DETERMINATION OF PLASMODIAL SPECIES IN PATIENTS RECEIVED AT COTONOU BONI CLINIC DURINGRAINNING SEASON IN 2022

Comment [AK1]: RAINY

Abstract :

Introduction: Malaria is a life-threatening disease caused by parasites transmitted by bites from infected female anopheles. It is a preventable and treatable illness. It remains a recurring disease among public health diseases that exposes many people to a risk of infection, including children under the age of 05 in Benin.

Methods: To determine the prevalence of malaria and different plasmodial species at the Dr Pierre BONI Clinic, we performed venous and capillary samples on 731 patients for the realization of thick drops and blood smears between June and July 2022.

Results: Three plasmodial species were identified in 228 patients identified as malaria: *Plasmodium falciparum* (95.5%), *Plasmodium malariae*(2.85%), *Plasmodium ovale*(1.65%). We note double species in some patients namely: *Plasmodium falciparum+ Plasmodium malariae* and *Plasmodium falciparum+ Plasmodium ovale.* The majority of patients have the presence of trophozoites at Plasmodium falciparum, *95.5%*. The parasitic density of *P. falciparum* is higher than that of *P. malariae*and that of *P. ovale.*

Conclusion: Although evaluated during a period of low transmission, malaria remains a real public health problem. The distribution of the disease is closely related to the presence in the blood of plasmodial species.

Keywords: Malaria, *Plasmodium ovale, Plasmodium falciparum, Plasmodium malariae,* Cotonou.

INTRODUCTION

Malaria remains a major cause of disease and mortality in the majority of tropical areas. It is endemic in 106 countries around the world. It is an endemic-epidemic parasitosis. In 2020, out of a total of 241 million malaria cases compared to 229 million in 2019, the annual number of malaria-related deaths, estimated at 627,000, is observed in Africa, mainly affecting children under five (86%) (WHO, 2021). It is characterized by the presence in the body of a unicellular parasite (protozoa) of the genus *Plasmodium*, transmitted to humans by

the bite of the female of a haematophage mosquito of the group of infected anopheles vectors associated with clinical signs. Plasmodium *infects the* liver cells of infected subjects and then circulates in the blood by colonizing the red blood cells and destroying them. It is *caused by five parasitic species of the genus* Plasmodium*: Plasmodium falciparum* (Alphonse, 1880), *Plasmodium vivax* (Grassi&Feletti, 1890), *Plasmodium ovale* (Stephens, 1922), *Plasmodium malariae*(Feletti&Grassi, 1889) and *Plasmodium knowlesi* (Sinton & Mulligan, 1933). Two of these five species of plasmodia responsible for human malaria are particularly dangerous: *P. falciparum,* the parasite causing the most death which is also the most widespread on the *African continent, and* P. vivax, the dominant species in most countries outside sub-Saharan Africa (WHO 2020).

In Benin, malaria represents 44.2% of the causes of health care use in health facilities and is the major disease affecting communities (SNIGS/MS, 2020). Based on 2021 data, 45.5% of visits and hospitalizations were related to this condition. 2,521,966 isolated cases of malaria have been reported with 197,642 serious cases. The number of deaths in 2021 is 3,509 (SNIGS/MS, 2021). Indeed, in areas endemic to malaria in Africa, the majority of clinical malaria attacks are attributed to *Plasmodium falciparum.* While *P. malariae* and *P. ovale* are reported to be widely distributed throughout Africa and other endemic regions around the world. Their epidemiology remains much less studied than that of *P. falciparum.* Although generally considered benign, *P. malariae*and *P. ovale* have the potential to cause significant morbidity (Razafindrakoto 2004).

It is for this purpose that we have taken an interest in this study, carried out at the BONI clinic in Cotonou with the aim of evaluating the profile of plasmodial species in patients with malaria in the said clinic in rainy periods.

MATERIAL AND METHODS

Framework for the study

Our study was conducted at the Akpakpa district of the Dr Pierre BONI Clinic (6°21'55.68'N 2°27'10.07'E). It is located in the municipality of Cotonou at PK2, Porto-Novo road on the right. It is next to the SociétéBéninoise de Brasserie (SOBEBRA) and opposite the Cid Super

Décor. This study is a descriptive cross-sectional study based on direct observations of the Thick Drops slides of patients in the clinic.

Sampling

Our study population consisted of 731 people suspected of malaria (fever with or without other symptoms) regardless of age or sex. Thus, patients who went to the laboratory of the Dr Pierre BONI clinic for an examination of Gout Epaisse/Blood smear were included in the study.

Biological material

The biological material used was blood. An Olympus® CX23 optical microscope (Olympus, Granges, Switzerland) was used to read colour slides at Giemsa (Cypress Diagnostics, Hulshout, Belgium).

Analysis of blood samples

We possessed at a venous sample on EDTA tube and then we made a thick drop and a blood smear on a holder blade. The prepared blades were then dried, fixed and coloured in Giemsa (Cypress Diagnostics, Hulshout, Belgium) diluted to 1/10th for 10 minutes. The reading was done using the Olympus® CX23 optical microscope (Olympus, Granges, Switzerland) at the X100 lens with a drop of immersion oil. When a parasite is identified in the microscopic field, the result is positive. If, on the contrary, after covering 100 microscopic fields with no parasites, the examination is considered negative.

