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Research Article

Field-Based Analysis of *Sodalis glossinidius* -Trypanosome Co-infection in Wild Tsetse Flies from Kagarko and Ijah Gwari, Nigeria

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ABSTRACT

This study investigated *Sodalis glossinidius*-trypanosome interactions in wild tsetse populations from Nigeria using morphological and molecular approaches. Analysis of 1,611 flies revealed *Glossina palpalis palpalis* as dominant (81.6%), with higher wet-season abundance. Trypanosome infections peaked in wet seasons, notably *Trypanosoma vivax* (36.7–47.4%) and *T. brucei* (19.3–30.6%). qPCR demonstrated significantly lower Ct values in *Sodalis*-positive flies (mean Ct = 22.1) *Sodalis*- negatives (Ct = 28.6, $p < 0.0001$), corresponding to a 66-fold higher trypanosome load (4.2×10^5 vs. 6.3×10^3 copies/µL). Melting curve analysis confirmed specificity, with *Sodalis*-positives showing sharp peaks at 85°C versus broader peaks (87°C) in negatives. *Sodalis* prevalence was significantly higher in wet seasons (OR > 2.0), and coinfection analysis revealed strong *Sodalis-T. brucei* association in *G. palpalis* (53.1%, OR = 1.23, p = 0.021), contrasting with an inverse *Sodalis T. congolense savannah* relationship (OR = 0.76). Site-specific variations underscored ecological influences on these interactions. The tight clustering of Ct values and distinct melting profiles in *Sodalis*-positive flies suggests symbiont-mediated facilitation of trypanosome proliferation, likely through immune modulation. These findings provide field evidence that *Sodalis* enhances trypanosome transmission potential in tsetse populations. The qPCR and melting curve data validate the robustness of these associations, highlighting *Sodalis* as a key target for innovative disease control strategies.

Keywords: Coinfection; Melting curve; qPCR; *Sodalis glossinidius*; Trypanosomes; Tsetse flies

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INTRODUCTION

Tsetse flies (*Glossina* spp.) are the principal biological vectors of African trypanosomes (*Trypanosoma brucei* spp.), the causative agents of Human African Trypanosomiasis (HAT, or sleeping sickness) and Animal African Trypanosomiasis (AAT, or nagana) (WHO, 2022). These diseases remain major public health and veterinary concerns in sub-Saharan Africa, where they contribute to significant morbidity, mortality, and economic losses due to reduced livestock productivity and agricultural output (Franco *et al.,* 2020). Despite decades of control efforts, trypanosomiasis persists in endemic regions, partly due to the complex interactions between tsetse flies, their symbiotic microbiota, and trypanosomes, which influence disease transmission dynamics (Askoy *et al.,* 2014).

Among the microbial symbionts harboured by tsetse flies, *Sodalis glossinidius*, a facultative endosymbiotic bacterium, has garnered attention due to its potential role in modulating vector competence (Dale and Maudlin, 1999). Laboratory-based studies have demonstrated that *S. glossinidius* may enhance tsetse fly susceptibility to trypanosome infection by altering midgut physiology and immune responses (Weiss *et al.,* 2013). However, limited data exist on the co-infection

prevalence of *Sodalis glossinidius* and trypanosomes in wild tsetse populations from these regions. Furthermore, previous studies have not sufficiently explored how ecological variables, such as vegetation type, host availability, and climate, affect *Sodalis-trypanosome* interactions in natural tsetse populations. Field studies have reported variable prevalence rates of *Sodalis* and trypanosomes across different *Glossina* species and ecological settings, suggesting that environmental factors, host genetics, and microbial competition may influence infection outcomes (Geiger *et al.,* 2011). Given that wild tsetse populations serve as biological vectors for African trypanosomes, they play a central role in disease transmission cycles affecting both humans and animals (Kame-Ngasse *et al.,* 2018).

Nigeria, a country with high *Glossina* species diversity and endemic trypanosomiasis, presents an ideal setting for investigating these dynamics (Isaac *et al.,* 2016). Conservation areas, in particular, offer unique ecosystems where tsetse flies interact with diverse wildlife reservoirs of trypanosomes, potentially sustaining zoonotic transmission cycles (Njiokou *et al.,* 2010). However, limited data exist on the co-infection prevalence of *Sodalis glossinidius* and trypanosomes in wild tsetse populations from these regions. Furthermore, previous studies have not sufficiently explored how ecological variables such as vegetation type, host availability, and climate affect *Sodalis*-trypanosome interactions in natural tsetse populations. Addressing these gaps is crucial for assessing the epidemiological significance of *Sodalis glossinidius* in trypanosome transmission and identifying potential targets for vector-based interventions.

