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Molecular diagnosis of *Plasmodium falciparum* in three active foci of human African trypanosomiasis (Boffa, Dubreka and Forecariah) in Guinea

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ABSTRACT

Objectives: the overall objective of this study was to determine the prevalence of *Plasmodium (P.) falciparum* infection in the three foci of human African trypanosomiasis (HAT) (Boffa, Dubreka and Forecariah) in coastal Guinea using the polymerase chain reaction (PCR).

Methodology and results: DNA from blood samples was extracted and amplified using pf1/2 primers. A total of 1001 individuals were included. The population was predominantly male (57.34 %) and female (42.66%) with a sex ratio of 1.34. The mean age of the study population was 34 ± 16.5 years. The overall molecular prevalence of *P. falciparum* infection was 17.78 % (178/1001). In the three HAT foci, the prevalences were 10.37 % (31/299) in Boffa, 20.12 % (99/492) in Dubreka and 22.86 % (48/210) in Forecariah. Women (71/178 or 39.89 %) were significantly less infected than men (107/178 or 60.11 %) (*p-value* = 0.0013).

Conclusion and application of results: These results showed the positive PCR results in the three HAT foci. The diagnosis of malaria could be improved by using PCR to determine the true extent of the disease, but this test is higher cost comparing to classical methods. The malaria/HAT co-infection in this coastal area could be a factor of aggravation of the sleeping sickness, and should be taken into account by the HAT control programme in Guinea.

Key words: PCR diagnosis, prevalence malaria, human African trypanosomiasis, coastal Guinea

RESUME

Objectifs : l'objectif général de cette étude était de déterminer la prévalence de l'infection à *Plasmodium* (*P.*) *falciparum* dans les trois foyers de trypanosomiase humaine africaine (THA) (Boffa, Dubréka et Forécariah) en Guinée côtière en utilisant la réaction d'amplification en chaîne (PCR).

Méthodologie et résultats : l'ADN des échantillons de sang a été extrait et amplifié en utilisant les amorces *pf1/2*. Au total, 1001 individus ont été inclus. La population était constituée essentiellement de 57,34 % d'hommes et de 42,66 % de femmes, soit un sexe ratio H/F de 1,34. L'âge moyen de la population d'étude était de 34 ans ± 16,5. La prévalence moléculaire globale de l'infection à *P. falciparum* était de 17,78 % (178/1001). Dans les trois foyers de THA, les prévalences ont été de 10,37 % (31/299) à Boffa, de 20,12 % (99/492) à Dubréka et de 22,86 % (48/210) à Forécariah. Les femmes (71/178 soit 39,89 %) étaient moins infectées que les hommes (107/178 soit 60,11 %) (*p-value* = 0,0013).

Conclusion et application des résultats : Nos résultats ont montré des résultats positifs de la PCR dans les trois foyers de THA. Le diagnostic du paludisme pourrait être amélioré en utilisant la PCR pour déterminer l'étendue réelle de la maladie, mais ce test est plus coûteux que les méthodes classiques. La co-infection paludisme/THA dans cette zone côtière pourrait être un facteur d'aggravation de la maladie du sommeil, et devrait être prise en compte par le programme de lutte contre la THA en Guinée.

Mots-clés : PCR diagnostic, prévalence du paludisme, trypanosomiase humaine africaine, Guinée côtière.

INTRODUCTION

Malaria is the first worldwide parasitic disease (WHO, 2021a). Thus, the World Health Organization (WHO) estimated in 2019 that there were 229 million cases of malaria in the world. In 2009, the deaths due to malaria were 409,000. Children under five years are the group more affected by this disease. The malaria caused in 2019, 274,000 children deaths. The most malaria burden is beared by the African continent. In 2019, 94 % of malaria cases and deaths occurred in this region (WHO, 2021a). In Guinea, malaria remains a major public health problem (INS *et al.*, 2017). It is the leading cause of hospitalization, accounting for 42 % among adults (all genders); 66 % and 57 % among under-fives in boys and girls respectively (Camara *et al.*, 2019). Furthermore, Guinea is currently the country most affected by Human African Trypanosomiasis (HAT) or sleeping sickness in West Africa (Simarro *et al.*, 2010; Kambiré *et al.*, 2012). In 2018, less than 100 cases of HAT were reported in Guinea (WHO, 2021b). Almost all cases are detected in mangrove areas, where environmental conditions favour the presence of humans, tsetse flies, mosquitoes, sleeping sickness and malaria. Thus, any interaction between these two conditions could have significant public health consequences. Both diseases occur along the Guinean coastline and have almost the same clinical signs and symptoms making clinical diagnosis difficult (Maina *et al.*, 2010). Moreover, the presence of the malaria parasite, *Plasmodium* (*P.*), could be a factor in the aggravation of HAT disease. Diagnosis of malaria disease is carried out by a microscopic examination or using a rapid diagnostic test. These diagnostic techniques sometimes lack sensitivity and specificity due to low parasitaemia (Gaye *et al.*, 1998), which can lead to a bias in the estimation of malaria epidemiology. The limitations of these methods have necessitated the development of new techniques to provide a reliable diagnosis of malaria parasite. The recent development of

