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(RESEARCH ARTICLE)

Prevalence of *Plasmodium falciparum* identified in people using microscopy, rapid diagnostic test and PCR in the prefecture of Siguiriya/Guinea

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Abstract

Introduction: Malaria remains one of the leading causes of medical consultations in the tropics. In Guinea, this disease is primarily caused by *Plasmodium falciparum*. Objective: To determine the prevalence of malaria in a population.

Material and Methods: This is a prospective study lasting eight months, from October 5, 2022, to May 30, 2023. Diagnostic of malaria was done using microscopy, rapid diagnostic tests, and polymerase chain reaction (PCR).

Results: A total of 4352 people were included in the study, 50% of whom were children under 5 years of age. The rapid diagnostic test (RDT) for malaria was positive for *Plasmodium falciparum* in 5.10% of cases. The diagnosis of *Plasmodium* by thick blood smear (SBS) was positive in 5.85% of cases (255/4352), and the thin blood smear allowed the determination of the *Plasmodium falciparum* species in all SBS-positive cases. The molecular method (PCR) applied to the 255 SBS-positive samples showed the presence of *Plasmodium falciparum* DNA in 96.47% of the SBS-positive samples (246/255). Extrapolation of PCR results positive for *Plasmodium falciparum* DNA to all 4352 subjects in the study gave a prevalence of 5.65% (246/4352). DNA prevalence according to age groups after PCR showed that 118 children under 5 years of age (47.96%) were carriers of *Plasmodium falciparum* DNA compared to 128 individuals over 5 years of age (52.03%) also carriers of Plasmodium falciparum.

Conclusion: The results of this present study showed a significant prevalence of *Plasmodium falciparum* in the population studied, and malaria diagnosis by microscopy remains the reference method; therefore, the rapid diagnostic test serves as a quicker means of diagnosis and the PCR technique is not usually used routinely because of the generally higher costs of molecular methods.

Keywords: Prevalence; Plasmodium Falciparum; Antifolate Resistance; Mutations; Republic Of Guinea

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1. Introduction

Malaria remains one of the main causes of medical consultations, morbidity, and mortality worldwide for more than half a century. It is a parasitic disease caused by five species of the genus Plasmodium: Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale and Plasmodium knowlesi, which is mainly prevalent in Southeast Asia [1-5]. Plasmodium falciparum is the most formidable and dangerous species of the genus Plasmodium responsible for cases of morbidity and mortality in tropical and equatorial countries [2,3,5]. Thus, this species is often considered to be one of the Plasmodium species associated with the most formidable forms of malaria in the world. Moreover, this parasite has developed resistance to most antimalarial drugs, significantly complicating the treatment and control of the disease. Children under 5 years of age represented 76% of the fatalities [7].

Hence, the treatment and eradication of this parasitosis necessitate a precise biological diagnosis. Currently, in Guinea, as well as globally, various techniques are employed for malaria diagnosis. These diverse methods commonly utilized in biomedical analysis laboratories for malaria diagnosis include rapid diagnostic tests (RDT), thick blood drop (BG) and blood smears, as well as molecular techniques like loop-mediated isothermal amplification (LAMP) [8,9]. However, molecular methods are not generally used in routine malaria diagnosis in most countries of the world.

Malaria diagnosis remains a concern for all medical biology laboratories. On a daily basis, this diagnosis poses strategic problems: on the one hand, errors or delays in diagnosis can have dramatic consequences. Furthermore, the use of a single diagnostic method is not without consequences. In recent years, the commercialization of new immunochromatographic techniques known as “rapid diagnostic tests” (RDTs) has opened up new perspectives in malaria diagnosis [9].

After decades of dramatic reductions in malaria cases and deaths worldwide, progress toward malaria control and elimination had stalled before the COVID-19 pandemic [10] and malaria cases and deaths increased in 2020 [11]. Further erosion of recent progress in malaria control will lead to resurgences, at great cost to health, lives, and economies in the world's poorest countries [12]. Chemoprevention strategies currently recommended by the World Health Organization (WHO) include intermittent preventive treatment in pregnancy (IPT), intermittent preventive treatment in infants (IPTNI), seasonal malaria chemoprevention (SMC), and mass drug administration (MDA) to reduce disease burden in emergency settings [13]. Having an efective treatment for malaria is a key to achieve the goals set by the WHO through the Global Technical Strategy for Malaria 2016–2030. In recent years, several studies have demonstrated the appearance of strains resistant to antifolates in endemic areas. Artemisinin-based combination treatment combines short-acting artemisinin with a longer-acting partner drug to reduce the emergence of resistance [14].

