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Prevalence and Associated Risk Factors of Trypanosomiasis in Southern Ethiopia: A Cross-Sectional Study of 4,436 Individuals

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Annotation: Background: Trypanosomiasis remains a significant vector-borne disease in sub-Saharan Africa, particularly in Ethiopia. Its epidemiology varies by region and is influenced by multiple socio-demographic and environmental factors. Objective: To determine the prevalence of Trypanosoma infection and identify associated risk factors among rural populations in Southern Ethiopia. Methods: A cross-sectional study involving 4,436 participants was conducted in Southern Ethiopia. Participants were screened for Trypanosoma infection using microscopy. Data on socio- demographic characteristics, environmental sanitation, income, education, and proximity to tsetse fly habitats were collected using structured questionnaires. Logistic regression and t-tests were applied to identify associations and differences. Results: The prevalence of Trypanosomiasis was 22.3%, with higher rates among females (24.3%) compared to males (20.3%). Participants living in rural areas had a slightly lower prevalence (21%) compared to urban residents (25%). Poor environmental sanitation showed a strong positive association with infection (OR = 12.95, $p < 0.001$). Illiteracy (OR = 3.95, $p < 0.001$) and low income (≤1,000 birr/month) were significant risk factors. Proximity to tsetse fly breeding areas was not statistically associated with infection. Interestingly, participants spending less time in bush areas (<1 hour/day) were more likely to be infected (OR = 1.39, $p < 0.001$). Infected individuals had significantly lower hemoglobin levels (mean difference = −2.09 g/dL, $p < 0.001$), indicating a strong link between Trypanosomiasis and anemia. Conclusion: Trypanosomiasis is prevalent in Southern Ethiopia, with sanitation, education, and income levels being the primary predictors of infection. Microscopic diagnosis remains a reliable method in low-resource settings. Community-based interventions focusing on sanitation and awareness are essential to control disease transmission.

Keywords: Trypanosomiasis, prevalence, Southern Ethiopia, risk factors, hemoglobin, anemia, sanitation

Introduction

Trypanosomiasis, commonly referred to as sleeping sickness in humans and nagana in animals, is a vector-borne disease caused by protozoan parasites of the genus Trypanosoma, primarily transmitted by Glossina (tsetse) flies (World Health Organization [WHO], 2023). This disease remains a significant public health concern in sub-Saharan Africa, disproportionately affecting rural communities dependent on agriculture and livestock (Matzefinowos et al., 2022).

In Ethiopia, human African Trypanosomiasis is endemic in lowland regions, where the disease contributes

to both morbidity and socioeconomic hardship. Despite significant global progress, with a reported >97% decline in cases over recent decades, localized endemic zones in rural Africa remain poorly characterized (WHO, 2023). Accurate prevalence data and identification of population-level risk factors are essential for designing targeted interventions.

Microscopic examination of blood smears continues to be the primary diagnostic method in many endemic settings due to its affordability and field feasibility (MacLean et al., 2004). Beyond detection, Trypanosoma infection induces anemia through multiple mechanisms, including red cell destruction, suppressed erythropoiesis, and cytokine-induced iron sequestration (Magez et al., 2007; WHO, 2001).

However, a significant research gap exists in Ethiopia: most Trypanosomiasis research focuses on livestock (Matzefinowos et al., 2022), and little is known about human-specific predictors such as sanitation, income, education, or behavior. Past studies in West Africa have shown environmental and socioeconomic factors to be critical predictors of parasitic infection burden (Lover et al., 2014; Haidar & Pobocik, 2009), but robust community-level studies in high-risk districts in Southern Ethiopia are lacking.

There is limited epidemiological evidence on Trypanosoma prevalence in humans in rural Africa, hindering the implementation of effective control programs. This study fills a vital knowledge gap by assessing Trypanosoma prevalence, anemia status, and associated demographic and environmental risk factors through a large-scale cross-sectional approach in Southern Ethiopia. This study aims to (1) determine the prevalence of Trypanosoma infection among residents of Southern Ethiopia, (2) identify key socio-demographic and environmental risk factors, and (3) assess the impact of infection on hemoglobin concentrations.