molecular diagnostics has allowed further characterisation of the parasite. Thus, the more sensitive and specific polymerase chain reaction (PCR) (Gaye *et al.*, 1998), is a useful tool in the molecular diagnosis of malaria. The general objective of this study was to survey the individuals infected with *P. falciparum* in the three foci of HAT in coastal Guinea using the PCR technique.

MATERIALS AND METHODS

Study area and sample collection: Blood samples were collected during HAT medical surveys from 2005 to 2013 in three active foci in Guinea (Boffa, Dubreka and Forecariah) which are located along the Guinean coastline (Figure 1). For each patient, sex, principal activities and age were collected.

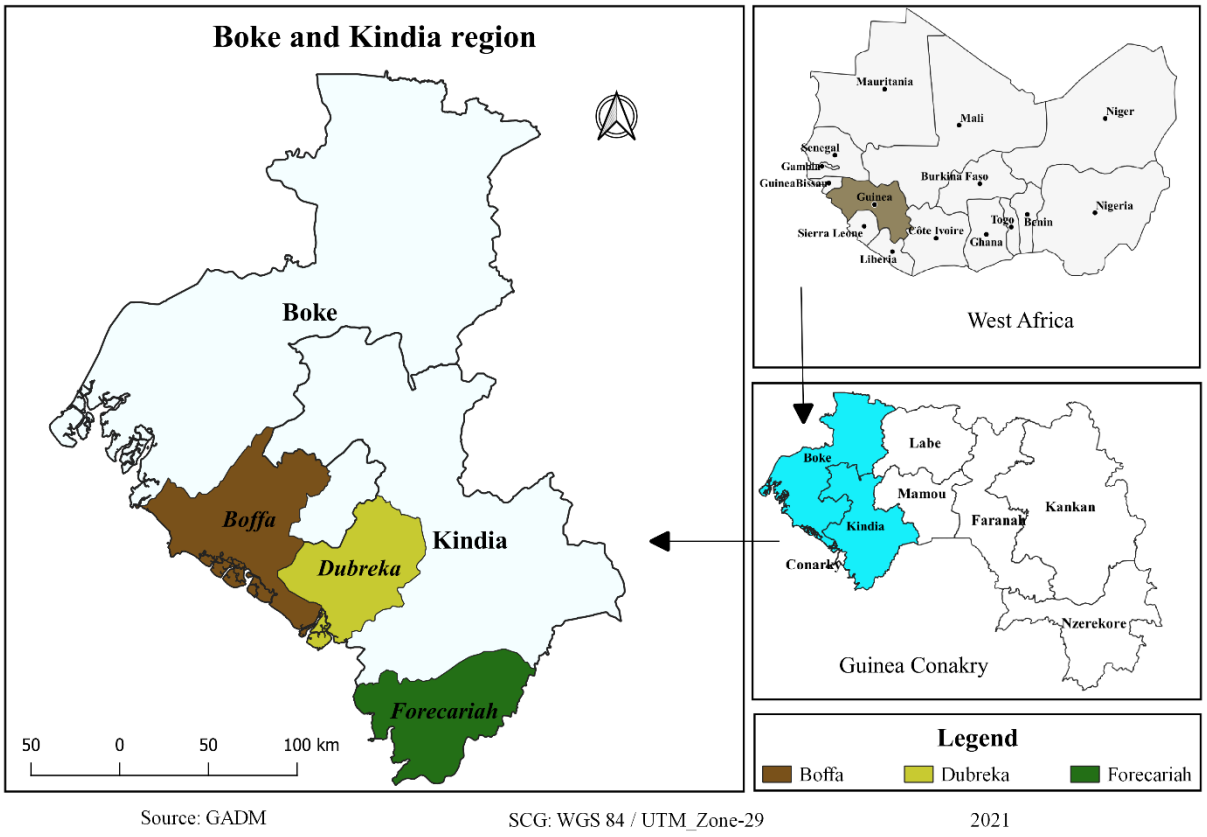

Figure 1: Location of the study area

***DNA extraction*:** DNA was extracted from the blood samples using the Qiagen DNA extraction kit according to the manufacturer's instructions. These extracts were stored at -20˚C. After, DNA extract was quantified by the NanoDrop 2000 (Thermo-Fisher-Scientific, 2009) (Koffi *et al.*, 2006).