In Guinea, like in other sub-Saharan African countries, malaria is endemic and remains one of the leading causes of consultations in health facilities. The awareness campaigns, screening, and mass treatment organized by the Ministry of Health and Public Hygiene of Guinea through the national malaria control program, supported by development partners, illustrate its endemic nature. The aim of this present study was to determine the prevalence of Plasmodium. falciparum in the population of the prefecture of Siguiri in the Republic of Guinea.

2. Materials and Working Methods

This study was conducted in the Republic of Guinea. The Microbiology laboratories of the Faculty of Sciences and the Faculty of Health Sciences and Technology of Gamal Abdel Nasser University in Conakry, and the biomedical laboratory of the China-Guinea Friendship Hospital of Kipé in Conakry, served as the framework for this study. This is a prospective study lasting eight months, from October 5, 2022, to May 30, 2023. The sampling was simple random, and the sample size (n=4352). All children aged 3 to 59 months were included in this study on the one hand and people aged over 5 years on the other. All these people were residents in the seasonal malaria chemoprevention (SMC) zones in the Republic of Guinea.

2.1. Variables under study

- Biological variables Thick blood smear, Blood smear, Mutations
- Epidemiological variables: Age, Sex

II-4 Biomaterial Our biomaterial consisted of blood samples, three drops collected on filter paper or confetti (Whatmann 3MM), with a total volume of 50 µL each. The number of participants in our study was 4,352, including 4,152 in the seasonal malaria chemoprevention zone of Siguiri Prefecture, which is densely populated due to mining and is one of the first prefectures to benefit from the SMC since 2015. In addition, 200 samples were collected from health centers in other prefectures benefiting from the SMC.

3. Working Method

3.1. Type and Duration of Study

This is a prospective study lasting eight (8) months, from October 5, 2022, to March 30, 2023.

3.2. Study Population

Our research involved a total sample of 4,352 individuals, including children aged 3 to 59 months and individuals aged over 5 years, distributed as follows: 4,152 samples collected in the community and 200 samples collected in health facilities.

3.3. Sampling

In communities and health facilities, sampling was simple random, and the sample size (n=4352) was obtained using the Schwartz formula (2016) based on the prevalence of malaria in the Republic of Guinea (P=15%).

The sample size collected in the study was calculated using the formula.

- n= t2 x p (1-p)/m2
- t= 95% risk of error (1.96)
- P= national malaria prevalence (15%)
- m= margin of error (5%)
- n= 1.962 x 0.15(1-0.15)/0.052 = 196

Our sample size (n=4352) is well above the minimum accepted sample size, making our sample highly representative.

3.4. Selection Criteria

3.4.1. Inclusion Criteria

This study included all children aged 3 to 59 months, as well as individuals aged over 5 years and residing in seasonal malaria chemoprevention (SMC) areas in the Republic of Guinea.

3.4.2. Exclusion Criteria

All individuals who did not meet our inclusion criteria were excluded from our study.

3.5. Study Variables

3.5.1. Biological Variables

The biological variables in our research are: Goutte épaisse, Fortis sanguins, and mutations in Pfdhfr and Pfdhps.

3.5.2. Epidemiological Variables

The epidemiological variables in this research are: Age, Sex

4. Operational Variables

4.1. Sampling

The samples were taken from the third or fourth finger of the left hand on the side, which is less sensitive than the fingertip. The punctured areas were first disinfected using a cotton swab soaked in 70° alcohol, followed by a dry swab to remove all traces of alcohol. After a quick prick with a vaccinostyle, the first drop of blood was wiped off with a dry cotton swab. The following drops of blood were spread thickly in the middle of a slide. This drop of blood was spread with the corner of a clean slide until uniformly thickened.

On a second slide, a second drop of blood was gently placed at one end of the slide. Holding the slide in one hand, the edge of the ground slide was placed just in front of the blood drop with the other hand, then slid until it touched the blood drop. The blood was then spread all along the edge of the ground slide. With a gentle, even movement, the ground slide was pushed to the end of the smear slide. The sample smears were then dried at room temperature, before being stored in storage boxes for Giemsa staining and reading under a microscope. Rapid diagnostic tests were performed on all samples. From each RDT-positive sample, three drops of 50µL of blood each were collected on filter papers or confetti for the search for molecular markers. Each sample collected on confetti filter paper was packed in a plastic bag with silica gel to protect it from moisture.

