World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Updating of entomological and parasitological parameters in Hypoendemic HAT foci in San Pedro and Soubré (Côte d'Ivoire)

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World Journal of Advanced Research and Reviews, 2025, 26(03), 587-596

Publication history: Received on 16 April 2025; revised on 29 May 2025; accepted on 01 June 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.3.2059

Abstract

Context: Human African Trypanosomiasis was eliminated as a public health problem in Côte d'Ivoire in December 2020. To preserve the gains from vector control in Bonon and Sinfra, surveys were conducted in hypoendemic foci. This study aimed to assess disease risk in San Pedro and Soubré through entomological and parasitological data, to better understand transmission dynamics and guide post-elimination surveillance toward interruption of transmission by 2030.

Methods: Tsetse flies were trapped in San Pedro and Soubré using 116 Vavoua traps, then identified, dissected, and tested for trypanosomes by microscopy and PCR. Fly age and infection rates were assessed, and statistical analyses were performed to compare the apparent density per trap (ADT) and Glossina infection rates between the two study sites.

Results: Two tsetse species were identified: Glossina palpalis palpalis (predominant) and G. pallicera s.l. (rare). Tsetse density was higher in San Pedro (3.05/trap/day) than in Soubré (1.97/trap/day), likely due to wildlife. Molecular analysis detected Trypanosoma vivax (16 cases) and T. congolense forest-type (22 cases), the latter being predominant. No T. brucei s.l. was found. The high number of young (nulliparous) flies increases transmission risk due to early feeding. PCR was more sensitive than microscopy, confirming its usefulness in early detection.

Conclusion: This study highlights the predominance of G. p. palpalis and T. congolense forest-type. The absence of T. b. gambiense, despite historical relevance, is notable. The findings underscore the value of molecular tools in early surveillance and the need for vigilance in San Pedro. Investigation of animal reservoirs is recommended to identify potential parasite sources.

Keywords: Tsetse Flies; Trypanosomes; Glossina; Trypanosomiasis; Hypoendemic Foci

1. Introduction

Tsetse flies (Glossina spp.) are blood-sucking insects found only in tropical Africa. They are the cyclic vectors for flagellated protozoa of the genus Trypanosoma, responsible for diseases affecting humans and animals. Human African Trypanosomiasis (HAT), also known as sleeping sickness, is endemic in parts of Africa. It can cause irreversible sequelae or even death if not treated quickly. This disease is caused by trypanosomes of the Trypanosoma brucei especially T. b. gambiense in West and Central Africa, and T. b. rhodesiense in East Africa [1]. In addition, African Animal Trypanosomiasis (AAT), known as " Nagana ", is the main livestock disease hindering the development of livestock in sub-Saharan Africa. This disease has a significant impact on the economy and food security and is caused by species

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such as Trypanosoma vivax, Trypanosoma congolense, Trypanosoma brucei brucei, Trypanosoma equiperdum and Trypanosoma suis [2].

Intensive screening and treatment campaigns have successfully limited the devastating HAT epidemics of the twentieth century [3]. These efforts have led to a significant reduction in the number of HAT cases. In 2009, for the first time in 50 years, the number of cases fell below 10,000 (9,878 cases). This trend continued, reaching less than 1,000 reported cases (663 cases) in 2020, well below the original target of 2,000 cases set for 2020 [4]. Building on this progress, the World Health Organization (WHO) has set a new target in its 2021-2030 roadmap for Neglected Tropical Diseases: to eliminate HAT transmission in T. b. gambiense, with the ultimate goal of reducing the number of reported cases to zero [3].

Côte d'Ivoire, one of West Africa's economic engines, was reported in 2013 as the second West African country to report the most cases of HAT, according to a WHO report [5]. In response, control activities, including active screening, patient treatment, and vector control programs [6; 7], were conducted. In order to carry out the strategy put in place for the elimination of HAT, entomological and parasitological surveys were initiated throughout the country between 2017 and 2018 in old and hypoendemic outbreaks. These surveys aimed to assess the status of these foci and ensure they did not pose a threat to control activities in Sinfra and Bonon, which were active HAT foci at the time. All of these actions led, in December 2020, to the elimination of HAT as a public health problem [8]. However, these interventions have mainly focused on HAT, while TAA, which threatens domestic animals and compromises food security, remains an unresolved challenge. In addition, Côte d'Ivoire is aiming for an even more ambitious goal, which is to achieve a permanent interruption of the transmission of T. b. gambiense by 2030, in line with the WHO roadmap 2021–2030. Data collected in former transmission foci, such as San Pedro and Soubré, could play a key role in guiding post-elimination surveillance strategies and preventing potential disease re-emergence. These historical HAT foci remain poorly documented in terms of interactions between tsetse flies, the animal reservoir in the case of AAT, and the pathogens. This study aims to document the distribution of tsetse flies and trypanosomes in the departments of San Pedro and Soubré, in order to better understand transmission dynamics and guide post-elimination surveillance strategies within the context of achieving sustainable interruption of transmission by 2030.