PCR diagnostic: A *P. falciparum*-specific PCR (*Pf*1 5'-GGAATGTTATTGCTAACAC-3' and *Pf*2 5'-AATGAAGAGCTGTGTATC-3') was performed on the DNA extracts to identify sporozoite positive samples (Morassin *et al.*, 2002). Amplification of *P. falciparum* DNA was performed in the Applied Biosystems™ 2720 thermal cycler. The final volume of a sample was 15 µL with a composition of 2 µL (1-5 ng/µl) purified DNA, 1X Taq, 0.5 mM magnesium chloride ($MgCl_2$),

0.2 Mm deoxyribonucleotide dNTPs, 5 pmoles of each primer and 0.5 Taq DNA polymerase. DNA amplification in this PCR followed the following cycles: 94°C for 3', 94°C for 30", 50°C for 1'15", 68°C for 1' and 68°C for 5' and is repeated 32 times. Migration was performed on 2 % agarose gel. The expected band size was 501 base pairs.

Data analysis: The prevalence of malaria infection is calculated by dividing the number of positive PCR by the study population size. The χ^2 test was used to compare the prevalences of infection of the different foci and according to gender using the JMP® Pro 14.0.0 software (SAS-Institute, 2018) The significance level was 5%.

RESULTS

***Socio-demographic characteristics of the study population*:** 1001 individuals were included, consisting mainly of 57.34 % males and 42.66 % females and giving a sex ratio of 1.34. The study population was 34 ± 16.5 years old. The 20-30 age group was most represented in Boffa (Figure 2), while the 30-40 age group was most represented in the other two foci. The main activities are fishing, salt extraction, agriculture (rice, fonio, corn) and trade (fish, salt, millet, corn, rice and fonio).