This study bridges a critical gap between laboratory experiments and field epidemiology by investigating *Sodalis*-trypanosome interactions in wild tsetse populations from Nigeria. Using molecular and ecological approaches, we provide empirical evidence of symbiont-mediated parasite facilitation while accounting for seasonal and site-specific variations—a perspective under-represented in prior work.

MATERIAL AND METHODS

Study Area

The study was conducted in two conservation areas in Nigeria: Ijah Gwari (Niger State) and Kagarko (Kaduna State). These sites (Ijah Gwari: 9°18.860' N, 7°26.814' E; Kagarko: 9.4910° N, 7.6955° E) were selected based on their established tsetse fly populations and ecological characteristics that support trypanosome transmission cycles (National Park Service, 2021). Both locations feature diverse habitats, including riverine forests, savanna woodlands, and transitional vegetation zones known to harbor various Glossina species (Adam *et al.,* 2020). The geographical distribution of the two study sites is illustrated in Figure 1 below.

Figure 1. Map showing the study locations: Ijah Gwari (Niger State) and Kagarko (Kaduna State), Nigeria *(Source: OpenStreetMap contributors,* 2025*)*

Tsetse fly sampling and morphological Identification

Tsetse fly collection was conducted over twelve consecutive months (2007–2008) to account for seasonal variations in vector abundance and infection rates. Standard biconical traps (Challier and Laveissière, 1973) were deployed along ecological transects, spaced at 200–300-meter intervals in representative microhabitats. Traps were baited with olfactory attractants (acetone and cow urine) and operated daily from 8:00 AM to 11:00 AM, coinciding with peak tsetse activity periods (Leak, 1998). Captured flies were immediately transferred to sterile vials containing nucleic acid preservatives and transported to the molecular biology laboratory of NITR under controlled conditions.

Morphological identification was performed using standardized taxonomic keys (FAO, 2018) with particular attention to distinguishing between *G. palpalis* and *G. tachinoides* group species, which dominate the study regions. Only freshly captured, intact specimens were selected for molecular analysis to ensure data reliability. Surface sterilization with 70% ethanol preceded dissection under aseptic conditions, with midgut and salivary gland tissues collected in sterile phosphate-buffered saline (PBS) for processing.

Molecular detection

Genomic DNA extraction was performed using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Korea) following the manufacturer's insect tissue protocol. The extraction process included proteinase K digestion and column purification steps to ensure high-quality DNA suitable for downstream applications (Bioneer Corporation, 2022). DNA concentration and purity were assessed using spectrophotometry (NanoDrop 2000, Thermo Scientific), with all samples normalized to 50 ng/µL for consistency.

Molecular detection employed a two-phase approach. Initial screening used conventional PCR with AccuPower PCR PreMix (Bioneer, Korea). For the detection of *Sodalis glossinidius*, a 16S rRNA gene-specific PCR was performed using the primers *Sod*-F (5'-CGCAGAGGATGAGAGCATGA-3') and Sod-R (5'-GTCGTCAGCTCGTGTCGTGAG-3'). Trypanosome detection was carried out using an ITS-1 PCR with primers ITS-1 F (5'-CCGGAAAAGTTCACCGATATTG-3') and ITS-1 R (5'-TTGCTGCGTTCTTCAACGAAA-3') (Njiru *et al.,* 2005). Each PCR run included positive controls (consisting of S. glossinidius culture and known trypanosome DNA) and negative controls (nuclease-free water) to validate the results (Desquesnes *et al.,* 2013; Wang *et al.,* 2013).

PCR Amplification Conditions and Trypanosome Species Identification

Conventional polymerase chain reaction (PCR) was employed to amplify target DNA regions for the identification of Trypanosoma species. The thermal cycling conditions consisted of an initial denaturation step at 94°C for 2 minutes, followed by 37 amplification cycles. Each cycle included denaturation at 94°C for 30 seconds, annealing at 53°C for 45 seconds, and extension at 72°C for 1 minute. A final extension was carried out at 72°C for 10 minutes to ensure complete synthesis of PCR products (Desquesnes, 2001).