2. Material and methods

2.1. Study area

This study was conducted in the Bas-Sassandra district, specifically in the departments of San Pedro and Soubré (Fig. 1), Côte d'Ivoire. The district covers an area of 25800 km^2 and had 2,687,176 inhabitants in 2021. Historically, the department of San Pedro was linked to the Tabou health sector, but it was declared free of HAT in 1941 and was later closed. On the other hand, the department of Soubré has been successively attached to the health sectors of Sassandra, Gagnoa and Daloa and has always been classified as a hypoendemic area for HAT.

The Bas-Sassandra district, which includes the department of Sassandra, is now a region of high migration in Côte d'Ivoire. The influx of foreign populations, combined with the presence of tsetse flies, vectors of trypanosomes, makes this district a high priority area for regular surveillance to prevent possible outbreaks of sleeping sickness. The presence of free-ranging pigs (Sus scrofa domesticus) was observed during the survey, particularly in the department of San Pedro, specifically in the localities of Prollo, Pounié, Oueleké, and Boubelé (Fig. 1).

Although much of the dense, moist intermediate evergreen forest has been replaced by oil palm, rubber and cocoa plantations, significant forest areas remain, including the UNESCO World Heritage-listed Taï National Park (307,257 hectares) and the classified forests managed by SODEFOR. The district has swamp forests, mangrove-like ecosystems, and is crossed by large rivers such as the Sassandra and the Cavally. The climate is sub-equatorial, with annual rainfall between 1,200 and 2,200 mm and average temperatures between 26 and 28°C.