4.1.1. DNA Extraction from Blood Samples Collected on Filter Papers Using Qiagen automated DNA Extraction Machine

Blood samples collected on filter papers (confetti) are first scanned on a computer in barcode order and in batches of 96 samples according to the test plates containing 96 reaction wells.

-DNA extraction machine or DNA extraction machine (DNA extraction machine).

4.1.2. Procedure

The filter papers containing the blood sample drops were cut using a scissor punch previously treated with 70% methanol and flamed with a Bunsen burner for each sample. The filter papers containing the blood samples were cut into circles with a diameter of 2 mm. Each sample (small circle) was collected in a well of a reaction microplate containing 96 samples. A volume of 180 µL of DNA extraction buffer solution and 20 µL of protein kinase K enzyme were added to each well corresponding to a sample.

The microplate was then placed in a thermo-mixer for 15 min at 56°C and 900 rpm. The reaction microplate was then transferred to the DNA extraction room. The reagents were placed in the Qiagen automated DNA extraction machine. The reagent and consumables boxes were opened successively. The reagents and consumables were placed in the corresponding boxes. The operation was validated by pressing the scan button. The eluent box was opened, along with a new 96-well reaction microplate intended to collect the DNA from the samples after extraction. The wash solution collection reservoir was checked and emptied, if necessary, then scanned.

DNA extraction was performed in the 96-well microplates for 4 hours after the introduction of all the necessary process elements. The DNA extraction products were then collected in a new microplate before being brought to the PCR room.

4.2. Working Method

4.2.1. Study Design

The workflow of this research was designed. The starting point of this work was the collection of blood samples in the CPS areas of Guinea, according to the approval of the Ethics Committee for Science and Research in Guinea. A total of 4352 blood samples were collected in the community and health centers. All participants underwent the rapid diagnostic test (RDT). Subsequently, these samples were analyzed by microscopy (thick drop and blood smear), this allowed us to establish the parasite density and to know the Plasmodium species involved. Molecular methods were applied to all positive thick drop samples.

Then we collected three drops of 50µL of blood each from the same microscopic-positive samples on filter papers or confetti for the search for molecular markers. From the blood drops collected on filter paper we extracted genomic DNA for each sample using Nucleospin Blood mini kit. Then all samples were subjected to a DNA amplification technique.

4.3. Primer sequences and reaction conditions for PCR and nested PCR for Pfdhfr and Pfdhps genes.

For the Pfdhfr gene, the primers used for the detection of SNPs in codons 50, 51, 59, 108, and 164 were: forward primer (5'-TTTATGATGGAACAAGTCTGC-3') and reverse primer (5'-CTAGTATATACATCGCTAACA-3'); the PCR product size was 650 bp. The amplification conditions used were: 95°C for 5min, 94°C for 30s, (54°C for 60s, 65°C for 60s) x 41 cycles; 65°C for 5min, 15°C for 5min. Nested F (5'-CTGGAAAAAATACATCACATTCATATG-3') and Nested R (5'-TGATGGAACAAGTCTGCGACGTT-3') and the amplification product size was 594bp. The amplification condition: 95°C for min, (93°C for 30s, 68°C for 75s) x 30 cycles, 75°C for 5min.

For the Pfdhps gene amplified for the detection of SNPs in codons 50, 51, 59, 108 and 164, the primers used were Forward primer (5'-GATTCTTTTTCAGATGGAGG-3') and Reverse primer (5'-TTCCTCATGTAATTCATCTGA-3') for an amplified product size of 770 bp. The amplification conditions were: 93°C for 5min, (93°C for 20 seconds, 55°C for 30 seconds, 68°C for 75 seconds) x 40 cycles; 68°C for 5min, 15°C for 5min; Nested F primer (5'-AACCTAAACGTGCTGTTCAA-3'), Nested R (5'-AATTGTGTTGATTTGTCCACAA-3'). The amplified sequence size was 711bp with the amplification conditions were 93°C for 5min, (93°C for 30 seconds, 56°C for 30 seconds, 68°C for 75 seconds) x 30 cycles, 72°C for 5min, 15°C for 5 min.

