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Prevalence of *Trypanosoma* Among Cattle Found in Grazing Hotspots in Jalingo Metropolis, Taraba State, Nigeria

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Abstract

Bovine trypanosomiasis, commonly referred to as nagana, poses a significant threat to cattle populations across various regions in Africa, particularly in Nigeria. This disease caused by protozoan parasites of the genus Trypanosoma and transmitted by infected tsetse flies, results in extensive economic losses due to decreased productivity, fertility issues, and increased mortality rates among livestock. This study focuses on the prevalence of bovine trypanosomiasis in Jalingo, Taraba State, Nigeria a critical hub for cattle trade amidst the challenges posed by varied ecological factors and management practices that influence disease dynamics. Our research assessed 113 cattle samples collected from different grazing hotspots using both microscopy and polymerase chain reaction (PCR) techniques. The overall prevalence was found to be 17.7%, with notable concentrations of positive cases in specific locations. Factors such as age, breed, and the educational background of herders were significantly associated with infection rates. Notably, all positive cases were reported among herders lacking formal education, indicating a potential link between educational attainment and effective disease management. Furthermore, the White Fulani breed exhibited a higher susceptibility to the disease compared to other breeds evaluated. The results emphasize the necessity for targeted intervention strategies, including enhancing diagnostic capabilities, public awareness campaigns, and educational initiatives aimed at cattle herders. Strengthening healthcare infrastructure and ongoing research into disease transmission dynamics are essential for controlling bovine trypanosomiasis and mitigating its impact on the livestock industry in Nigeria.

Keywords: Bovine Trypanosomiasis; *Trypanosoma*; Cattle; Jalingo, Nigeria; PCR; Microscopy; Prevalence; Risk Factors

INTRODUCTION

Bovine trypanosomiasis, commonly known as nagana, is a significant parasitic disease affecting cattle populations in various regions of Africa, including Nigeria (Maichomo *et al.,* 2021). The disease is caused by protozoan parasites belonging to the genus Trypanosoma and transmitted through the bite of infected tsetse flies. Trypanosomiasis leads to substantial economic losses in the livestock industry due to reduced productivity, impaired fertility, and increased mortality among affected cattle (Maichomo *et al.,* 2021; Tessema and Dana, 2022). Nigeria, being one of the major countries dependent on agriculture and livestock rearing, faces considerable challenges in managing this disease (Presicce, 2020). The prevalence of trypanosomiasis in Nigeria across different regions of the country due to variations in ecological factors, vector abundance, livestock management practices, and the presence of susceptible cattle breeds (Franco *et al.,* 2022, Habeeb *et al.,* 2021).

The prevalence of bovine trypanosomiasis has been found to be particularly high in the northern and central regions of Nigeria, where tsetse flies, the main vectors of the disease, are more abundant (Majekodunmi *et al.,* 2013; Isaac *et al.,* 2016; Odeniran and Ademola, 2018; Weber *et al.,* 2019). These regions typically experience higher temperatures and humidity, which create favorable conditions for tsetse fly survival and reproduction (Alsan, 2015). Prevalence of bovine trypanosomiasis in Nigeria ranged from 10% to 70% in some areas (Yusuf *et al.,* 2015; Habeeb *et al.,* 2021). These figures highlight the significant impact of the disease on cattle populations and the livestock industry's overall productivity.

Additionally, it is essential to note that underreporting and lack of comprehensive surveillance systems may contribute to an underestimation of the true prevalence of trypanosomiasis in some regions. Also, the prevalence was higher in free ranching cattle than in institutional cattle (Odeniran *et al.,* 2019).

The presence of trypanosomiasis has several consequences on livestock health and productivity. Infected cattle often suffer from weight loss, reduced milk production, anemia, and reproductive disorders, leading to considerable economic losses for farmers and pastoralists (Swallow, 2000; Holmes *et al.,* 2004). Moreover, the movement of infected cattle from one region to another can facilitate the spread of the disease to previously uninfected areas (Ngongolo *et al.,* 2019; Ngari *et al.,* 2020; Signaboubo *et al.,* 2021). Jalingo, the capital city of Taraba State in northeastern Nigeria, is an important center for cattle trade (Bitrus *et al.,* 2021). However, limited research has been conducted to assess the prevalence of bovine trypanosomiasis in this specific region, despite its critical significance in the livestock industry (Gado *et al.,* 2023). Hence the need to study the Prevalence of Trypanosome among Cattle found in grazing hotspots in Jalingo Local Government Area of Taraba State, Nigeria.