METHODS

Study Setting and Design

This study is a secondary analysis of de-identified cross-sectional survey data from rural Demographic Surveillance Sites (DSS) in the Meskan, Mareko, and Silti Districts of the Gurage Zone, Southern Nations, Nationalities, and Peoples' Region (SNNPR), Ethiopia. The DSS include nine rural units and one urban administrative unit established to generate longitudinal health and demographic data. Using the original survey dataset, I assessed the prevalence of Trypanosoma infection and evaluated socio-demographic and environmental risk factors in this population.

Study Population

In the original survey, investigators enrolled a total of 4,436 participants from both rural and urban communities. Eligible individuals met the following inclusion criteria: residing within the DSS area, being available during the survey period, and providing informed consent.

Investigators applied no restrictions based on age or sex. All eligible participants were screened for Trypanosoma infection using microscopy and interviewed using a structured questionnaire.

Measurement and Data Collection

In the original study, trained fieldworkers conducted face-to-face interviews using standardized and pre-tested questionnaires. The data collected included socio-demographic characteristics (age, sex, residence, income, education, occupation), environmental sanitation status, exposure to tsetse fly breeding sites, and time spent in bush areas. We categorized environmental sanitation into three levels: very clean, few waste materials, and unsanitary.

Laboratory Testing

Fieldworkers collected capillary blood samples using sterile lancets via finger pricks. They prepared thin and thick blood smears on microscope slides, fixed them with methanol, and stained them with Giemsa. Microscopic examinations were performed by trained laboratory technicians using ×1000 magnification to identify the presence of Trypanosoma parasites. Quality control was assured by having a second technician re-examine a random subset of slides.

Hemoglobin Determination

Hemoglobin (Hb) levels were measured to assess the impact of the Trypanosoma infection on anemia status. Blood samples were analyzed within two hours of collection using a calibrated hematology analyzer. The mean hemoglobin concentration between infected and non-infected participants was compared.

Outcome Definition

The primary outcome was infection with the Trypanosoma species, determined by the microscopic detection of parasites in blood smears. Participants were classified as infected (positive) or uninfected (negative). Hemoglobin concentration served as a secondary outcome to assess clinical impact.

Statistical Data Analysis

Data were entered and analyzed using IBM SPSS. Descriptive statistics summarized demographic and clinical characteristics. Chi-square tests were used for initial associations. Logistic regression was conducted to determine univariate associations between Trypanosoma infections and explanatory variables. Crude odds ratios (COR) with 95% confidence intervals (CI) were reported. Multivariate logistic regression was performed using backward elimination to control for potential confounders. Independent sample t-tests were used to compare mean hemoglobin levels between infected and uninfected groups. A p-value < 0.05 was considered statistically significant.

Ethical Approval

Ethical clearance was obtained from the Department of Medical Laboratory Science at Addis Ababa University and approved by the Ethiopian Ministry of Health Ethical Review Board. Written informed consent or assent (via signature or thumbprint) was obtained from all participants or their guardians. Confidentiality was maintained by assigning unique numerical identifiers. All participants testing positive for Trypanosoma infection were referred for appropriate clinical care at no cost.

RESULTS

Social demographic characteristics of study participants:
The study included 4,436 participants residing in Southern Ethiopia. As shown in Table 1, the gender distribution was nearly equal, with 2,230 (50.3%) males and 2,206 (49.7%) females.
Most participants (66.7%) resided in rural areas, while 33.3% were from urban settings. Regarding educational attainment, 1,744 participants (39.3%) were illiterate, 2,557 (57.6%) had basic literacy, and 135 (3.0%) reported informal education only. Their monthly income was predominantly low, with 3,616 participants (81.5%) earning less than 1,000 Ethiopian birr. Only nine individuals (0.2%) reported a monthly income above 5,000 birr.
Environmental sanitation varied, with just 365 participants (8.2%) reporting their surroundings as very clean, while 3,334 (75.2%) lived in areas with few waste issues, and 737 (16.6%) in dirty environments. Only 661 participants (14.9%) reported proximity to known tsetse fly breeding grounds. Occupationally, the most common roles were students (31.6%), farmers (11.9%), and daily laborers (10.5%), with the remainder classified in various other roles (39.3%). (Table 1)

Table 1. Distribution of social demographic characteristics of study participants in Southern Ethiopia