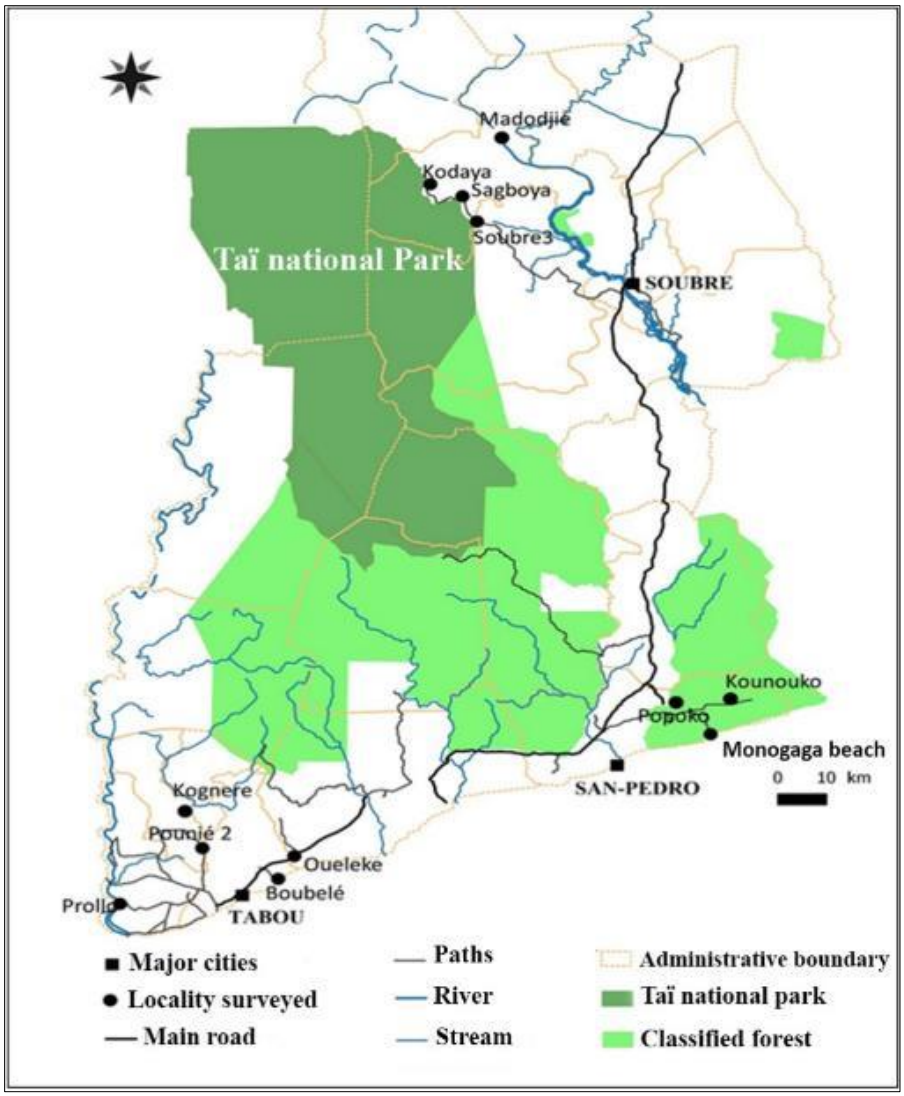

Figure 1 Study area (Source: Institut Pierre Richet-Bouaké, 2018, published with permission)

2.2. Field sampling and sample preparation

A prospective cross-sectional study was conducted in March 2018 in the Bas-Sassandra district, more specifically in the departments of San-Pedro and Soubré, in Côte d'Ivoire. Monoconical "Vavoua" traps were placed in different ecological areas, including natural and artificial water sources, intersections of paths and streams, fishing and bathing areas, breeding areas (pigs and cattle) and around dwellings. Traps have also been placed in mangrove areas. A total of 116 "Vavoua" traps were set in the localities studied, with 75 traps in San Pedro and 41 in Soubré. In each village, 4 to 15 traps were set up for 48 hours. Tsetse flies were collected every 24 hours, and used cages were replaced with new ones. The sites surveyed in San Pedro included Prollo, Pounié, Kognéré, Boubélé, Ouelléké, Popoko, Monogaga and Kounouko, while in Soubré, the traps were placed in Kodaya, Sagboya, Soubré 3 and Madodjié. Captured tsetse flies were identified and counted by species and sex according to Pollock's (1982) identification key and then dissected under a binocular magnifying glass to isolate the digestive organs (proboscis, midgut, and salivary gland) for examination under a light microscope for trypanosome infection.

For further molecular analysis, if any one of the three organs tested positive for trypanosomes under the microscope, all three organs from the same tsetse fly were individually collected and placed in Eppendorf tubes containing 25 µL of distilled water (Kazadi et al., 1994), and stored at -20°C for subsequent molecular identification.

2.3. Determination of the physiological age of female tsetse flies

The ovarian age of female tsetse flies was determined during dissections using the method of Challier (1965) which classifies females into three physiological age groups according to the size of the ovaries and the presence or absence

of follicular remains: Nulliparous group 0 for tsetse flies aged 1 to 10 days, young pares of groups I, II, III for tsetse flies aged 11 to 42 days and old pares of groups IV, V, VI, VII for tsetse flies aged 43 to 82 days. This classification provided important information on the reproductive dynamics and potential longevity of tsetse flies in the areas studied.

2.4. DNA extraction procedure

DNA extraction from the organs of the dissected tsetse flies was performed using the 5% Chelex 100® method (Chelating Ion Exchange Resin, Biorad, CA, USA). Specifically, 50 µl of Chelex 100® was added to 1.5 mL Eppendorf tubes containing 50 µl of distilled water and the infected organs. The organs were then crushed using a previously sterilized pestle. The samples were incubated at 56°C for 1 hour, followed by heat denaturation at 96°C for 30 minutes. They were then centrifuged at 13,000 rpm for 3 minutes, and the supernatant was used as the template in the PCR assays.

2.5. Conditions de la PCR

PCR amplification was performed using specific primers for T. brucei s.l. (TBR1-2) [9], T. congolense savannah type (TCS1-2) [10], T. congolense forest type (TCF1-2) [10], and T. vivax (TVW1-2) [11]. To detect T. b. gambiense, the TgsGP1/2 primers [12], targeting the TgsGP gene, were used in a single-round PCR for all samples that tested positive with the TBR1-2 PCR. PCR reactions were performed in a thermal cycler (Eppendorf Mastercycler nexus) with a final volume of 25 µl, containing 1X Qiagen HotStarTaq Master Mix, 20 pmol of each primer and 5 µl of DNA template. Negative extraction controls were systematically included throughout the procedure. The PCR products obtained were visualized by electrophoresis on a 2% agarose gel (MP Biomedical, Eschwege, Germany), stained with Red Gel (Interchim, Montluçon, France), and visualized on a 2% agarose gel (MP Biomedical, Eschwege, Germany).