METHODOLOGY

Study Area

Jalingo, the capital of Taraba State is our study area. It is located at coordinates 8°54'N 11°22'E in Taraba State, northeastern region of Nigeria. It is characterized by a mix of urban and peri-urban landscapes. The area is influenced by a tropical climate, with distinct wet and dry seasons. Jalingo Local Government Area exhibits diverse altitudinal variations characterized by its varied topography and river distribution. The altitude in Jalingo Local Government Area ranges from approximately 150 meters to 500 meters above sea level. This variation in altitude contributes to different ecological zones and climatic conditions within the region. The lower altitudes, closer to the Benue River valley, tend to have a relatively warmer and drier climate, reflecting the characteristics of the Sahel zone. As the altitude increases towards the central and northern parts of the local government area, the climate becomes more temperate and conducive to agriculture. The landscape is intersected by several rivers and streams that play a significant role in shaping the local environment, economy, and livelihoods.

The presence of rivers in the area also influences vegetation patterns, with riverine vegetation often being lusher and more diverse compared to areas farther away from the watercourses. The Benue River although not located within the study area, is one of the major rivers in Nigeria and flows along the western boundary of Jalingo Local Government Area. It serves as an important water resource for agriculture, fishing, transportation, and other activities. The river supports irrigation practices and contributes to the fertility of the surrounding lands. The proximity of Jalingo to water bodies, vegetation, and human settlements creates an environment conducive to the proliferation of various vectors. This region is of significant interest due to its unique geographical and socio-economic characteristics, which make it a potential hotspot for vector-borne diseases.