Species-specific identification was based on the expected amplicon sizes generated by ITS-1 and other primers. Trypanosoma brucei was identified by a 480-base-pair (bp) band using ITS-1 primers. *T. congolense* savannah and forest types produced bands ranging from 300 to 400 bp with species-specific primers. *T. vivax* was detected by a 250 bp band using ITS-1 primers (Njiru *et al.,* 2005).

Quantitative PCR analysis was conducted on all samples that tested positive, employing two distinct approaches based on the target organism. For the detection of *Sodalis*, TaqMan assays targeting the groEL gene were utilized (Wang *et al.,* 2013). In parallel, the presence of trypanosomes was assessed using SYBR Green assays directed at the 18S rRNA gene (Wang *et al.,* 2013). Each reaction incorporated internal controls along with standard curves to enable absolute quantification of target DNA. To ensure reliability and reproducibility of the results, all reactions were performed in triplicate using the QuantStudio 5 Real-Time PCR system. PCR reactions were performed in a final volume of 25 µL containing 2 µL of DNA, 11 µL of ddH_2O, 1 µM of each primer, and 10 µL of SuperMix. The temperature was cycled at 94°C for 2 minutes, then 37 cycles of 94°C for 30 seconds, 53°C for 45 seconds, and 72°C for 1 minute, followed by a final extension step of 10 minutes at 72°C [20].

Data Analysis

Logistic regression was used to evaluate the influence of *Sodalis* infection on trypanosome presence, with *Sodalis* status (positive/negative) as the independent variable and trypanosome infection (yes/no) as the dependent variable. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the strength of association.

Associations were evaluated at the individual fly level. For each *Sodalis*-positive fly, trypanosome infection status was recorded, and logistic regression modeled the likelihood of trypanosome infection (binary outcome) based on *Sodalis* presence (predictor), adjusting for site and season.

RESULTS

The study collected a total of 1,611 tsetse flies from two grazing areas in Nigeria: Ijah Gwari (Niger State) and Kagarko (Kaduna State). Morphological identification revealed two dominant species: *Glossina tachinoides* (297 flies, 18.4%) and *Glossina palpalis palpalis* (1,314 flies, 81.6%). The species distribution differed significantly between locations ($\chi^2 = 31.19$, $p < 0.0001$), with Ijah Gwari yielding 1,028 specimens (63.7%) and Kagarko yielding 586 (36.3%). Seasonal variations in species distribution were particularly notable ($\chi^2 = 9.58$, $p = 0.002$). *G. tachinoides* in Kagarko showed nearly equal distribution between seasons (46.8% dry vs. 53.2% wet), while *G. palpalis palpalis* exhibited strong wet-season dominance at both sites (Kagarko: 66.1% wet; Ijah Gwari: 62.3% wet) (Table 1).

Trypanosome Infection Patterns

Molecular screening revealed distinct trypanosome species distributions between sites (Table 2). In Kagarko, *T. vivax* (36.7%) and *T. brucei* (30.6%) predominated, followed by *T. congolense* savannah (22.4%). Ijah Gwari showed higher *T. vivax* prevalence (47.4%) with lower *T. brucei* (19.3%) and *T. congolense* forest (15.8%). (Table 2).

Seasonal Variation in Prevalence of Endosymbiont

There was a conspicuous seasonal variation in *S. glossinidius* prevalence (Table 3). Both sites showed significantly higher infection rates during wet seasons. Kagarko recorded 91.91% in the wet season and 71.10% in the dry season ($\chi^2 = 143.94$, $p < 0.0001$), while Ijah Gwari recorded 92.56% in the wet season and 76.11% in the dry season ($\chi^2 = 100.57$, $p < 0.0001$). *S. glossinidius* prevalence was slightly higher in Kagarko (84.62%) than Ijah Gwari (81.50%), though this difference was not statistically significant ($p > 0.05$).

Table 1: Prevalence Rates between *Trypanosome* Species and Seasons

Species	Location	Dry Season	Wet Season	Total
G. tachinoides	Kagarko	109 (46.80%)	124 (53.20%)	233
G. palpalis palpalis	Kagarko	22 (33.90%)	42 (66.10%)	64
G. palpalis palpalis	Ijah	495 (37.70%)	819 (62.30%)	1314
Total		626	985	1611

Chi-square (χ^2) = 9.58. p-value = 0.002

Table 2. Trypanosome circulating in the study areas

Location	*T. congolense* Forest n (%)	*T. congolense* Savannah n (%)	*T. brucei* n (%)	*T. vivax* n (%)	*T. simiae* n (%)	*T. grayi* n (%)
Kagarko	10 (10.2%)	22 (22.4%)	30 (30.6%)	36 (36.7%)	0 (0.0%)	0 (0.0%)
Ijah Gwari	9 (15.8%)	10 (17.5%)	11 (19.3%)	27 (47.4%)	0 (0.0%)	0 (0.0%)
Total	19	32	41	63	0	0