Variables	Frequency	Percent
Sex		
Male	2230	50,3
Female	2206	49,7
Place of residence		
Rural	2960	66,7
Urban	1476	33,3
Environmental Sanitation		
very clean	365	8,2
few waste materials	3334	75,2
dirty	737	16,6
Nearby Tsetse fly breading area		
yes	661	14,9
no	3775	85,1
Education status		
illiterate	1744	39,3
literate	2557	57,6
informal education	135	3
Family monthly income		
less than 1000	3616	81,5
1001-2000	393	8,9
2001-3000	239	5,4
3001-4000	103	2,3
4001-5000	76	1,7
more than 5000	9	0,2
Occupation		
Unemployed	100	2,3
Farmer	528	11,9
Merchants	38	0,9
Student	1401	31,6
Daily Laborer	467	10,5
Others	158	3,6
Not applicable	1744	39,3

Distribution of Trypanosoma in Southern Ethiopia:

Out of the total sample, 990 individuals (22.3%) tested positive for Trypanosoma spp. as determined by microscopy (Figure 1). The prevalence was marginally higher in females (24.3%) than in males (20.3%), and more pronounced in urban residents (25%) compared to rural residents (21%). (Figure 1)

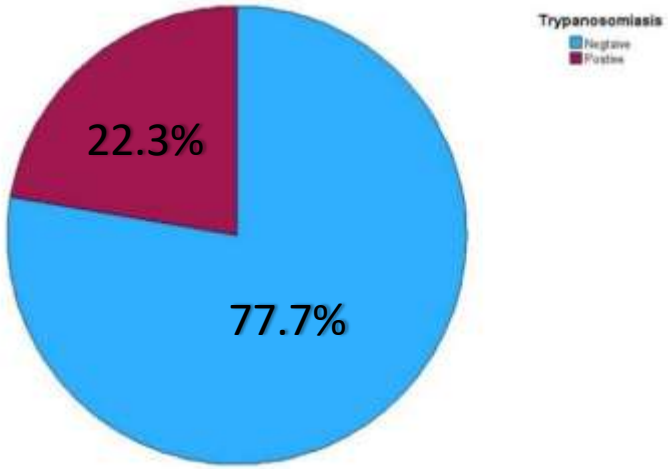

Figure 1. Prevalence of Trypanosomiasis in Southern Ethiopia out of 4436 samples.

Association between demographic and lifestyle characteristics with Trypanosoma:

Bivariate analyses demonstrated significant associations between participant characteristics and Trypanosoma infection (Table 2). Female participants had a higher infection prevalence (24.3%) compared to males (20.3%) ($p = 0.001$). Rural residents were slightly less affected (21%) than their urban counterparts (25%) ($p = 0.002$). Environmental conditions were strongly linked to infection: participants in dirty environments showed the highest prevalence (26.7%), followed by those in areas with few waste materials (23.5%), and very clean regions (2.7%) ($p < 0.001$).

In terms of education, illiterate participants had the highest infection rate (26%), followed by those with basic literacy (20.6%) and informal education (8.1%) ($p < 0.001$). Interestingly, there was no significant association between infection and proximity to tsetse fly breeding grounds ($p = 0.094$). Income stratification showed that those earning less than 1,000 birr had a prevalence of 22.6%. People earning between 3,001–4,000 birr reported the highest prevalence (26.0%), with inconsistent trends across groups (p-value for trend not significant). (Table 2)

Table 2. Overall Association between demographic and lifestyle characteristics with Trypanosoma among Southern Ethiopia

	Trypanosoma				
Variable	**Pos, N%**	**Neg, N%**	**Chi square**	**DF**	**P-value**
Sex					
Male	453 (20,3%)	1777 (79,7%)	10,383	1	.001
Female	537 (24,3%)	1669 (75,7%)			
Residence					
Rural	621 (21%)	2239 (79%)	9,182	1	.002
Urban	369 (25%)	1107 (75%)			
Environmental Sanitation					
very clean	10 (2,7%)	355 (97,3%)	91,596	2	.001
few waste materials	783 (23,5%)	2551 (76,5%)			
dirty	197 (26,7%)	540 (73,3%)			
Educational Status					
illiterate	453 (26%)	1291 (74%)	33,588	2	.001
literate	526(20,6%)	2031 (79,4%)			
informal education	11 (8,1%)	124 (91,9%)			
Nearby tsetse fly breading area					
yes	188 (20,3%)	739 (79,7%)	2,805	1	.094
no	802 (22,9%)	2707 (77,1%)			
Family monthly income					
less than 1000	816 (22,6%)	2800 (77,4%)	9,468	5	.092
1001-2000	84 (21,4%)	309 (78,6%)			
2001-3000	38 (15,9%)	201 (84,1%)			
3001-4000	27 (26,2%)	76 (73,8%)			
4001-5000	22 (28,9%)	54 (71,1%)			
more than 5000	3 (33,3%)	6 (66,7%)			
Stay in Bush area					
less than 1 hour	688(20,8%)	2621(79,2%)	17,486	1	<.001
more than 1 hour	302(26,8%)	825(73,2%)			