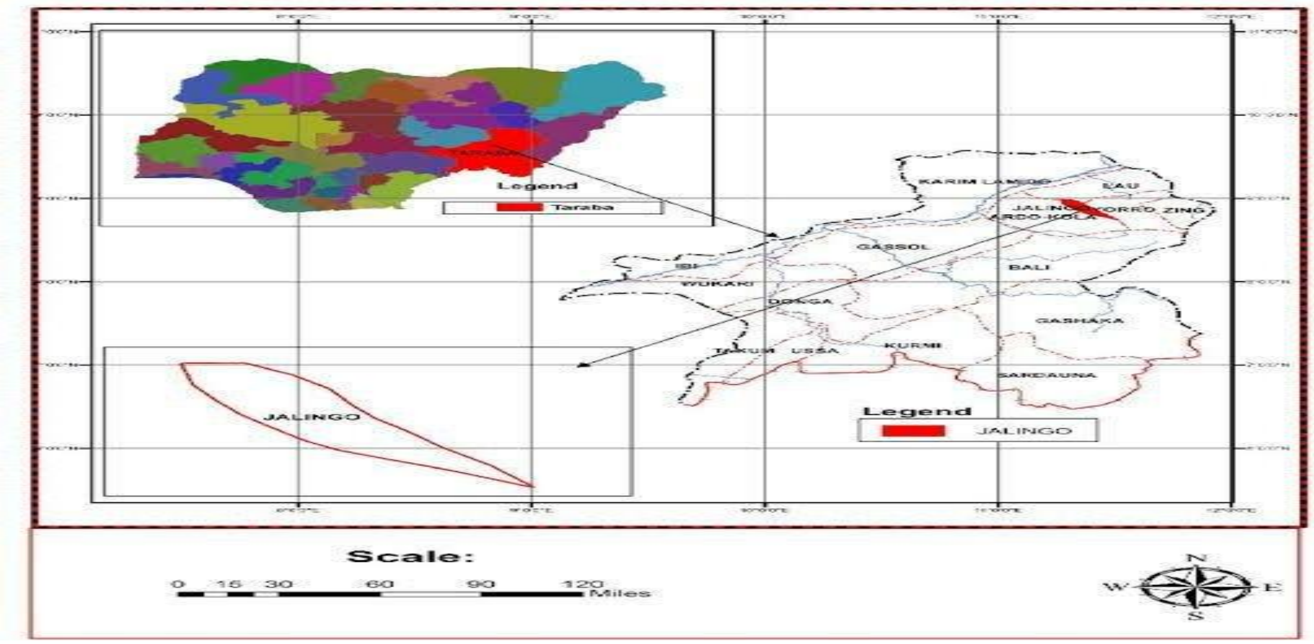

Figure 1: Map of Taraba State Showing Jalingo Local Government Area (Source: Taraba State, Ministry of Land and Survey, 2021)

Table 1: Names of Sampling Locations and their Coordinates

Key	Location Name	Coordinate
A	Jeka da Fari	8.891072, 11.373479
B	Bagobiri Slaughter house	8.919196, 11.361328
C	Nyabun kaka	8.917198, 11.368101
D	TSU Farm, Jal	8.894208, 11.321948
E	ATC (Kurnahi)	8.910543, 11.319194

Key	Location Name	Coordinate
F	Jetters (sumbu)	8.954788, 11.385994
G	TC Quarters	8.907128, 11.360305
H	Unguwan Kasa	8.934163, 11.357093

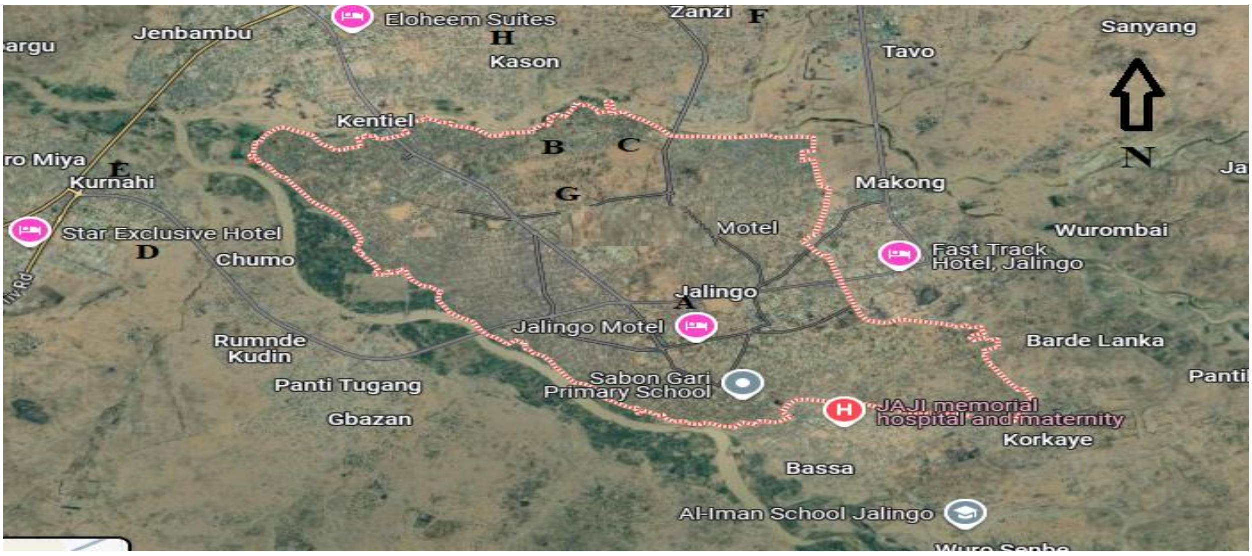

Figure 2: Sampling Location on the Map of Jalingo Metropolis

Ethical Clearance

This study was carried out in strict compliance to the guideline for the use of biological samples. The animals were handled according to the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals. Also, Ethical clearance was sort from the University Ethical Committee as well as the Department for the protection of animals used for experimental and other scientific purposes, Ministry of Agriculture, Taraba State, Nigeria. Also, informed consent from the cattle herd owners were sort, where the purpose, study's objectives, procedures, and potential benefits of the research were clearly explained.