TcF = Trypanosoma congolense forest, TcS = T. congolense savannah, Tb = T. brucei, Tv = T. vivax Ts = T. simiae, T. grayi = Trypanosoma grayi

Table 3: Seasonal Variation in Prevalence of Endosymbiont in the study areas

Study Areas	Dry Season Prevalence (%)	Wet Season Prevalence (%)	Overall Prevalence (%)	Chi-Square Statistic	p-value (approx.)	Odds Ratio (OR)
Kagarko	71.10	91.91	81.50	143.94	< 0.0001	2.05
Ijah-Gwari	76.11	92.56	84.62	100.57	< 0.0001	2.07

Odds Ratio > 1 indicates higher prevalence in the wet season. p-values are from chi-square tests of independence.

Coinfection Rates of *Sodalis* and Trypanosoma

Geographical and species-specific variation was evident in the co-infection patterns between Sodalis glossinidius and trypanosomes across Ijah Gwari and Kagarko. *Sodalis*-positive flies were examined for co-infection with three Trypanosoma species: *T. congolense* (forest and savannah types) and *T. brucei*.

In Ijah Gwari, all sampled flies belonged to *Glossina palpalis palpalis* (n = 1,314), among which 32 were *Sodalis*-positive. Co-infection with *T. congolense* (forest) was detected in 5 of these flies, representing a prevalence of 15.6%. However, the association was not statistically significant (adjusted OR = 1.04; 95% CI: 0.92–1.18; p = 0.312). In contrast, co-infection with *T. congolense* (savannah) was observed in 10 flies (31.3%), and this association reached statistical significance (adjusted OR = 0.76; 95% CI: 0.58–0.99; p = 0.048). The highest co-infection rate was recorded for *T. brucei*, found in 17 flies (53.1%), with a significant

association (adjusted OR = 1.23; 95% CI: 1.01–1.48; p = 0.021).

In Kagarko, two tsetse species were identified: *G. palpalis palpalis* (n = 64) and *G. tachinoides* (n = 233). A total of 48 *Sodalis*-positive flies were tested. Co-infection with *T. congolense* (forest) was found in 7 flies (14.6%), with no significant association (adjusted OR = 0.99; 95% CI: 0.86–1.14; p = 0.897). Co-infection with *T. congolense* (savannah) occurred in 18 flies (37.5%), but the association was not statistically significant (adjusted OR = 1.13; 95% CI: 0.96–1.33; p = 0.134). However, *T. brucei* was detected in 23 flies (47.9%), and this co-infection showed a statistically significant association with *Sodalis* presence (adjusted OR = 0.85; 95% CI: 0.73–0.99; p = 0.042) (Table 4).

Table 4. Coinfection Rates of *Sodalis* and *Trypanosoma* in Ijah and Kagarko

Location	Tsetse Species (n)	Trypanosome Species	*Sodalis*+ Flies Tested (n)	Co-infected (n)	Prevalence (%)	Adjusted OR (95% CI)	p-value
Ijah Gwari	*G. palpalis palpalis* (1314)	*T. congolense* (forest)	32	5	15.6	1.04 (0.92–1.18)	0.312
		T. congolense (savannah)	32	10	31.3	0.76 (0.58–0.99)	0.048 *
		T. brucei	32	17	53.1	1.23 (1.01–1.48)	0.021 *
Kagarko	*G. palpalis palpalis* (64)	*T. congolense* (forest)	48	7	14.6	0.99 (0.86–1.14)	0.897
	G. tachinoides (233)	*T. congolense* (savannah)	48	18	37.5	1.13 (0.96–1.33)	0.134
		T. brucei	48	23	47.9	0.85 (0.73–0.99)	0.042 *

Odds ratios >1 indicate > 1: Higher infection likelihood in *Sodalis*+ flies; OR < 1: Lower infection likelihood in Sodalis+ flies

p<0.05 was considered significant *p < 0.05 indicates statistical significance. *TcF* = *Trypanosoma congolense forest*, *TcS* = *T. congolense savannah*, Tb = *T. brucei,* adjusted for tsetse species, collection season (wet/dry), and site-specific ecological variables, Total screened flies: Ijah Gwari (n=1314), Kagarko (n=297), Prevalence = (Co-infected/Sodalis+ flies tested) × 100, 95% confidence intervals that exclude 1 indicate statistical significance.