2.6. Data analysis

Statistical analyses were performed using R version 4.3.2. Prior to statistical analysis, bulk density per trap per day (ADT) was calculated to estimate the abundance of tsetse flies in each region, expressed as the number of tsetse flies caught per trap per day. A generalized linear model (GLM) was then used to compare the ADT between the San Pedro and Soubré regions. The Fisher exact test was used specifically to assess differences in infection rates between the two regions. Statistical significance was set at a risk threshold of 5% (α=0.05). The sex ratio, which reflects the proportion of men to women, was calculated. The infection rate was analyzed as the percentage of tsetse flies infected with trypanosomes out of all tsetse flies dissected.

3. Results

3.1. Captured tsetse fly species and apparent density per trap (ADT)

A total of 620 tsetse flies were captured at the study sites, 458 in the department of San Pedro and 162 in Soubré. Morphological identification revealed the presence of a single subspecies, Glossina palpalis palpalis, in Soubré, while in San Pedro, G. p. palpalis represented 99.8% of the population, with only one individual of Glossina pallicera s.l. identified. The total apparent density of tsetse flies was 2.67 flies/trap/day, with densities of 3.05 flies/trap/day in San Pedro and 1.97 flies/trap/day in Soubré. This difference is statistically significant (GLM test, p = 0.001) (Fig. 2).

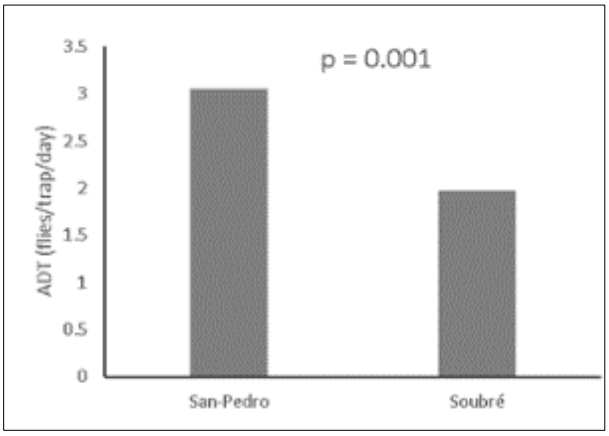

Figure 2 Apparent density of tsetse flies captured in the departments of San-Pedro and Soubré

ADT: Apparent Density per Trap; flies/trap/day: tsetse flies per trap per day.

The location of the different species of Glossina captured is presented in Fig. 3. A reduction in tsetse species diversity was observed compared to historical data. Only G. p. palpalis and G. pallicera s.l. have been identified in San Pedro, while only G. p. palpalis has been found in Soubré.