Blood Sample Collection from Cattle in the Farm

Sampling interval was determined by dividing the total number of cattle in the farm within the specified period by the desired sample size to obtain the sampling interval (K).

$$K = \frac{Total\ Number\ of\ Cattle\ in\ a\ Farm}{Sample\ Size}$$

Using a sample size of 14 cattle per farm, the K_{th} for a farm with average cattle population of 100 cattle is 7^{th}. Using a random number generator, the start points between 1 and K were randomly selected and the first animal to be sampled. Starting from the random start point, every selected Kth animal from the sample frame was systematically selected until the desired sample size is reached. Five (5) mL of blood were collected from the jugular vein of each randomly selected animal with the aid of a sterile Vacutainer syringe and needle and placed into tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. Each sample collected was identified by a unique number to represent the name of the location, herd cluster and sample number. The samples harvested were transported in an ice pack to the Laboratory in the Department of Biological Sciences, Taraba State University, Jalingo for microscopy and preliminary investigations. The processed samples were then transported in ice part to the DNA Labs, Kaduna Kaduna, Nigeria for molecular analysis. Other demographic data such as age, sex, and origin of the animal are to be collected alongside each sample.

Demographic Data Collection

A structure datasheet was used to record environmental factors and demographic information such as; humidity, closeness of tsetse fly catch area to river, temperature, vegetation type, etc. as well as the age, weight, sex and origin of the animal.

RBC lysis and concentration of trypanosomes

Exactly 9 mL of RBC Lysis Solution (Qiagen, catalogue #158902) was added to 3 mL of whole blood. After gentle mixing, samples were allowed to stand for 10 min at room temperature and spun at 1000×g for 15 min. After discarding most of the supernatant, the pellet was re-suspended in the residual volume of supernatant (200μL) and used to prepare smears for microscopy.

Preparation of slides for microscopy

Thick smears were prepared on glass slides from each sample using 5μL of whole blood and 5 μL, and 10 μL of lysed blood. Smears were allowed to dry for 20 min. Each set of slides were stained with Giemsa. For Giemsa staining, slides were incubated in 10% Giemsa (Sigma–Aldrich) solution prepared with phosphate buffered saline pH 7.4 (PBS) for 45 min, then rinsed with PBS and air dried.

Examination of Smears

Slides were examined using microscope for trypanosomes with a 40×objective (total magnification: 400×). Smears stained with Giemsa were examined using the bright field illumination.

Polymerase Chain Reaction (PCR) Amplification

For PCR amplification was performed using AccuPower Hotstart PCR premix from Bioneer, a reaction mixture was prepared by adding template DNA, specific primers, and water to the premix. For a 20 µl reaction, 16 µl of dH_2O, 1 µl each of primer 1 and primer 2 (Bioneer), and 2 µl of template DNA were used. PCR was performed in a thermal cycler (PTC 100, MJ Research) under the following conditions: an initial denaturation at 95℃ for 5 minutes, followed by 35 cycles of denaturation at 94℃ for 40 seconds, annealing at 54℃ for 40 seconds, and extension at 72℃ for 40 seconds. A final extension was performed at 72℃ for 5 minutes. The PCR products were then analyzed by running them on a 2% agarose gel.

Data Analyses

The collected data were analyzed using a combination of statistical, molecular, and bioinformatics approaches for accurate interpretation of results. The analyses were carried out in three primary stages: descriptive statistics, molecular sequence analysis, and phylogenetic analysis. Descriptive statistics were used to summarize demographic, environmental, and prevalence data. The prevalence of *Trypanosoma* species among the sampled cattle was calculated as: The association between trypanosome infection and demographic/environmental factors such as age, sex, herd size, and proximity to riverine areas was analyzed using Pearson's Chi-square test (χ^2) for categorical variables and logistic regression for continuous variables. Data were processed and analyzed using SPSS (Version 27.0) A p-value of <0.05 was considered statistically significant.

RESULTS

Determination of the Prevalence of Bovine Trypanosome Infection in Jalingo Metropolis.