In these techniques we used plasmids like, Puc-18-Pfdhfr-TM/8.2 containing wild type Plasmodium falciparum as negative control and Puc-18-Pfdhps-V1/S containing mutant type Plasmodium falciparum as positive control.

4.3.1. Ethical Considerations

Before initiating data collection and analysis, our research protocol was submitted to the Ethics Committee of the Directorate of the Doctoral School of Life Sciences, Health and Environment at Gamal Abdel Nasser University in Conakry. The research team guaranteed the confidentiality of the information collected and the anonymity of the study participants.

4.3.2. Limitations and Challenges

The high cost of materials and reagents, and the high travel costs for analyzing our samples in molecular biology laboratories were some of the challenges we encountered.

5. Results

The application of the Plasmodium falciparum research methodology resulted in the following results, presented in the form of tables, figures, and graphs. These results were interpreted, commented on, and discussed based on literature data.

5.1. Biological diagnosis of Plasmodium falciparum using the different methods used

Table 1 Determination of sample size according to age groups

N°	Age groups (years)	Number	Percentage
1	< 5years	2176	50
2	5 years and older	2176	50
Total		4352	100

In total, a total of 4,352 people were included in our study, including children under 5 years old (2,176) and individuals aged 5 years and over (2,176), i.e. 50% on both sides.

5.2. Biological diagnosis of Plasmodium falciparum by the different methods used

Table 2 Determination of the prevalence of Plasmodium falciparum in the subjects surveyed according to the rapid diagnostic test (RDT)

N°	TDR	Number	Percentage
1	Positive	222	5,10
2	Negative	4130	94,89
Total		4352	100

Table 2 shows that out of a total of 4352 people tested by the rapid malaria diagnostic test (RDT) method, we obtained 222 positive cases for Plasmodium falciparum (5.10%) against 4130 negative (94.89%).

Table 3 Determination of the prevalence of Plasmodium falciparum in subjects surveyed by thick drop

N°	Thick drop	Number	Pereengage
1	Positive	255	5,85
2	Negativet	4097	94,14
Total		4352	100

After carrying out the thick drop we found 255 positive people (5.85%) against 4097 negative people (94.14%).

Table 4 Determination of the surveyed prevalence of Plasmodium falciparum in the subjects based on thin smear

N°	Thin smear	Number	Percentage
1	Positive	255	5,85
2	Negative	4097	94,14
Total		4352	4352

In this table we see a concordance between the results of the thick drop and those of the thin smear: 5.85% positive versus 94.14% negative.

Table 5 Prevalence of Plasmodium falciparum by PCR method in the 255 subjects positive by thick smear

N°	PCR	Number	Percentage
1	Positive	246	96.47
2	Negative	9	3.52
Total		255	100

Table 5 explains that after PCR in 255 samples positive by thick drop, 246 were found to be positive for Plasmodium falciparum DNA (246/255 or 96.47%). Thus, extrapolation of the PCR results to all 4352 subjects included in the study shows that 5.65% of the subjects were found to be positive for Plasmodium falciparum DNA.

Table 6 Distribution of the prevalence of Plasmodium falciparum in the subjects surveyed according to age groups after PCR

N°	Age groups (years)	Number	Percentage
1	< 5 years	118	47,96
2	Plus, de 5 years	128	52,03
Total		246	100

This table indicates that following PCR, 118 children under 5 years old (47.96%) were positive carriers of plasmodium falciparum compared to 128 individuals over 5 years old, i.e. 52.03% also carriers of plasmodium falciparum.

6. Discussion

In total, a sample of 4,352 people was included in our study, including children under 5 years old (2,176) and individuals over 5 years old (2,176), i.e., 50 percent on both sides. Our results are comparable to those of Beshir et al., (2023) who tested 29,274 children under 5 years old and individuals aged 10 to 30 years old in 2016 and 28,546 children under 5 years old and individuals aged 10 to 30 years old in 2018 from seven countries in the Sahel region of Africa [15].

We tested the 4352 people by the rapid malaria diagnostic test (RDT) and we obtained 222 positive cases for plasmodium falciparum (5.10%) against 4130 negative (94.89%). Our results are lower than those of Tarama et al., (2023) who found a malaria prevalence by RDT of 29.1% (338/1134) against 33.7% (382/1134) for microscopy in 2023 this difference could be explained by the size of the samples tested on both sides or better Guinea has been using SMC since 2015 and is a means of reducing the prevalence of malaria in Guinea [16].

