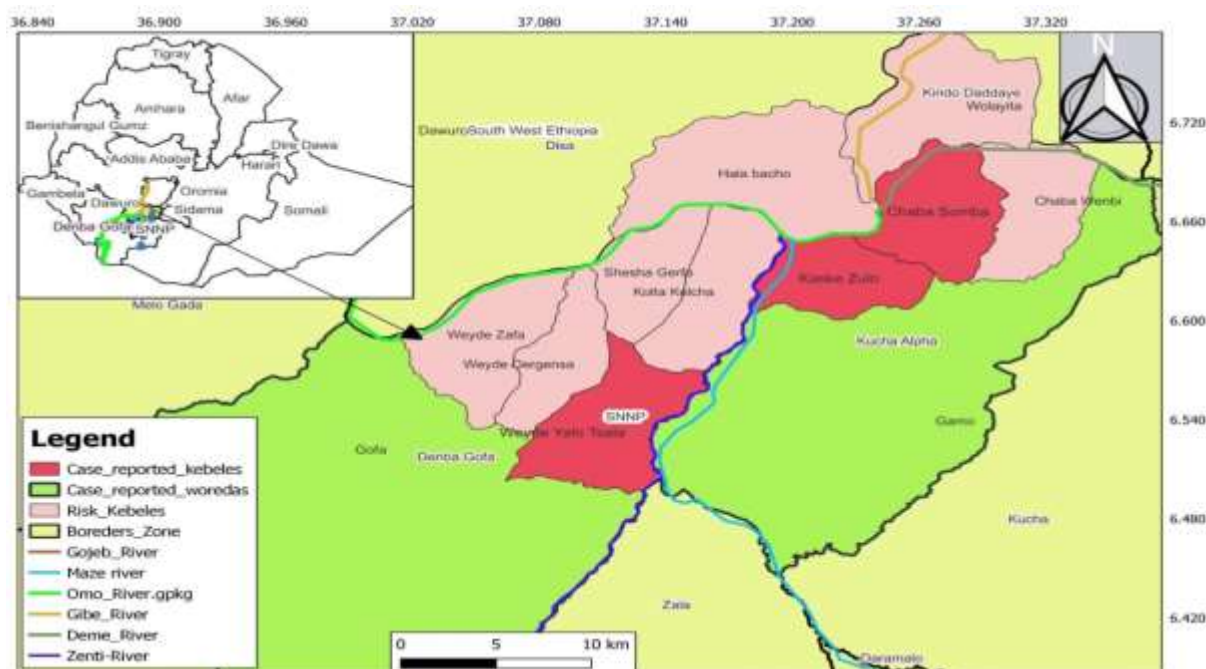


Figure 1: Map of outbreak areas in Ethiopia, SNNP and HAT investigation sites (Kucha Alpha and Demba Gofa), 2022

Animal Sample Collection and parasite detection:

Animal sample collection and parasite identification Methods were used to collect animal samples and identify trypanosomal parasites. Samples were collected from different regions of Ethiopia where the disease had recurred. 301 cattle were randomly selected by local breeders based on different body types and colors. Cattle were the most common livestock in the study area, and interestingly, cattle were mainly kept on the outskirts of villages or along rivers flowing through villages and forests. They also discovered that the area was infested with tsetse flies, making the livestock vulnerable to various parasites. All necessary precautions were taken during animal sampling to ensure the safety of both the animals and the research team. Blood samples were taken from each animal and carefully collected. About 10 µl of blood was collected from the peripheral ear vein using

a heparinized microhematocrit capillary (Rosemary et al. 2017). All blood samples were analyzed using the buffy coat method (BCM). After unscrewing the screw cap and closing the centrifuge, he centrifuged the sample at 12,000 rpm for 5 minutes (Ngomtcho et al. 2017). After centrifugation, the tubes were placed in a hematocrit reader and measurements were expressed as a percentage of red blood cells relative to total blood volume. Animals with PCV = 25 were considered not anemic. Finally, he examined the slides for parasitic movement's using a 40x objective and a 10x eyepiece (Adugna et al. 2017).