Table 2: Prevalence by Sampling Hotspots

Location Name	Total Examined	Positive (%)
Jeka da Fari	12 (10.60)	0 (0.00)
Bagobiri Slaughter house	45 (39.80)	20 (17.70)
Nyabun kaka	1 (0.90)	0 (0.00)
TSU Farm, Jal	26 (23.00)	0 (0.00)
ATC	10 (8.80)	0 (0.00)
Jetters	8 (7.10)	0 (0.00)
TC Quarters	6 (5.30)	0 (0.00)
Unguwan Kasa	5 (4.40)	0 (0.00)
Total	113 (100.00)	20 (17.70)

Table 2 presents the distribution of PCR test results across nine sampling hotspots, with a total of 113 samples collected. Out of these, 93 tested negative and 20 tested positive, yielding an overall positivity rate of approximately 17.7%. The data clearly show that the Transient location accounts for all 20 positive cases, indicating a notably high prevalence at this specific site.

In contrast, all other locations tested negative, suggesting no detectable infections in those areas or potential limitations in detection. Among these locations with only negative results, Taraba State University Farm, Jal, reported the highest number of negative samples (26), followed by Bagobiri Slaughter House (13) and Jeka da Fari (12). The remaining locations—ATC, Jetters, TC Quarters, Unguwan Kasa, and Nyabun Kaka—recorded fewer negative results, ranging from 10 to 1.

Table 3: Prevalence by Educational Background of the Herders

Educational Background	Number Examined	Positive
None	87 (77.00)	20 (23.00)
Masters and PhD Level	26 (23.00)	0 (0.00)
Total	113 (100.00)	20 (17.70)

Table 3 presents the prevalence of PCR test results among herders based on their educational background. Among the 113 herders sampled, 87 reported having no formal education and 26 held a Master's or PhD level qualification. All 20 positive PCR results came exclusively from the group with no formal education, while none of the highly educated herders tested positive. This distribution indicates that herders with no formal education accounted for 100% of the positive cases, suggesting a potential link between lower educational levels and higher infection rates.

Table 4: Prevalence by Sex of Cattle Sampled

Sex	Number Examined	Positive
Male	44 (38.90)	7 (15.90)
Female	69 (61.10)	13 (18.84)
Total	113 (100.00)	20 (17.70)

Table 4 presents the prevalence of PCR test results based on the sex of the cattle sampled. Out of the 113 cattle tested, 44 were male and 69 were female. Among the 20 positive cases, 7 (35.0%) were male, while 13 (65.0%) were female. Although more female cattle tested positive than males, the overall distribution of infection between sexes appears relatively balanced, with no significant disparity.

Table 5: Prevalence by Age of Cattle Sampled

Age Group (Years)	Number Examined	PCR Test Positive
0 – 2	30 (26.50)	0 (0.00)
2 – 5	34 (30.10)	2 (5.88)
6 – 8	29 (25.70)	6 (20.68)
9 – 11	20 (17.70)	12 (60.00)
Total	**113 (100.00)**	**20 (17.70)**

The table 5 groups cattle by age into four categories and shows their PCR test results. In the youngest group, aged 0–2 years, all 30 cattle tested negative, with no positive cases recorded. The next group, 2–5 years, consisted of 34 cattle, of which only 2 were positive, representing a low positivity rate.

In the 6–8 years group, there were 29 cattle with 6 positive cases, indicating a moderate level of infection. Notably, the oldest group, 9–11 years, had the highest positivity rate with 12 out of 20 cattle testing positive—60% of all positives—despite representing a smaller portion of the total sample.

Table 6: Prevalence by Breed of Cattle Sampled

Breed	Total Examined	Positive
White Fulani	57 (50.40)	14 (24.56)
Red Bororo	15 (13.30)	3 (20.00)
Sokoto Gudali	6 (5.30)	1 (16.67)
White Bororo	29 (25.70)	0 (0.00)
Black	6 (5.30)	2 (33.33)
Total	113 (100.00)	20 (17.70)

Table 6 displays the distribution of PCR test results among cattle breeds. Out of the 113 cattle sampled, White Fulani constituted the largest group at 50.4% and represented a striking 70% of the positive cases with 14 positives. In contrast, although White Bororo made up 25.7% of the sample, none of these animals tested positive. The remaining breeds—Red Bororo, Sokoto Gudali, and Black—had smaller representations, accounting for 15, 6, and 6 cattle respectively, with positive cases of 3 (15%), 1 (5%), and 2 (10%). This pattern suggests that the White Fulani breed may be more susceptible to infection, whereas the White Bororo breed showed no evidence of infection in this sample.