After performing the thick drop and thin smear, we found 255 positive people (5.85%) against 4097 negative people (94.14%). Our results are lower than those of [17] who found in their study in children aged 0 to 5 years a prevalence of 305/320 or 95.31% against 4.68% of negative children in 2021 (64). Our results are also lower than those of Tarama et al., (2023) who found by microscopy 33.7% (382/1134) of positive cases in 2023 [16].

After PCR, out of 4353 samples, only 246 were found to be positive for Plasmodium falciparum DNA (246/4352) or 5.65%, compared to 94.34% (4106/4352) of non-carriers of Plasmodium falciparum. Our results are lower than those reported by Beshir et al., (2023) who found an overall prevalence of malaria by PCR in children under 5 years and individuals aged 10 to 30 years respectively 17.5% in 2016 compared to 7.6% in 2018 [15].

The PCR results (Table 6) indicate that 118 children under 5 years old (47.96%) were carriers of Plasmodium falciparum compared to 128 individuals over 5 years old, i.e. 52.03% also carriers of Plasmodium falciparum. Our results are superior to those of Beshir et al., (2023) who, in their study published in 2022, found prevalences in each country among children under 5 years of age in 2016 for Gambia 51/1752 (2.9%), Guinea 418/1996 (20.9%), Mali 704/2189 (32.2%), Burkina Faso 161/2281 (7.1%), Niger 725/2146 (33.8%), Nigeria 601/1786 (33.7%) and Chad 184/2195 (8.4%) and among individuals aged 10 to 30 years in Gambia 85/2012 (4.2%), Guinea 305/1893 (16.1%), Mali 585/2176 (26.9%), Burkina Faso 65/2275 (2.9%), Niger 570/2317 (24.6%), Nigeria 495/2062 (24.0%) and Chad 181/2194 (8.2%) [15]. These authors in their 2022 publication demonstrated having found in 2018 in children under 5 years of age different prevalence of Plasmodium falciparum in seven African countries including Gambia 53/1383 (3.8%) Guinea 128/2182 (5.9%) Mali 154/2156 (7.1%) Burkina Faso 73/2264 (3.2%) Niger 143/1783 (8.0%) Nigeria 224/2110 (10.6%) and Chad 26/2141 (1.2%) and in individuals aged 10 to 30 years including in Gambia 62/2159 (2.9%) Guinea 134/2191 (6.1%) Mali 322/2106 (15.3%) Burkina Faso 106/2168 (4.9%) Niger 232/2586 (9.0%) Nigeria 391/1770 (22.1%) and Chad 128/1547 (8.3%) [15].

According to data from the World Health Organization, approximately 247 million cases of malaria occurred worldwide in 2021 with 619,000 deaths [18]. The World Health Organization (WHO) African Region estimates that approximately 234 million cases of malaria occurred in Africa, representing approximately 95% of global cases, and that deaths recorded in Africa accounted for approximately 96% of malaria-related deaths [18]. Approximately 76% of all malaria-associated deaths in 2021 occurred in African children under five years of age [18]. In 2020, Burkina Faso was identified by WHO as a "high burden, high impact" (HBHI) country. Despite efforts to accelerate the reduction of malaria incidence and mortality through prevention, diagnosis, and treatment, the decline in malaria incidence is slow. Malaria continues to pose a public health threat as it remains the leading cause of consultation (37.2%) in Burkina Faso [19].

It should be noted that the incidence of malaria varies in the country according to the epidemiological facies and is higher in children under 5 years of age (1237‰) than in the general population (568‰) [19]. Malaria is permanent in the southern and southwestern regions. This disease is seasonal with a long duration of 4 to 6 months in the center and a short duration of 2 to 3 months in the north of the country. More than 12 million cases of malaria have been identified in Burkina Faso, including 605,504 cases of severe malaria and 4,355 deaths in 2021 [19]. Anti-malarial drugs are important components of malaria control and prevention programmes [20]. Tey are used in the prevention of the disease through chemoprophylaxis and in curative treatment to prevent the progres sion of severe disease [20].

7. Conclusion

These results show that malaria diagnosis by microscopy remains the reference method; therefore, the rapid diagnostic test serves as a quicker means of diagnosis. Moreover, malaria diagnosis by the molecular method (PCR) is costly, and the RDT is less sensitive compared to microscopy. The latter can be beneficial and more convenient for conducting malaria screening in large populations, especially in rural areas lacking equipment or electricity.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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