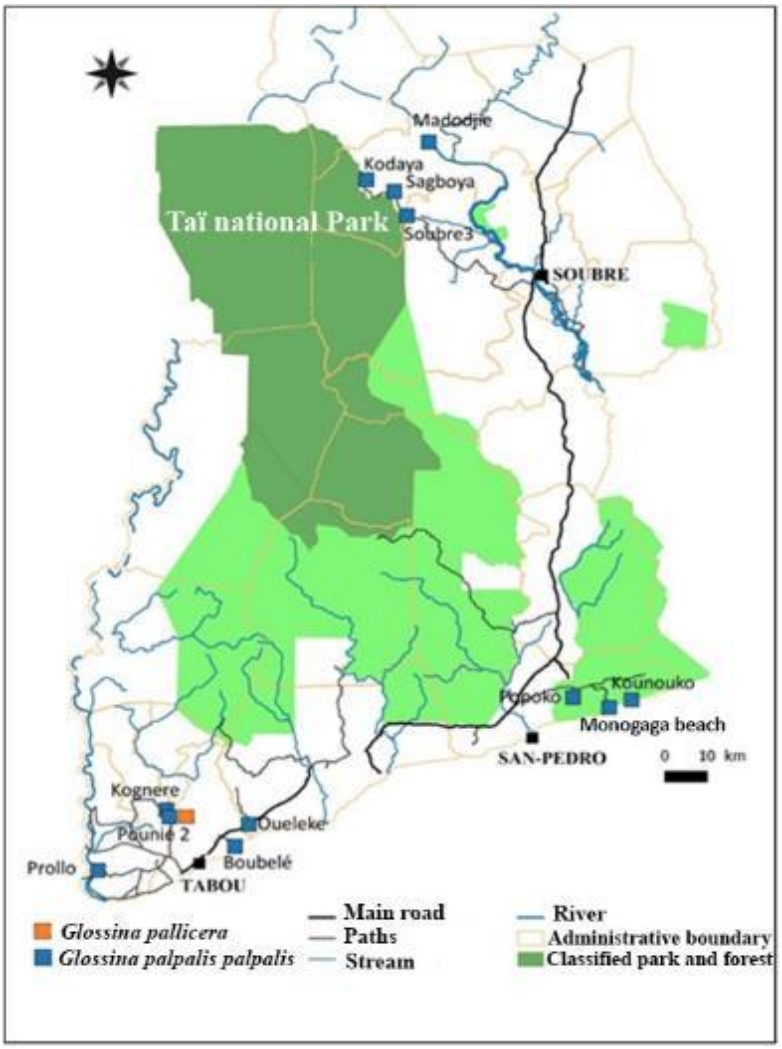

Figure 3 Location of the different species of Glossina captured (Source: Institut Pierre Richet-Bouaké, 2018)

3.2. Sex ratio of tsetse flies captured

The overall sex ratio (M/F) of tsetse flies captured at all study sites is 0.602. This indicates a higher proportion of females (62.41%) compared to males (37.57%) in the total population. For G. p. palpalis, which accounted for the majority of catches, the sex ratio was 0.603, with 233 males (37.64%) and 386 females (62.35%). In G. pallicera s.l., only one female was identified, therefore, the sex ratio could not be calculated for this species.

3.3. Profiling the physiological age of female tsetse flies

Of the 620 tsetse flies captured, 264 were dissected, of which 181 (68.6%) were identified as females. Among these females, 9 (4.97%) were nulliparous, 144 (79.56%) were young parous, and 28 (15.47%) were old parous. In San Pedro, 7 nulliparous flies (5.93%), 93 young parous flies (78.81%), and 18 old parous flies (15.25%) were identified. In Soubré, 2 nulliparous flies (3.17%), 51 young parous flies (80.95%), and 10 old parous flies (15.87%) were recorded (Fig. 4).

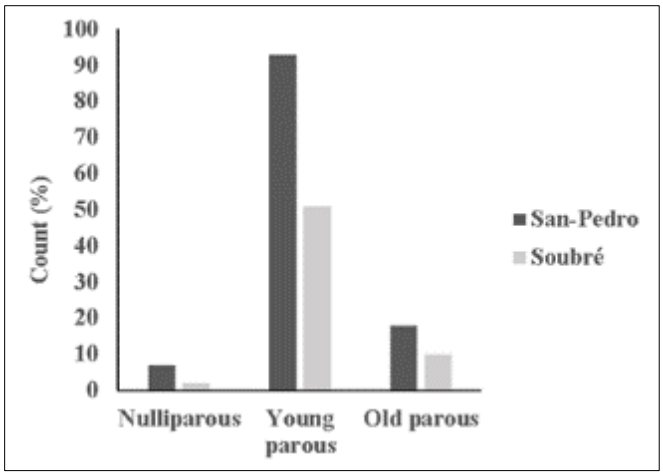

Figure 4 Physiological age of dissected female tsetse flies

3.4. Infection rates and trypanosome species identified

Of the 620 tsetse flies captured in San Pedro and Soubré, 264 (42.58%) were randomly selected for dissection. Of these, 177 (67%) came from San Pedro and 87 (33%) from Soubré. The overall infection rate was 13.25%, with 28 tsetse flies infected (80%) in San Pedro and 7 (20%) in Soubré. The infection rate in San Pedro (15.82%) was higher than in Soubré (8.04%), although the difference was not statistically significant (p=0.665). Infections were mainly localized in the midgut (MG) with 25 cases, followed by proboscis (Pr) with 9 cases and only one case of mixed infection was identified in the organs of a tsetse fly in the midgut and proboscis (MG/Pr). No infections were observed in the salivary glands (GS) (Table 1).