Method of statistical analysis: Data analysis began with data entry and cleaning using Microsoft Excel 2019, followed by exporting to SPSS version 23 for further analysis. Descriptive statistics were used to summarize the data based on various variables such as gender and abdominal condition, as well as animal and

laboratory results. Independent variables such as location, trap type, tsetse species, vegetation type, and collection were also analyzed for the distribution of vector and species ecological variation. Tsetse catches were considered as the dependent variable and tsetse fly population density (Apparent Density (AD)) was calculated using fly/trap/day (Adungo et al. 2020). Researchers used cross-tabulations, chi-square tests and logistic regression to conduct statistical analyses. The overall prevalence rate or infection rate was determined by dividing the number of positive results by the total number of animals tested and multiplying by 100 (Mekonnen et al. 2017; Nakamura et al. 2021). In addition, logistic regression was used to assess the association between trypanosomal infection and associated risk factors, and chi-square tests were used to determine prevalence based on gender, age, and body condition scores. Mean PCV values between *Trypanosoma*-positive and -negative animals were compared using two-sample t tests. In all analyses, the 95% confidence interval level was used and a P value of less than 0.05 was considered significant. These analytical techniques allowed us to generate reliable and comprehensive statistical data that served as the basis for the research results and conclusions.

Ethics statement

The blood collection from cattle was carried out as part of the epidemiological surveillance activities of the World Organization for Animal Health. Local authorities do not require an ethics statement. As a responsible organization, we understand the importance of conducting research in a way that ensures the welfare of all animals involved. Blood sampling was carried out exclusively for the purpose of epidemiological surveillance and with the permission of the livestock farmer. It was conducted as part of prophylactic or diagnostic campaigns in response to an outbreak of African trypanosomiasis in humans. No samples were collected for purposes other than outbreak response screening and diagnostic procedures. To ensure that owners were fully informed about the objectives of the study, we clearly explained the procedures and obtained their consent for animal sampling. In addition, a veterinarian from the Ethiopian Public Institute and the Animal Health Institute conducted vein samples to ensure the animals were properly cared for. Finally, all animals examined received free deworming treatment. Those who tested positive for trypanosomiasis were treated immediately to ensure they received the appropriate care they needed.

Results

HAT case reports: Of the 5 confirmed cases, 4 resulted in death, resulting in a CFR of 80%. This high

mortality rate underscores the urgency of identifying and treating cases as quickly as possible. The age distribution of confirmed cases shows that one case found 0-4 years, 2 cases occurred in the 5- to 14-year-old age group and 2 cases occurred in the 15- to 44-year-old age group. Interestingly, all but one of the cases were female, with only one female case reported between 0 and 4 years of age. The results of HAT case reports in Kucha Alpha and Damba Gofa districts revealed an alarming attack rate (AR) of 38 and 37 per 100,000 population respectively. These statistics demonstrate the potential for a large-scale outbreak of HAT in these regions and require immediate attention from health authorities.

Fly composition, density, Trap efficacy and abdominal status of tsetse flies: A total of 429 flies from three genera were captured: tsetse flies 329 (76.7%), stomoxis 76 (17%) and tabanids 27 (6.3%). Within this genus, two species have been identified as *G. pallidipes* and *G. fuscipes*. *G. pallidipes* was the more dominant species with 259 individuals captured (60.4% of all flies captured). This was followed by *G. fuscipes*, which accounted for 16.3% of all flies captured. Among the other genera captured, Stomoxis accounted for 17% of all flies captured, while Tabanids accounted for only 6.3%. Our findings are particularly important given the outbreak reported in the region. The fact that the predominant species of tsetse fly captured was *G. pallidipes*, which is a known vector for trypanosomiasis, suggests that control efforts should target this species. Furthermore, the high number of flies caught in such a short period of time (129.5 AD and 35 AD for *G. pallidipes* and *G. fuscipes*, respectively) highlights the urgency of controlling the outbreak.

A total of 329 tsetse flies, with 89.4% (294) captured by biconical traps and only 10.6% (35) captured by NGU traps. The effectiveness of the biconical trap was clearly demonstrated in these results. Furthermore, this study revealed a high population density of *G. pallidipes* (a notorious vector of African trypanosomiasis) in the biconical traps, with 70.5% (232) of the total 329 tsetse flies captured belonging to this species. The biconical trap was clearly the preferred choice of *G. pallidipes* as this type of trap captured a large number of unfed tsetse flies (283 or 86% of the total). The average density (AD) of the population was also remarkably high at 116. A relatively high number of 39 (11.8) fed tsetse flies were collected using biconical traps, particularly *G. pallidipes* 32 (9.7%).