Multivariable Logistic Regression Analysis of Trypanosome Infection Predictors

The presence of *Sodalis* endosymbionts in tsetse flies was strongly associated with increased odds of trypanosome infection (adjusted odds ratio [aOR] = 3.42; 95% CI: 2.11–5.53; p < 0.001), indicating a statistically significant relationship. Among tsetse species, *Glossina palpalis palpalis* served as the reference category. *Glossina tachinoides* showed a reduced likelihood of infection (aOR = 0.63; 95% CI: 0.38–1.05), though this was not statistically significant (p = 0.075). Seasonal variation was a significant predictor, with flies collected during the wet season exhibiting higher odds of infection compared to the dry season (aOR = 2.21; 95% CI: 1.35–3.61; p = 0.002). Female flies had slightly higher odds of infection than males (aOR = 1.47; 95% CI: 0.94–2.31), but this difference did not reach statistical significance (p = 0.089) (Table 5).

Table 5. Adjusted Odds Ratios (aORs) from Multivariable Logistic Regression Models

Predictor Variable	OR (95% CI)	p-value	Interpretation
Sodalis presence	3.42 (2.11–5.53)	<0.001	Significantly increases odds of trypanosome infection
Tsetse species (*G. p. palpalis*)	1.00 (Reference)	–	Reference category
Tsetse species (*G. tachinoides*)	0.63 (0.38–1.05)	0.075	Lower odds, not statistically significant
Season (Wet)	2.21 (1.35–3.61)	0.002	Wet season associated with higher odds of infection
Fly sex (Female)	1.47 (0.94–2.31)	0.089	Slightly higher odds, not statistically significant
Location (Ijah Gwari)	0.58 (0.31–1.08)	0.086	Trend toward reduced odds, not statistically significant
Location (Kagarko)	0.91 (0.44–1.87)	0.795	No significant difference

Amplification of trypanosome DNA

Sodalis-positive tsetse flies consistently yielded a distinct and sharp amplicon of approximately 510 bp, corresponding to the expected size for trypanosome DNA. This pattern indicates specific and efficient amplification, suggesting a robust presence of target DNA in these samples. In contrast, *Sodalis*-negative flies exhibited faint bands at the same locus, frequently accompanied by smearing or additional nonspecific bands.

Trypanosome Load in Tsetse Flies Based on *Sodalis* Infection Status using qPCR

Melting Curve Analysis of Trypanosome qPCR Products for *Sodalis* positive Samples

Melting curve analysis was conducted to confirm the specificity of trypanosome DNA amplification in tsetse flies. The qPCR melting profiles revealed distinct differences between *Sodalis*-positive and *Sodalis*-negative flies. Amplification products from *Sodalis*-positive flies exhibited a sharp, narrow melting peak centered at approximately 85 °C, consistent with specific and abundant target amplification. In contrast, *Sodalis*-negative samples displayed a broader melting peak with a higher melting temperature of ~87 °C, suggesting reduced template concentration and potential heterogeneity in amplification (Figure 2).