Univariate and Multivariate Regression Analysis

In logistic regression analysis (Table 3), several variables showed statistically significant associations with Trypanosoma infections. Female participants were 1.26 times more likely to be infected than males (COR = 1.26, 95% CI: 1.09–1.44). Participants from rural areas had 1.25 times higher odds of infection than urban residents (COR = 1.25, 95% CI: 1.08–1.45). Those living in environments with few waste materials and dirty settings had significantly higher odds of infection (COR = 10.89 and 12.95, respectively; $p < 0.001$ for both) compared to those in clean environments.

Educational status was also predictive: illiterate individuals were nearly four times more likely to be infected than those with informal education (COR = 3.95, 95% CI: 2.1–7.3). Participants who spent less than one hour daily in bush areas were paradoxically more likely to be infected (COR = 1.39, 95% CI: 1.2–1.6; $p < 0.001$). Income levels were inconsistently associated with risk, with

only the 3,001–4,000 birr group showing significantly reduced odds compared to the highest income bracket (COR = 0.64; $p = 0.017$). (Table 3)

Table 3. Univariate and multivariate analysis for the correlation between demographic and lifestyle factors with Trypanosomiasis in Southern Ethiopia

	Trypanosoma			
Variable	**Pos, N%**	**Neg, N%**	**COR (95% CI)**	**P-value**
Sex				
Male	453 (20,3%)	1777 (79,7%)	1 (reference)	
Female	537 (24,3%)	1669 (75,7%)	1,26 (1,09-1,45)	.001
Residence				
Rural	621 (21%)	2239 (79%)	1,25(1,08-1,45)	.002
Urban	369 (25%)	1107 (75%)	1 (reference)	
Environmental Sanitation				
very clean	10 (2,7%)	355 (97,3%)	1 (reference)	
few waste materials	783 (23,5%)	2551 (76,5%)	10,89(5,78-20,53)	<.001
dirty	197 (26,7%)	540 (73,3%)	12,95 (6,76-24,79)	<.001
Educational Status				
illiterate	453 (26%)	1291 (74%)	3,95 (2,1 - 7,3)	<.001
literate	526(20,6%)	2031 (79,4%)	2.91 (1,5 - 5,4)	<.001
informal education	11 (8,1%)	124 (91,9%)	1 (reference)	
Nearby tsetse fly breading area				
yes	188 (20,3%)	739 (79,7%)	1,16(0,974-1,39	0,094
no	802 (22,9%)	2707 (77,1%)	1 (reference)	
Family monthly income				
less than 1000	816 (22,6%)	2800 (77,4%)	1,71(0,42-6,87	0.446
1001-2000	84 (21,4%)	309 (78,6%)	1,39(0,84-2,31)	0.191
2001-3000	38 (15,9%)	201 (84,1%)	1,21(0,78-1,90)	0.384
3001-4000	27 (26,2%)	76 (73,8%)	0,64(0,45-0,92)	0.017
4001-5000	22 (28,9%)	54 (71,1%)	0,93(0,72-1,20)	0.591
more than 5000	3 (33,3%)	6 (66,7%)	1 (reference)	
Stay in the bush area				
more than 1 hour	688(20,8%)	2621(79,2%)	1,39 (1,2-1,6)	<.001
less than 1 hour	302(26,8%)	825(73,2%)	1 (reference)	