Ecological distribution of Tsetse fly in each study sites:

The study results showed that Demba Gofa was the most productive area for collecting tsetse flies as 188 (51.1%) of all flies were caught in this region. Further analysis revealed that 153 (41.6%) of these flies were identified as *G. pallidipes*, indicating a higher concentration of this species in Demba Gofa.

The average density of *G. pallidipes* in this area was 76.5 AD. Interestingly, the study also revealed that there were significant numbers of *G. pallidipes* in Kucha Alpha district, with 28.8% (n=106; AD =53) of total *G. pallidipes* were collected at this site (**Figure 2**).

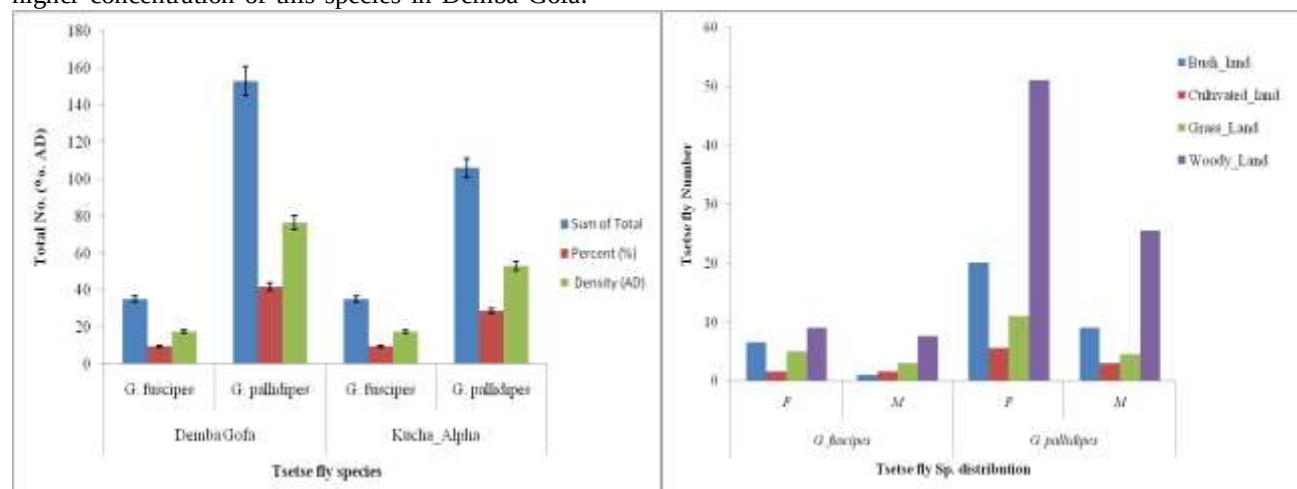


Figure 2: Distribution of Tsetse fly species in fly sampling sites (Demba Gofa and Kucha Alpha), SNNPR, Ethiopia

The AD of female tsetse fly species was found to be high in each ecological variant: forestland (51), shrubland/bush-land (20), and grass-land (11). A high AD of male tsetse flies (*G. pallidipes*) and a high number of females were observed in the forest. In general, the AD (93) of tsetse fly was high in forest areas, mainly in *G. pallidipes* (76.5), relatively followed by shrubland/bush-land (36.5) with the same species, *G. pallidipes* (Figure 2).

This study found that female tsetse fly species had high abundance density (AD) in all ecological variations, namely forest, shrubland and grassland. Surprisingly, forests had the highest AD of female tsetse flies at 51, while shrublands and grasslands had lower numbers at 20 and 11, respectively. Furthermore, AD numbers of male tsetse flies (*G. pallidipes*) were also high in forest areas, with densities similar to female numbers. The AD of tsetse flies in general was highest in forested areas, with a whopping 93 counts. *G. pallidipes* was the most abundant species in this variant with a density of 76.5, followed relatively closely by the same species in the shrubland with a density of 36.5 (**Figure 2**).