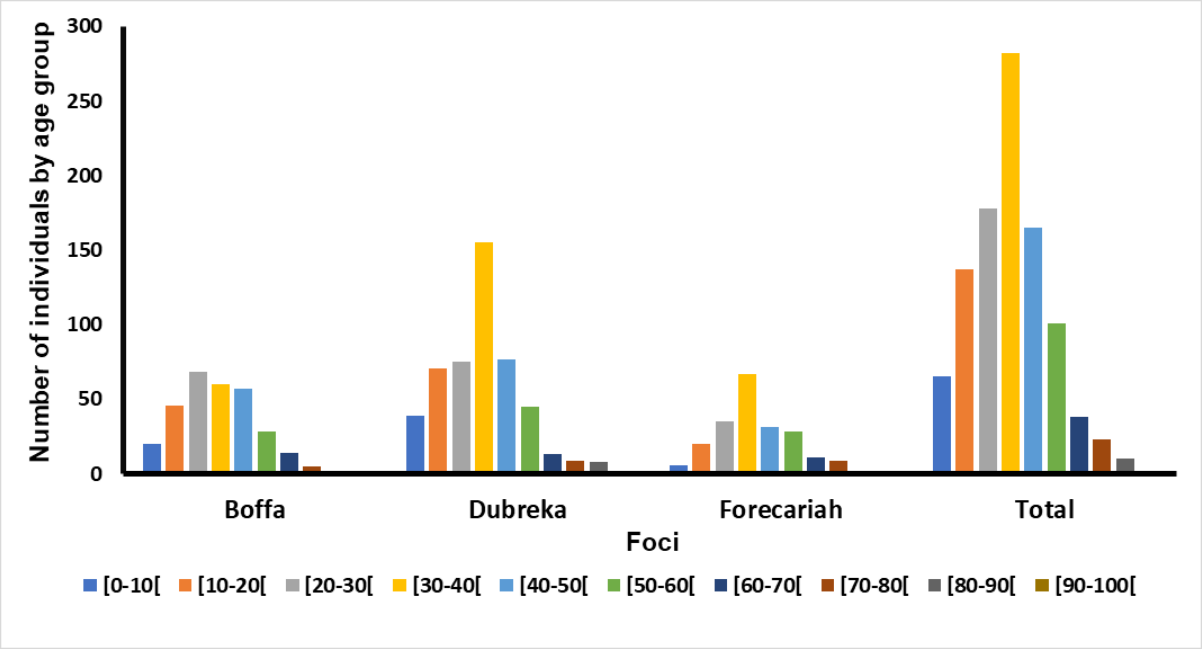

Figure 2: Age groups per focus in the study population

***Molecular prevalences of P. falciparum infection*:** The overall molecular prevalence of infection in the three foci was 17.78 % (178/1001), i.e., 178 PCR positive individuals. In Boffa, a prevalence of 10.36 % (31/299) was noted. In Dubreka, the prevalence was 20.12 % (99/492). Finally, in Forecariah, it was 22.86 % (48/210) (Table 1). No significant difference was noted between the prevalences of Dubreka and Forecariah (*p-value* = 0.4176). On the other hand, significant differences were noted between the prevalences of Boffa/Dubreka (*p-value* = 0.0002) and Boffa/Forecariah (*p-value* = 0.0001). Women (71/178 or 39.89 %) were less infected with malaria than men (107/178 or 60.11 %) (*p-value* = 0.0013).

Table 1: Molecular prevalence of *Plasmodium falciparum* infections in human African trypanosomiasis foci

Foci	PCR positive	Prevalence	Total
Boffa	31	10.37	299
Dubreka	99	20.12	492
Forecariah	48	22.86	210
Total	178	17.78	1001

DISCUSSION

The sex ratio was 1.34 in our study, which contrast with the Guinean population where the sex ratio is 0.94 (INS, 2019). This could be explained by the fact that the fishing and agricultural activity zones attract more young men, whose average age is 34. This same age is confirmed by a study in Conakry (Guinea) on malaria-HIV co-infection (Baldé *et al.*, 2010). The overall molecular prevalence of infection in the three foci was 17.78 %, which is slightly higher than the estimated seroprevalence of 15 % according to the Guinean population demographic survey (MICS, 2016). In the three study areas, the molecular prevalences were 10.37 % in Boffa, 20.12 % in Dubreka and finally 22.86 % in Forecariah. These prevalences are higher than those of the multiple indicator cluster survey where malaria seroprevalence was 6 % in Boke department (Boffa) and 10 % in Kindia department (Dubreka and Forecariah). Another study found that malaria seroprevalence was 38 % in Dubreka (Sayre *et al.*, 2021). This difference in prevalence between these zones could be explained by the control methods that have influenced malaria prevalence. Moreover, in Dubreka and Forecariah where molecular prevalences are higher (20.12 % and 22.86 % respectively), 49 % of households own at least one insecticide-treated net compared to 53 % in Boke department in Boffa (MICS, 2016). This difference in prevalence could also be explained by the fact that malaria can be both over-diagnosed and under-diagnosed (Yaméogo *et al.*, 2011). It could also reveal problems of sensitivity and specificity of serological screening tests (Ogouyèmi-Hounto *et al.*, 2013; Ba *et al.*, 2017; Houzé, 2017), that are used routinely. Thus, cross-reactions have been highlighted with viral and bacterial infections that can give false positives (Houzé, 2017). The use of PCR for malaria diagnosis has a high sensitivity. The sensitivity and specificity are closer to 100 % (Gaye *et al.*, 1998). However, the use of molecular techniques requires a good technical platform and competent and trained personal. This price is higher than other techniques (Berry *et al.*, 2005). Men (60.11 %) were more infected than women (39.89 %). Although malaria affects both men and women, gender roles and dynamics give rise to different vulnerabilities, such as exposure patterns (Cotter *et al.*, 2013). Thus, traditional gender roles may lead to men doing in the field activities after dark or women fetching water early in the morning, that expose them more to mosquito bites (Cotter *et al.*, 2013). In addition, along borders and in hard-to-reach areas where transmission is more focused, mobile and migrant populations favour transmission as they often sleep outdoors without protection. Men are generally at higher risk in these settings (Guyant *et al.*, 2015). For social and biological reasons, women (especially pregnant women) and children may also be at higher risk of contracting malaria in high and low endemicity areas (Duffy and Fried, 2005; Mbonye *et al.*, 2006).

CONCLUSION AND APPLICATION OF RESULTS

The present study shows that malaria is endemic in these three HAT foci in coastal Guinea, with the overall molecular prevalence of 17.78 %. This pathology has clinical diagnosis difficult because these symptoms could be confused with those of other pathologies like HAT disease. Serological diagnosis is also confronted with a lack of sensitivity and specificity, which can lead to cross-reactions with other pathogens. The reference techniques for the diagnosis of malaria remain the blood smear and the thick drop, which have also shown their limitations. Thus, the use of molecular diagnostic methods by PCR is becoming an asset for epidemiological studies, but present higher costs than these classical methods. Finally, the malaria should be taken into account in the control of HAT in these coastal foci because it could be a factor in the aggravation of sleeping sickness.

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