Table 1 Infection rate of tsetse flies identified by dissection

Study localities	Tsetse flies caught	Dissected tsetse flies	Infected tsetse flies	Infection rate (%)	Infected organs		
					MG	Pr	MG/Pr
San Pedro	458	177	28	15,82%	21	6	1
Soubré	162	87	7	8,04%	4	3	0
Total	620	264	35	13,25 %	25	9	1

MG: midgut; Pr: Proboscis

Molecular analysis of the 177 dissected tsetse flies identified two species of trypanosomes: Trypanosoma vivax and T. congolense forest-type, while T. brucei s.l. and T. congolense savannah-type were not detected. T. vivax was found mainly in probocsis (15 isolates) while T. congolense forest-type was more common in the midgut (13 isolates) and also present in proboscis (9 isolates). On the other hand, in Soubré, no trypanosome was identified by PCR in tsetse flies, despite the presence of microscopic infections (Table 2).

Table 2 Distribution of identified trypanosome species

Site	Infected Organ	T. vivax	T. congolense forest-type
San Pedro	Pr (Proboscis)	15	9
	MG (Midgut)	0	13
	GS (Salivary glands)	0	0
Soubré	Pr (Proboscis)	0	0
	MG (Midgut)	0	0
	GS (Salivary glands)	0	0

4. Discussion

This study has updated knowledge on the distribution of tsetse flies and trypanosomes in the Bas-Sassandra region, more specifically in the departments of San Pedro and Soubré. It also explored the current dynamics of trypanosomiasis transmission and contributed to the formulation of targeted strategies for the management of the associated risks. The results confirm the presence of two subspecies of tsetse flies of the subgenus Nemorhina (palpalis group), namely G. p. palpalis and G. pallicera s.l., which is consistent with previous knowledge on the distribution of these vectors in this region [5; 13]. However, the absence of other species of the subgenus Austenina (fusca group), such as G. fusca, G. nigrofusca and G. medicorum, previously described in the region [14; 15; 16], raises important questions about human-induced habitat alteration. This observation suggests that anthropogenic pressures, such as intensive agriculture, especially rubber farming, and cocoa and coffee cultivation, have altered tsetse flies' habitats, favoring the adaptation of more resilient species such as G. p. palpalis at the expense of more sensitive species. However, the absence of these species cannot be attributed exclusively to anthropogenic factors. It could also result from the sampling time (48 hours), the locations surveyed or the choice of sampling sites, which may not have covered all the habitats favourable to these species. A more in-depth study including a larger sampling in terms of duration and diversity of sites would be necessary to better understand these dynamics.

The high density of tsetse flies observed in San Pedro compared to Soubré, although not entirely explained by geographical and climatic factors, could be linked to the presence of fauna in the San Pedro biotope, a factor that favors the survival and reproduction of tsetse flies [17]. In particular, the presence of stray domestic pigs in several localities of San Pedro, including Prollo, Pounié, Ouelléké and Boubélé, could be a key element influencing the density of tsetse flies. Indeed, the field observations corroborate those of other studies indicating that pigs may play a role in maintaining tsetse flies' populations as potential hosts or by promoting a suitable environment for them to breed [18; 19; 20]. This high presence of tsetse flies in San Pedro than in Soubré is confirmed by the calculated apparent density per trap (ADT), which was 3.05 flies/trap/day in San Pedro and 1.97 flies/trap/day in Soubré, with a statistically significant difference ($p = 0.001$). The overall ADT of this study (2.67 flies/trap/day) is of the same order as that reported by Konan et al. [21] in Abidjan (2.26 flies/trap/day), suggesting relatively similar population dynamics between the study sites in these cities. Despite the geographical and climatic similarities between San Pedro and Soubré, the higher density of tsetse flies in San Pedro can be attributed to the presence of abundant wildlife, lower anthropogenic pressures, and possibly the number of traps deployed in each area.