Animal investigation result: The results of this study showed that trypanosomiasis was present in 3% of the

301 cattle examined. This indicates that there is a significant risk of disease in the population studied. The prevalence of the parasites was low; only nine cattle were infected. Of these, two types of trypanosomes were identified, namely *T. congolense* and *T. vivax*, with 6 and 1 cases, respectively. In addition, two suspected cases of *Brucei* were also reported. It is interesting to note that the risk prevalence of trypanosomiasis was higher in cattle with poor body condition (Bcs).

A total of 4 cattle were affected, the prevalence was 4.6%. This highlights the importance of maintaining the health and condition of cattle to reduce the risk of disease. In addition, a higher risk of trypanosomiasis was observed in the 5–9-year age group, with 7 cases detected, accounting for 5.3% of all cases. This suggests that older cattle are more susceptible to the infection and require additional care and monitoring (**Table 1**). The results of our study showed that trypanosomiasis is widespread in black cattle in Demba Gofa region with a detection rate of 6.7%. This is in stark contrast to the prevalence rate in Kucha Alpha, where the prevalence in white colored cattle was only 3.3% (**Table 1**).

Table 1: Results showing association of potential factors for the prevalence of Trypanosomiasis, Damba Gofa and Kucha Alpha districts, SNNP, Ethiopia

Factors	Level	Total tested	-ve	Positive (%)			Total +ve
				Brucei_suspected	T_congo	T_vivax	
Sex	Female	139	134	1(0.7)	4 (2.9)	0 (0)	5 (3.6)
	Male	162	158	1(0.6)	2 (1.2)	1(0.6)	4 (2.5)
Bcs	Good	55	54	0 (0)	1(1.8)	0 (0)	1(1.8)
	Medium	159	155	1 (0.6)	2 (1.3)	1(0.6)	4 (2.5)
	Poor	87	83	1((1.1)	3 (3.4)	0 (0)	4 (4.6)
Age	1 - 4 yrs	160	158	1 ((0.6)	0 (0)	1(0.6)	2 (1.3)
	5 - 9 yrs	132	125	1(0.8)	6 (4.5)	0 (0)	7 (5.3)
	10 - 15 yrs	9	9	0 (0)	0 (0)	0 (0)	0 (0)
Grand Total		301	292	2 (0.7)	6 (2)	1(0.3)	9 (3)

	Animal color	Total tested	-ve	Positive (+ve)			Total +ve	IR (%)
				Brucei	T_congo	T_vivax		
Dambagofa		96	94	0	2	0	2	2.1
	Black	15	14	0	1	0	1	6.7
	Red	44	43	0	1	0	1	2.3
	White	37	37	0	0	0	0	0.0
Kucha Alpha		205	198	2	4	1	7	3.4
	Black	31	29	1	1	0	0	0.0
	Red	114	111	1	2	0	0	0.0
	White	60	58	0	1	1	2	3.3
Grand Total		301	292	2	6	1	9	3.0

Discussion

The problem of sleeping sickness is a growing problem in rural areas of southwest Ethiopia, particularly in Kucha Alpha and Demba Gofa. The confirmed cases of HAT, decade years after it emerged in Ethiopia in 1991, paint a grim picture of the seriousness of the situation. This study on human African trypanosomiasis in Ethiopia sheds light on the alarming increase in the number of cases in the outbreak areas.

All ages and both sexes are at risk, prevalence is higher in adults, and gender distribution varies with respect to sex-specific activities at risk (e.g., predominantly male hunting and fishing or predominantly female water fetching and small plant cultivation) (Elenga et al. 2022). With a mortality rate of 80%, the disease poses a significant threat to the population, particularly men between the ages of 15 and 44. The most affected by this disease is the working population, which consists mainly of farmers, ultimately affecting productivity. Studies from the Republic of Congo have confirmed that uneducated people and those over 15 years of age have a significantly higher risk of HAT infection. The majority of positive cases came from households involved in agriculture (livestock and agriculture). As previously mentioned, children are less likely to contract the disease than adults because they are less exposed to flies. However, children who accompany their parents in everyday activities are still at risk