Table 7: Total Prevalence Determined by PCR and Microscopy

	Number Examined	Percent
	Microscopy	
Negative	97	85.8
Positive	**16**	**14.2**
Total	113	100
	PCR	
Negative	93	82.3
Positive	**20**	**17.7**
Total	113	100

Table 7 compares the prevalence of infection as determined by microscopy and PCR. Out of 113 samples, microscopy identified 16 positive cases (14.2%), whereas PCR detected 20 positive cases (17.7%), indicating a higher sensitivity of PCR. Microscopy reported 97 negative cases (85.8%), while PCR identified 93 negatives (82.3%). This suggests that PCR may be more effective in detecting infections that microscopy might miss.

Table 8: Chi Square of Prevalence of Bovine Trypanosome Infection in Jalingo Metropolis

S/N		A. Sig	Ex. Sig2	Ex. Sig1
1	**Sampling Hotspots**			
	Pearson Chi-Square	0.000		
	Likelihood Ratio	0.000		
	Linear-by-Linear Association	0.000		
2	**Educational Background**			
	Pearson Chi-Square	0.007		
	Continuity Correction[b]	0.016		
	Likelihood Ratio	0.001		
	Fisher's Exact Test		0.006	0.003
	Linear-by-Linear Association	0.007		
	N of Valid Cases			
3	**Sex**			
	Pearson Chi-Square	0.691		
	Continuity Correction[b]	0.884		

S/N		A. Sig	Ex. Sig2	Ex. Sig1
	Likelihood Ratio	0.689		
	Fisher's Exact Test		0.803	0.447
	Linear-by-Linear Association	0.692		
4	**Age**			
	Pearson Chi-Square	0.000		
	Likelihood Ratio	0.000		
	Linear-by-Linear Association	0.000		
5	**Breed**			
	Pearson Chi-Square	0.058		
	Likelihood Ratio	0.008		
	Linear-by-Linear Association	0.048		

Keys: [b] Using asymptomatic Standard Error Assuming Null Hypothesis, [a] Have expected count of less than 5, Df – Degree of Freedom, A. Sig-Asymptotic Significance (2-sided), Ex. Sig2.- Exact Significance (2-sided) and Ex. Sig.1 -Exact Sig. (1-sided)

The cross-tabulation results (Table 8) provide insights into the relationships between PCR test outcomes and various categorical factors, including educational background, sex, age, and breed.

Educational background appears to have a significant association with PCR test results (p=0.007p = 0.007p=0.007). All 20 positive cases were among individuals with no formal education, while none of the individuals with a Master's or PhD tested positive. This suggests that education level may be linked to factors influencing infection risk.

Sex did not show a statistically significant association with PCR test outcomes (p=0.691p = 0.691p=0.691). While females had a slightly higher proportion of positive cases (65%) compared to males (35%), the distribution did not deviate significantly from expectations.

Age exhibited a strong association with PCR test results ($p<0.001p < 0.001p<0.001$). Notably, all positive cases were recorded among individuals aged 5 years and older, with the highest positivity rate (30%) observed in the 9-year age group. Younger individuals (0–4 years) tested entirely negative, suggesting a potential age-related risk factor for infection.

Breed showed a borderline statistical significance in relation to PCR test results ($p=0.058p = 0.058p=0.058$). The White Fulani breed accounted for the majority (70%) of positive cases, while no positives were recorded among White Bororo individuals. This trend suggests that breed differences may play a role in susceptibility, though further investigation is needed.