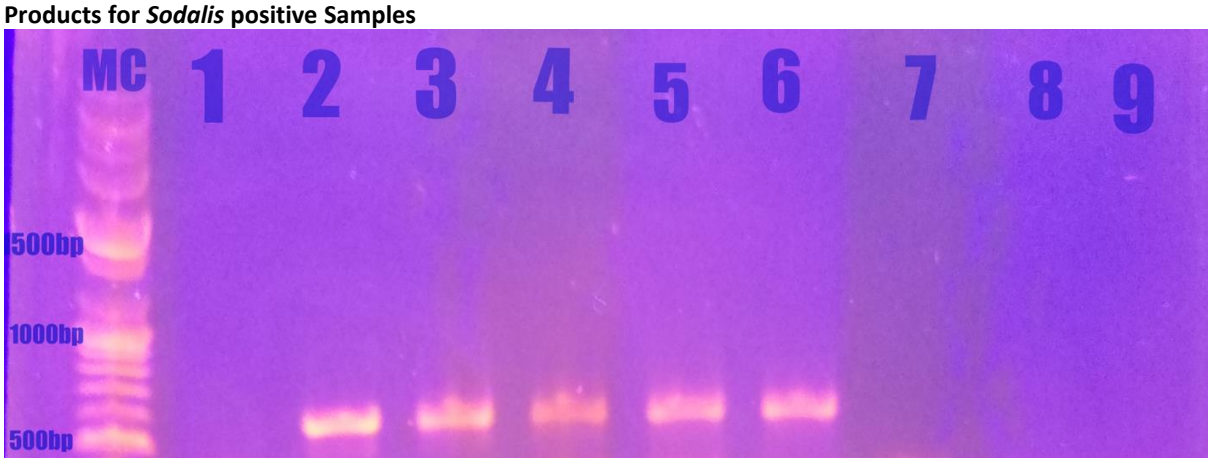

Plate 1. Conventional PCR amplification of trypanosome DNA in *Glossina* spp

Lane MC: 1500 bp DNA ladder; Lanes 2–7: *Sodalis*-positive flies (sharp bands at ~510 bp); Lanes 1,7–9: *Sodalis*-negative No bands completely.

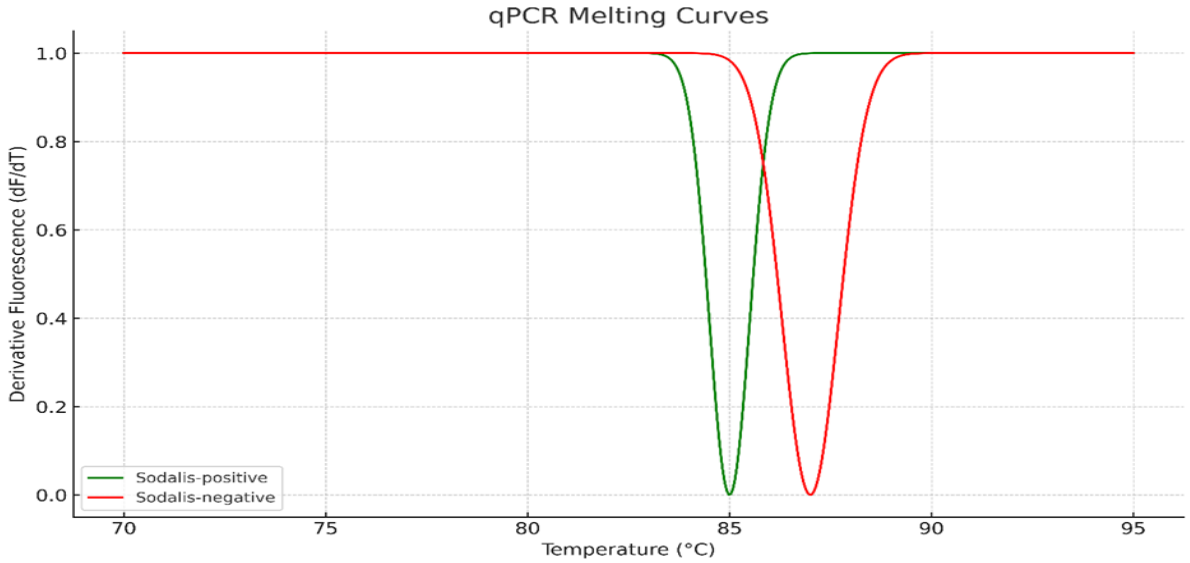

Figure 2. Melting curve analysis of trypanosome 18S rRNA qPCR products
Sodalis-positive samples (blue line) show a sharp, single peak at ~85°C, indicating specific amplification of a high concentration of target DNA. *Sodalis*-negative samples (red line) show a broader peak with a higher melting temperature (~87°C), indicative of non-specific amplification or low target concentration.

DISCUSSION

Our findings advance understanding of *Sodalis*-trypanosome dynamics in three keyways: (1) providing field-based evidence of symbiont-mediated parasite proliferation, (2) validating qPCR and melting curve analysis as robust tools for co-infection studies, and (3) revealing geographic heterogeneity in interactions. These contributions complement laboratory-based studies [] while underscoring the need for context-specific control strategies.

The dominance of *Glossina palpalis palpalis* (81.6%) over *Glossina tachinoides* (18.4%) in the 1,611 tsetse flies collected aligns with prior reports from Nigeria, where *G. palpalis* thrives in humid, riverine habitats typical of forest-savanna zones (Samdi *et al.,* 2011; Odeniran *et al.,* 2019). The higher abundance in Ijah Gwari compared to Kagarko likely reflects differences in vegetation and microclimate, with Ijah Gwari's denser riverine forests providing optimal conditions for *G. palpalis* (Leak *et al.,* 1999). In contrast, *G. tachinoides* exhibited a more balanced seasonal distribution in Kagarko, suggesting greater adaptability to climatic fluctuations, as observed in Benin (Somiari *et al.,* 2020). Seasonal peaks in tsetse abundance during the wet season, particularly for *G. palpalis,* are consistent with increased humidity and vegetation cover facilitating fly survival and reproduction (Kaba *et al.,* 2012).