(Efrem et al. 2018). The results of this study also suggest possible gender disparity in HAT cases, raising concerns about exposure and transmission patterns between different age and gender groups. The results of the HAT case reports in Kucha Alpha and Damba Gofa districts found an alarming attack rate (AR) of 38 and 37 per 100,000 population, respectively. Timely diagnosis and treatment of HAT cases is crucial to prevent serious illness, disability and even death (Bemba et al. 2022). This discrepancy could potentially influence future research and intervention efforts aimed at curbing the spread of the disease. The prevalence of HAT in older people observed in this study is alarming and highlights the need for targeted interventions in the most vulnerable age groups. It is important to recognize the far-reaching implications of HAT, beyond just the medical effects. This disease has a significant social and economic impact, disproportionately affecting some of the poorest and most vulnerable communities in endemic areas. It is estimated that around 70 million people (1.55 million km²) are at risk of HAT in Africa, accounting for 10% of the total population and 7.4% of the total area of endemic countries (Longbottom et al. 2021). While the study highlights the risks and potential spread of human African trypanosomiasis in Ethiopia, it emphasizes the need for ongoing surveillance and prevention efforts. At the territorial boundary of trypanosomiasis distribution, conservation areas shape

the patterns of HAT risk, particularly near the development corridor, livestock and agricultural intensification within the natural parks and forest of the Omo River and Gibe River Basin (Nakamura et al. 2021).

The results of the current study showed that two Glossina species, *G. pallidipes* and *G. fuscipes*, were the predominant species in the outbreak areas. The identification of these species in the regions where HAT cases have been reported suggests that animal reservoirs may be responsible for the spread of the diseases. It is not uncommon for the identified tsetse fly species to feed on both small and large wild and domestic animals, facilitating the transmission of HAT. The fact that these flies have a high degree of adaptation to their environment makes them difficult to control (Uba et al. 2016). It also poses a threat to both human and animal populations, making the management of HAT a complicated problem. The study results showed that Demba Gofa was the most productive area for collecting tsetse flies. Since the study zone is home to two species of tsetse flies - *G. pallidipes* and *G. fuscipes* as well as *T. vivax* and *T. congolense* - which dominate the cattle population, it is clear that measures are needed to control and prevent the spread of these vectors and parasites (Simo et al. 2014). Interestingly, the study also revealed that there were significant numbers of *G. pallidipes* in Kucha Alpha district, with 28.8% (n=106; AD =53) of the total *G. pallidipes* collected from this location. This suggests that Kucha Alpha district could also be a potential hotspot for collecting tsetse flies, particularly *G. pallidipes*. Finally, the study found that *G. fuscipes* was present at both study sites, although no specific numbers were provided.

The high apparent density of tsetse flies per trap per day of 164.5 is an alarming finding that cannot be ignored. This is in contrast to previous studies conducted in Benishangul Gumuz, Ethiopia, which reported lower densities of tsetse flies (Elenga et al. 2022). These results suggest the need for continued monitoring and surveillance of tsetse fly populations and their impacts on animal health and agriculture. The results of this study are relevant not only to the scientific community, but also to policymakers, ranchers, and communities whose livelihoods depend on livestock production. The results provide a basis for further research, interventions and development of strategies to control tsetse flies and trypanosomiasis in Ethiopia.

Understanding the importance of ecological variation on tsetse fly population density is an ideal step toward controlling the fly species. In this study, forest appears to be the most favorable habitat for these species,

which could lead to an increased risk of tsetse fly-borne diseases in this region. These findings could potentially help develop strategies to control tsetse fly populations and mitigate their impact on human and animal health in areas where they pose a threat. In the current study area, the abundance of *G. pallidipes* exceeded the previous study by Lemu et al. from Jima (Lemu et al. 2019). This increase in tsetse species can be attributed to several factors, including favorable vegetation and higher host densities (Gebre et al. 2022). Fluctuations in agroecology (Lemu et al. 2019) and bioclimatic conditions may occur during capture periods, leading to an increase in the tsetse fly population. The high density of flies in forests and the comparatively lower density in shrublands and grasslands may have several reasons, including climatic conditions, dispersal opportunities and the attractiveness of the vegetation. In fact, the availability of suitable vegetation appears to be the main factor responsible for these variations, according to the report by Mekonen et al (Golessa and Mekonnen 2017) from the Benishangul Gumuz region of Ethiopia. This study particularly highlights the importance of conserving natural habitats and maintaining vegetation cover to protect not only tsetse flies but the ecosystem as a whole (Gebre et al. 2022).