Hemoglobin Levels in Infected Versus Uninfected Individuals

An independent samples t-test was conducted to evaluate the difference in hemoglobin concentrations between individuals infected with Trypanosoma and those uninfected. The analysis revealed that the mean hemoglobin level among infected participants was significantly lower ($M = 10.37$ g/dL, $SD = 2.4$) compared to uninfected individuals ($M = 12.46$ g/dL, $SD = 2.7$). The mean difference of -2.09 g/dL was statistically significant ($t = -23.46$, $p < 0.001$), and the 95% confidence interval (-2.28 to -1.09 g/dL) did not cross zero, confirming the robustness of this difference. These results indicate a strong negative association between Trypanosoma infection and hemoglobin concentration, consistent with the anemia-inducing effects observed in similar parasitic infections (Magez et al., 2007; WHO, 2001).(Table 4)

Table 4. Mean difference in hemoglobin between Trypanosomiasis-infected and uninfected groups among Southern Ethiopia

	mean (s.d)	mean difference (m.d)	95% CI	p-value
Trypanosoma				
Positive	10,37 (2,4 s.d)	(neg) 2,094	(neg)2,28- (neg) 1,09	<.001
Negative	12,46 (2,7 s.d)	(neg) 2,094	(neg)2,27-(neg)1,91	<.001

Discussion

This cross-sectional study found a 22.3% prevalence of Trypanosoma infection, underscoring a considerable public health concern in the study area. The strongest determinant of infection was environmental sanitation: individuals living in dirty environments had over twelve times the odds of infection compared to those in very clean settings (OR = 12.95, $p < 0.001$). This highlights sanitation as a critical driver of transmission.

Infected participants also showed significantly lower hemoglobin levels than uninfected individuals (mean difference = −2.09 g/dL, $p < 0.001$), consistent with the anemia-inducing effects of trypanosomiasis. These findings mirror those from East African studies, including data from Malawi, where infected individuals had mean Hb levels of 8.96 g/dL compared to
12.17 g/dL in controls (MacLean et al., 2004). The biological mechanism—hemolysis and immune-mediated suppression of erythropoiesis—is well-documented (Magez et al., 2007; WHO, 2001), reinforcing the clinical relevance of anemia in Trypanosoma infections.
Notably, urban residents in our sample had a higher infection prevalence (25%) than rural residents (21%), diverging from WHO reports that identify rural areas as higher risk (WHO, 2023). One plausible hypothesis is that expanding peri-urban zones such as Butajira are experiencing deteriorating waste management and ecological changes that facilitate tsetse habitation. These peri-urban "fringe" zones may now mirror traditional rural risk environments. This pattern warrants further investigation, as it suggests an underrecognized vector ecology in transitional urban settings.

Our findings also align with veterinary research. Studies in Southern Ethiopia (e.g., Benatsemay) have documented high bovine trypanosomosis prevalence linked to poor environmental conditions. While direct comparison between human and animal data is limited, both emphasize the consistent role of environmental sanitation in vector-borne disease transmission.

One unexpected observation was that participants spending less time in bushy areas (<1 hour/day) were more likely to be infected (COR = 1.39). This may reflect short but frequent exposure to high-risk microhabitats—such as water collection sites or peridomestic vegetation— where tsetse fly activity may peak. These localized and time-sensitive exposures highlight the need for spatial and behavioral mapping in future studies.

Strengths and Limitations
This study's strengths include its large, community-based sample incorporating both urban and rural populations, and its multifactorial analysis of behavioral and environmental variables.
Microscopy-based diagnosis by trained technicians adds reliability, though it is less sensitive than molecular methods.
Limitations include the cross-sectional design, which restricts causal inference, and the use of self-reported behavioral data, which may be subject to recall bias. Additionally, microscopy may

miss early or low-intensity infections, suggesting future studies should incorporate PCR for improved diagnostic accuracy.

Conclusion and Recommendations

In conclusion, Trypanosomiasis remains a significant health problem with a 22.3% prevalence in Southern Ethiopia, having a substantial impact on hemoglobin levels. Critical risk factors include poor environmental sanitation, limited education, and peri-urban residence. Based on these findings, we recommend enhanced community-level sanitation campaigns, public health education efforts, and targeted vector control interventions in rural and urban-fringe areas. Future molecular diagnostics studies could further elucidate transmission dynamics and support elimination strategies to eradicate this neglected tropical disease.

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