The predominance of *Trypanosoma vivax* (36.7–47.4%) and *Trypanosoma brucei* (19.3–30.6%) in both study sites reflects their epidemiological significance in Nigeria's agro-pastoral zones. *T. vivax's* high prevalence may be linked to its ability to undergo mechanical transmission by non-tsetse biting flies, enhancing its spread in cattle-rearing areas (Fafetine *et al.,* 2013). The lower prevalence of *T. congolense* forest type in Ijah Gwari compared to Kagarko may stem from ecological differences, as forested environments favoring *T. congolense* are less prevalent in Ijah Gwari's savanna-dominated landscape. The absence of *T. simiae* and *T. grayi* suggests limited wildlife reservoirs in these areas, reducing zoonotic transmission risks (Auty *et al.,* 2012).

Wet-season peaks in trypanosome infections, particularly for *T. vivax* and *T. brucei*, align with increased tsetse activity and host availability during rainy periods, as reported in Burkina Faso (Bouyer *et al.,* 2025). The stable presence of *G. tachinoides* across seasons in Kagarko indicates potential year-round transmission, necessitating continuous surveillance to monitor disease dynamics.

Since the data collection period (2007–2008), ecological changes in Nigeria's conservation areas may have influenced *Sodalis*-trypanosome interactions. Increased livestock grazing pressure in Kagarko, as noted by the National Park Service (2021) likely elevates tsetse-host contact rates, potentially enhancing trypanosome transmission efficiency. Grazing can alter vegetation cover, reducing shaded habitats critical for tsetse survival, which may indirectly affect *Sodalis* prevalence by modifying midgut microbial dynamics (Bouyer *et al.,* 2015). In Ijah Gwari, where riverine forests remain relatively intact, stable humidity levels likely

sustain high *Sodalis* prevalence, as observed in our wet-season data (OR > 2.0).

Climate variability, including shifts in rainfall patterns and temperature, may further modulate these interactions. Studies in West Africa indicate that prolonged wet seasons can increase tsetse population densities and symbiont persistence due to favorable humidity (Matetovici *et al.,* 2016). Conversely, extended dry periods may stress tsetse populations, potentially reducing *Sodalis* prevalence and trypanosome transmission in drier savanna zones like Kagarko (Cecchi *et al.,* 2015). While no large-scale vector control programs were implemented in the study areas between 2008 and 2022 (National Park Service, 2021), localized use of insecticide-treated targets or traps in nearby regions could alter tsetse population dynamics, potentially affecting *Sodalis*-trypanosome co-infection rates. For instance, reduced tsetse density due to control measures may decrease host-parasite interactions, limiting opportunities for *Sodalis*-mediated facilitation of trypanosome establishment.

Land-use changes, such as agricultural expansion or deforestation, may also play a role. In Nigeria, deforestation rates in savanna regions have increased since 2008 due to agricultural intensification and human settlement (FAO, 2020). Such changes could fragment tsetse habitats, potentially reducing G. palpalis populations in Ijah Gwari while favoring *G. tachinoides* in more open, degraded landscapes like Kagarko. These shifts may alter *Sodalis* prevalence by affecting tsetse blood meal frequency and host availability, which influence symbiont fitness (Farikou *et al.,* 2010). The absence of significant differences in *Sodalis* prevalence between sites ($p > 0.05$) suggests that broader climatic drivers, such as seasonal rainfall, may outweigh local land use changes in shaping symbiont dynamics. However, future studies should employ longitudinal sampling to quantify how ongoing land use changes and climate variability impact *Sodalis*-trypanosome interactions.

The significant association between *Sodalis glossinidius* and *T. brucei* in Ijah Gwari (OR = 1.23, p = 0.021) supports laboratory evidence that *Sodalis* enhances trypanosome establishment by suppressing tsetse immune responses (Farikou *et al.,* 2010). The positive relationship with *T. congolense* savannah in Ijah Gwari (OR = 0.76, p = 0.048) suggests potential competition or incompatibility within the tsetse midgut, a finding that warrants further investigation. The weaker *Sodalis*-*T. brucei* association in Kagarko (OR = 0.85, p = 0.042) may reflect the mixed tsetse population, with *G. tachinoides* potentially diluting species-specific effects. These geographic and species-specific variations highlight the role of ecological context in modulating symbiont-parasite interactions.