The results of this study shed new light on the effectiveness of various traps in catching tsetse flies, particularly the species *G. pallidipes*, which is notorious for transmitting African trypanosomiasis. The biconical trap emerged as the clear winner, catching 89.4% of the total 329 flies collected in the study. In fact, an incredible 70.5% of all tsetse flies caught in the biconical trap were of the species *G. pallidipes*. This insight is crucial for the development of effective control measures against this vector-borne disease. It is also worth noting that a significant number (11.8%) of the fed tsetse flies were collected in the biconical trap, particularly from the species *G. pallidipes* (6.7%). This suggests that the trap could be used to monitor the disease, as the presence of fed flies indicates possible transmission of African trypanosomiasis. By highlighting the connection between human and animal trypanosomiasis and the various ecological factors, we can better assess the risks associated with specific habitats and design appropriate intervention measures (Ngomtcho et al. 2017).

Large numbers of unfed flies have been observed in the current study areas. This may affect the parasite density within the tsetse fly population and the transmission of trypanosomiasis. This may be an important factor in natural situations where the densities of hosts that commonly feed on tsetse flies may vary between periods (Golessa and Mekonnen

2017). The movement of the host population can determine the nutritional status of the vectors. In addition, when moving from protected areas to agricultural areas, host densities can fall below the level required to maintain tsetse fly populations, but lead to an increase in host densities in other areas (Shumago and Tekalign 2016). Likewise, host densities of wild and domestic hosts are significant factors in trypanosome transmission and affect vector density and mortality rates. The presence of hungrier flies in regions where wild hosts are scarce may have an impact on trypanosome transmission. First hungrier flies may be more likely to bite less preferred hosts, including humans, further increasing the risk of trypanosomiasis transmission.

Furthermore, the identification of pathogenic trypanosomes in these tsetse flies was crucial to understanding the potential risks associated with different biotopes. After carefully dissecting several tsetse fly species, we found that none of them were positive for the HAT parasite. While the lack of infected tsetse flies is encouraging news, it is important to note that this is not a guarantee that there will be no more infections in the region in the future. Furthermore, a zero prevalence of the HAT parasite would most likely not mean that there are no parasite-infected tsetse flies in the area (Aksoy et al. 2014; Golessa and Mekonnen 2017). Our results are consistent with a previous study by Vincent et al. (Vincent et al. 2015) in Ivory Coast, where a low natural infection rate of tsetse by trypanosomes of the Brucei complex was also found. However, further research is needed to better understand the mechanisms underlying the low prevalence of HAT parasite infections in tsetse flies, which remains very low. This information will be invaluable in guiding future efforts to control and prevent HAT infections in affected populations.

This study provided some interesting insights into the prevalence of trypanosomiasis in the Dembagofa and Kucha Alpha regions. According to this study, Bovine trypanosomiasis is widespread, with a risk prevalence of 3% in the population studied. The occurrence of the disease in only a small number of cattle indicates a significant threat to the population. It is therefore essential to maintain the health and condition of these animals. On a positive note, the prevalence of bovine trypanosomiasis in the present study was significantly lower than in the previously published study (Gebre et al. 2022). The study identified *T. congolense* and *T. vivax* of the parasite, with the former being more common. This is consistent with the results of previous studies and sheds light on the worrying reality of AAT transmission in these areas (Golessa and Mekonnen 2017). Animal health and welfare must continue to be

a priority, particularly in areas where the risk of transmission is higher. It is important to remember that trypanosomiasis does not only affect our animals. People can also become infected with this disease, leading to serious health complications. Therefore, it is important to monitor and combat any possible signs of this disease in both animals and humans. It is worth noting that poor body condition in cattle conferred a higher risk of trypanosomiasis, as 4 cattle, accounting for 4.6% of all cases, were affected. This is due to poor body condition, increased risk of infectious diseases in the animals, reduced animal presentation due to lack of essential nutrients and poor management (Ngomtcho et al. 2017). This suggests that there is a relationship between animal health and disease prevalence (Adugna et al. 2017). This therefore implies that measures to promote good husbandry practices, such as adequate feeding, regular health checks and maintaining hygiene, could reduce the incidence of the disease (Elenga et al. 2022). Additionally, the study observed a higher risk of trypanosomiasis in older cattle, with seven cases recorded between 5 and 9 years of age. This reinforces the need for regular monitoring of cattle, particularly older cattle, to facilitate early detection and treatment of the disease.