The transmission dynamics of trypanosomiasis, whether Human African Trypanosomiasis (HAT) or African Animal Trypanosomiasis (AAT), is strongly influenced by this variation in vector density, since G. p. palpalis, which feeds indiscriminately on humans, domestic animals and wildlife, is a major vector in this region. Molecular analysis of tsetse flies revealed the presence of two species of trypanosomes, T. vivax (16 cases) and T. congolense forest type (22 cases), the latter being predominant. This result contrasts with the studies of Bosson-Vanga et al. [22], Acapovi-Yao et al. [23] and Konan et al. [21], who identified T. vivax as the predominant species in the areas in which their work was carried out. Furthermore T. congolense savannah type was not identified in this study. Similar to the results of Bosson-Vanga et al. [22] and Acapovi-Yao et al. [23], T. brucei s.l. was also not detected in this study. However, Acapovi-Yao et al. [24] reported T. brucei s.l. in 7% of cases in the departments of Korhogo, Ferkessédougou and Boundiali. The absence of T. brucei s.l. in this study does not necessarily mean that it is absent in San Pedro and Soubré, as the infection rates of tsetse flies by T. brucei s.l., even in areas of high transmission, rarely exceed 1 in 1000 [25]. In addition, it is important to emphasize that not all tsetse flies were dissected in this study, which could have led to the non-detection of some trypanosome species.

The PCR results suggest that in San Pedro, at least 15 tsetse flies were infected in the proboscis and at least 13 tsetse flies in the midgut, which is discordant with the number of infected organs which is 6 for the proboscis and 21 for the midgut, indicating a higher sensitivity of molecular methods. Similar results have been reported by Bosson-Vanga et al. [22] and Acapovi-Yao et al. [23], highlighting the limitations of microscopy, which is highly dependent on the skill and fitness of the operator. However, in Soubré, the non-detection of pathogen in infected organs could be explained by the presence of other types of trypanosomes than those targeted. Indeed, some countries in sub-Saharan Africa have reported tsetse flies with proboscis infections indicative of T. vivax that did not produce PCR signals with the T. vivax, suggesting the possible circulation of strains of Duttonella not recognized [26]. Although PCR typically has higher detection rates than parasitological techniques, the choice of primers can have a significant impact on the results [27]. These results highlight the importance of careful primer selection and the risk of false negatives when PCR targets do not match local trypanosome strains. However, the use of molecular biology tools in surveillance systems represents a significant advance in accurately detecting subclinical infections and better understanding the transmission dynamics of trypanosomes.

The identification of high proportions of young tsetse flies (nulliparous and young parous) adds another level of risk for the transmission of trypanosomiasis. Young flies, especially tenerals, are more susceptible to infection during their first blood meal [28]. Once infected, these flies can become key players in the dissemination of trypanosomes, as the parasite's life cycle ends quickly in them, making the insects infectious to their hosts.

These findings support increased surveillance in areas at risk, particularly in areas with high density of tsetse flies, such as San Pedro, and in protected areas such as Taï National Park, which were not part of this study. However, the traps set at the edge of this park have captured infected tsetse flies. Control/surveillance strategies must be reconsidered to account for the specific ecological dynamics of each area, focusing interventions on the biotopes and tsetse fly species most involved in the transmission of trypanosomiasis. The integration of molecular biology into surveillance and control strategies could facilitate the early detection of trypanosomiasis outbreaks and allow for the implementation of more effective prevention measures adapted to local dynamics. In addition, understanding the molecular mechanisms underlying parasite transmission could open up new perspectives for the development of therapeutic and preventive strategies, thereby reducing the burden of trypanosomiasis on human and animal health in this region.

5. Conclusion

In conclusion, molecular analyses have detected two species of trypanosomes, T. vivax and T. congolense forest-type, the latter being predominant. No cases of T. b. gambiense have been detected, in contrast to its historical role in the transmission of Human African Trypanosomiasis (HAT) in this region. These results highlight the increased sensitivity of molecular methods, such as PCR, compared to traditional microscopic approaches, which are often subject to operator-related limitations. Analysis of tsetse flies revealed a predominance of G. p. palpalis, a species that is highly adaptable to environmental changes and known for its major role in the transmission of T. b. gambiense. The high proportion of young tsetse flies indicates active renewal of vector populations, increasing the potential risk of transmission in the event of circulation of pathogenic trypanosomes.

These results highlight the importance of integrating molecular tools into surveillance systems in order to detect active outbreaks early, even in cases of low prevalence. Such an approach would optimize prevention and control strategies while improving the understanding of transmission dynamics at the local level. It would also be relevant to carry out an animal reservoir survey, targeting domestic animals, to assess their role in the transmission of the disease. Finally, it is essential to maintain this vigilance in risk areas in order to prevent the potential re-emergence of HAT in the historic outbreaks of San Pedro and Soubré.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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