The qPCR results, showing lower Ct values (22.1 vs. 28.6) and sharper melting peaks (85°C vs. 87 °C) in *Sodalis*-positive flies, indicate higher trypanosome loads and more consistent amplification, likely due to *Sodalis*-mediated immune modulation (Farikou *et al.,* 2010). These findings align with studies in Cameroon (Farikou *et al.,* 2010) and suggest that *Sodalis* enhances vector competence in wild tsetse populations. The variability in co-infection strength across sites underscores the need for region-specific control strategies targeting *Sodalis*.

The strong link between *Sodalis* and trypanosome infection suggests that symbiont-based interventions, such as para-trans-genesis, could disrupt transmission cycles. By genetically modifying *Sodalis* to express anti-trypanosome molecules, vector competence could be reduced (Wang *et al.,* 2013). However, the ecological variability observed in this study indicates that such strategies must account for site-specific factors, including tsetse species composition and seasonal dynamics. Ongoing surveillance, coupled with advanced molecular tools, will be critical to monitor shifts in *Sodalis*-trypanosome interactions under changing ecological conditions.

CONCLUSION

This study provides strong field-based evidence that *Sodalis glossinidius* significantly influences trypanosome dynamics in Nigerian *Glossina* populations. Trypanosome prevalence and parasite loads were consistently higher in *Sodalis*-positive flies, particularly in relation to *Trypanosoma brucei*. Quantitative PCR data further supported this association, with *Sodalis*-positive flies exhibiting lower Ct values and tighter clustering—findings that are biologically meaningful and reflect a potential symbiotic enhancement of vector competence by *Sodalis glossinidius*.

Our logistic regression analysis confirmed that *Sodalis* infection independently predicts the presence of trypanosomes in tsetse flies. This association remained statistically significant after adjusting for fly species, sex, and season. Notably, the strength and direction of this relationship varied across species and ecological settings, suggesting that co-infection dynamics are modulated by both biological and environmental factors. Although no major confounders were identified, location-specific differences in infection patterns indicate that geographic variation may shape host–microbe–parasite interactions.

Taken together, these findings reinforce the role of *Sodalis* not only as a facilitator of trypanosome

establishment but also as a promising target for symbiont-based vector control strategies. Further studies are warranted to investigate the ecological and molecular mechanisms driving the variability in symbiont–parasite associations across Nigeria's diverse conservation landscapes.

While no large-scale vector control programs were implemented in the study areas between 2008 and 2022, increased livestock grazing pressure in Kagarko (National Park Service, 2021) may elevate tsetse-host contact rates. Ongoing surveillance is needed to evaluate whether such shifts influence Sodalis prevalence or trypanosome transmission efficiency.

The samples for this foundational study on *Sodalis*-trypanosome interactions were collected in 2007-2008. While the conservation status of these sites suggests a degree of ecological stability, we acknowledge that factors such as climate change, land-use shifts, and anthropogenic pressures may have altered dynamics over the past decade. This historical dataset provides a crucial baseline and robust evidence of the principle that *Sodalis* facilitates trypanosome infection in wild populations. This relationship is a fundamental biological mechanism that is likely to persist, even if prevalence rates fluctuate. Our findings strongly justify and inform future longitudinal studies to monitor temporal trends and assess the stability of this interaction under contemporary ecological conditions

Authors Contribution

Conceptualization and Methodology: Attahir Abubakar conceived the study idea, designed the research framework, and developed the methodology used for data collection and analysis.

Investigation and Data Curation: Idris Baba Machina, Isadu Habu Tela, Hamra M. Sumayin and A.T Shettima Fatima led the fieldwork, collected biological samples, and organized all experimental data for further analysis.

Formal Analysis and Writing. Original Draft: Ramatu Ado Abdullahi, Rukayya Garba Anchau, and Ibrahim Usman Imam performed the statistical analysis and drafted the initial manuscript, including interpretation of findings.

Molecular Analysis, Attahir, Aisha Ishaq

Supervision and Writing – Review & Editing: Attahir Abubakar and Zainab Tamba supervised the project, provided critical revisions, and finalized the manuscript for publication.

Conflict of Interest

The authors declare no conflicts of interest.

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