The black cattle in Dembagofa are more susceptible to this disease than white cattle in Kucha Alpha. These results have significant implications for cattle disease management strategies as they highlight the importance of animal color in determining susceptibility to trypanosomiasis (Nakamura et al. 2021). A possible explanation for this difference in susceptibility could be environmental conditions (Golessa and Mekonnen 2017). It is possible that the climate and other environmental factors in Dembagofa create a more hospitable environment for the disease-carrying vectors that transmit trypanosomiasis. Likewise, differences in geographical location between the two regions may contribute to the prevalence of trypanosomiasis. Another factor that may contribute to these differences is genetic predisposition. There is evidence that certain coat colors in animals may be associated with a higher susceptibility to disease. Therefore, breeders and livestock owners should pay close attention to animal color when selecting breeding animals or considering disease control strategies. It is also possible that there are differences in vector populations associated with different cattle breeds and colors (Boundenga et al. 2022). While further research is needed to fully understand the mechanisms underlying these differences in susceptibility to trypanosomiasis,

our study provides important insights that can inform disease management strategies (Regasa and Workine 2018).

Study Limitation

The study did not collect data from a diverse geographic area and was limited to the tsetse fly species used in the research. The data on seasonal variations were not included in the study's analysis. These limitations prove to be crucial when considering the current study results. Therefore, it is necessary to emphasize the need for future research to take these shortcomings into account when designing a similar study. Ultimately, acknowledging these weaknesses allows for a comprehensive overview of the results and ensures an informed and transparent assessment of the research.

Conclusion

In conclusion, the study has shown the complexity of controlling insect-borne diseases such as African trypanosomiasis (HAT) in humans and African trypanosomiasis (AAT) in animals. The results of this study suggest that *Glossina* species, particularly *G. pallidipes* and *G. fuscipes*, are potential vectors of these diseases, highlighting the need for sustained research and measures to address the risks. The complexity of insect populations requires a concerted effort by researchers, policymakers and communities to protect human and animal populations and the planet's natural heritage. It is essential to understand the dynamics of insect populations and in this case, proper vector identification is of utmost importance. The control and management of HAT must be carried out in collaboration between public health and animal health. Therefore, efforts must be made to coordinate interventions between the two sectors, leading to effective and sustainable management of HAT. Improving access to healthcare is imperative, particularly in rural areas where healthcare services may be limited. Additionally, it is important to conduct more research and development into effective treatment of sleeping sickness. Additionally, health authorities must prioritize surveillance activities such as active case finding to quickly identify new HAT cases and contain them if they lead to larger outbreaks. Tsetse flies in the regions should be regularly treated with insecticides to reduce the likelihood of transmission.

Funding statement

This research is part of routine government work as an outbreak investigation has not received external funding. This represents an important public health milestone as it shows that government agencies are prepared to fund vital work even in difficult economic times. Although funding is a crucial aspect of any

research, our team recognizes the importance of conducting this investigation with urgency and precision, regardless of the resources available to us. We approached the task at hand with the utmost care and professionalism, ensuring we left no stone unturned in the search for answers.

Data Availability Statement

All necessary data that we analyzed and used in our research are included in the manuscript and supporting information not included in the manuscript is available from the corresponding authors and will be provided upon reasonable request.

Ethical Approval

Not applicable

Consent for Publication

Not applicable

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

We would like to take this moment to express our sincere appreciation to the heads of the South Nation, the Nationalities of Peoples of Regional Bureau and the Health Department Office. Without their valuable collaboration and support, our data collection efforts would not have been successful. We also thank the village chief, health advisors and volunteers who helped us with data collection and without whom this project would not have been possible. We owe our data collectors a huge debt of gratitude for their unwavering dedication and commitment to the cause. We know that data collection is not an easy task, but their resilience and perseverance have gotten us to this point. Their contributions to this study are invaluable and without them we would not have been able to achieve our goal. We appreciate the hard work and collaboration of everyone involved and would like to express our deepest gratitude. This study will have a significant impact on the health and well-being of the community and is thanks to their tireless efforts.

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