DISCUSSION

The study found a diverse range of experience levels among cattle herders, with a significant proportion being new to cattle farming (0–10 years of experience). This trend aligns with previous studies in Nigeria, where high turnover in cattle farming has been attributed to economic constraints, environmental factors, and disease outbreaks (Akinmoladun *et al.*, 2022). Notably, 100% of positive PCR cases were recorded among herders with 0–10 years of experience, while those with more than 10 years had no recorded infections. This suggests that newer herders may have a higher risk of infection due to limited experience in disease management. Regarding herd size, 100% of the positive cases were reported in herders with smaller herds (0–10 cattle), whereas herders managing larger herds (11–150 cattle) had no positive cases. This could indicate that herders with small herds are more attentive to individual animal health, leading to more frequent disease detection. However, it could also suggest that larger herds have better management strategies or access to veterinary services.

The study revealed that 77% of herders had no formal education, with 100% of the positive PCR cases occurring in this group. In contrast, none of the herders with a Master's or PhD level education tested positive. This finding supports previous studies indicating that formal education enhances awareness and adoption of disease prevention strategies, leading to lower infection rates (Igbokwe *et al.*, 2021; Adebayo *et al.*, 2022). The association between education and infection rates underscores the need for targeted awareness campaigns and training for herders with no formal education. The overall prevalence of bovine trypanosomiasis detected by PCR was 17.7%, higher than the 14.2% detected by microscopy. This difference highlights the superior sensitivity of PCR in detecting subclinical or low-parasitemia infections, consistent with findings by Karshima *et al.* (2016) and Daniel *et al.* (1993).

The study also found that all 20 positive cases were recorded in the Transient sampling location, while all other locations had 0% prevalence. This suggests that specific environmental or management factors at the Transient site may contribute to higher infection rates, warranting further investigation. Sex and Age of Cattle: The study found no significant association between cattle sex and trypanosome infection ($p = 0.691$), although females had a slightly higher prevalence (65% compared to 35% in males). However, age showed a strong correlation with infection rates ($p < 0.001$), with the highest prevalence in the 9–11-year age group (60%). This aligns with previous studies indicating that older cattle may have prolonged exposure to infected tsetse fly bites, increasing their risk of infection (Karshima and Bobbo, 2011). Breed Susceptibility: The White Fulani breed constituted the highest proportion of positive cases (70%), while the White Bororo breed showed 0% infection. This suggests a possible breed-related susceptibility to trypanosomiasis, which has been observed in other studies where indigenous breeds exhibited varying resistance levels (Bitrus *et al.*, 2021).

CONCLUSION

In conclusion, this study offers a detailed examination of bovine trypanosomiasis in Jalingo Metropolis, revealing a 17.7% overall prevalence. The concentration of 100% of positive cases in the "Transient" location highlights a critical hotspot requiring focused intervention. Age and breed significantly impacted infection rates: cattle aged 9-11 years showed a 60% positivity rate, and White Fulani cattle accounted for 70% of positive cases, while White Bororo showed 0% infection. The 100% association of positive cases with herders lacking formal education underscores the need for targeted educational initiatives. Diagnostic accuracy was confirmed with PCR detecting 17.7% positive cases, compared to 14.2% by microscopy, demonstrating PCR's superior sensitivity. Despite 85% of herders reporting awareness of the disease, 0% had conducted diagnostic tests, and only 17.7% demonstrated adequate knowledge of symptoms and effects, revealing a significant gap between knowledge and practice. The 74.3% acknowledgement of tsetse flies, with 100%

of PCR-positive cases in this group, emphasizes the vector's role, despite 83.2% reporting control measures.

Recommendations

1. To strengthen disease control efforts, policy implementation should focus on enhancing healthcare regulations that integrate PCR-based testing alongside traditional microscopy. Establishing clear guidelines for routine screening and early detection will help reduce disease prevalence and improve diagnostic accuracy. Additionally, policymakers should ensure the availability of necessary laboratory infrastructure and trained personnel to support efficient disease surveillance.

2. Research and development should be encouraged to enhance diagnostic techniques and better understand disease prevalence. Studies focusing on molecular diagnostics can help improve sensitivity and specificity in detecting infections. Additionally, research into environmental and genetic factors influencing disease transmission can guide more targeted and effective interventions, ultimately leading to improved healthcare strategies.

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