Statistical analysis

The data was analyzed by the chi-square and Kruskall Wallis test using Microsoft Excel 2016 software. Results are expressed in mean standard deviation form. The variations were considered significant at 5%

RESULTS

- **Distribution of the population by sex**

731 people have been diagnosed with malaria in the study period. The figure below shows the distribution of this population by sex.

Figure 1 shows that men are in the majority with 52.8% compared to 47.2% for women. The sex ratio is 1.11 in favor of the male sex.

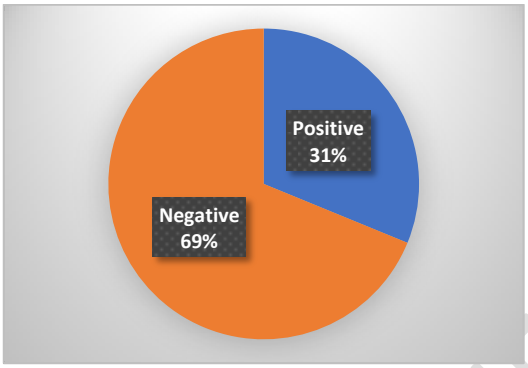


Figure 1: Distribution of subjects examined by sex.

Age distribution of the study population.

Figure 2 shows the age distribution of subjects examined. This breakdown shows that patients aged [20-30[years are 21.5%; those aged [30-40[years are 21.05%; those aged [10-20[years are 18.86%; those aged [0-10[years are 15.35%; those aged [40-50[years are 12.72%; those aged [50-60[years are 7.89%; and those aged [60-90]years are 2.63%. Note that the most represented range is [20-30[years.

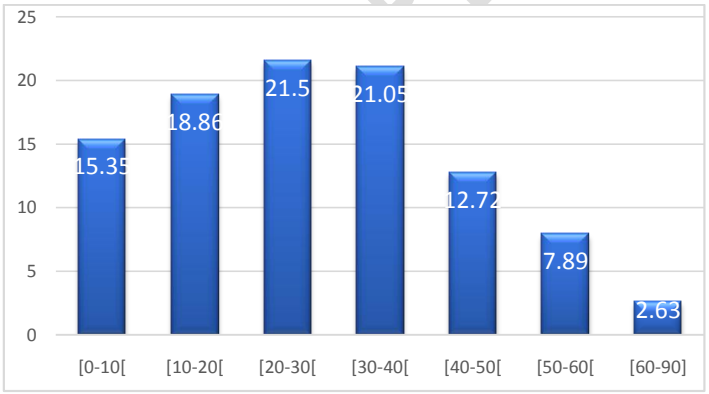


Figure 2: Age distribution of patients examined.

- **Distribution of patients by percentage of positive and negative cases**

Figure 3 shows the proportion of positive and negative cases. Of the 731 patients received, 228 were diagnosed with thick-drip. Thus, patients diagnosed with malaria accounted for 31.19% and patients diagnosed with no malaria accounted for 68.81%.

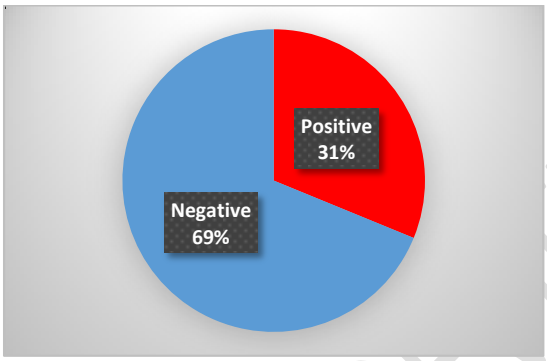
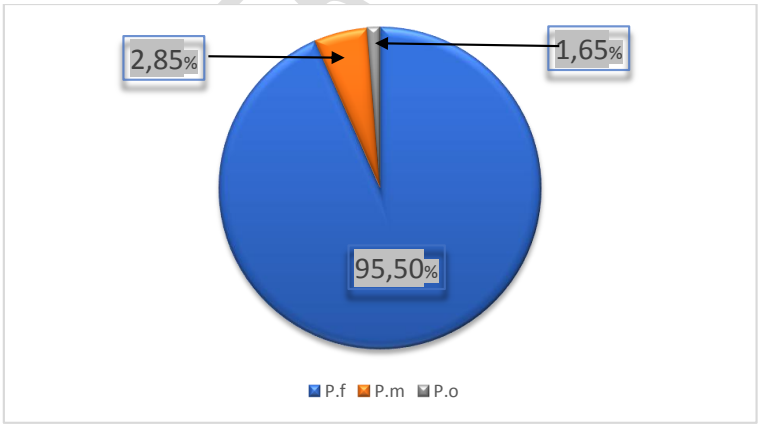


Figure 3: Proportion of positive and negative cases

- **Proportion of *Plasmodium species* identified**

The proportion of *Plasmodium species* identified is shown in Figure 4. Of the 228 malaria patients, three *Plasmodium*species were identified. Then *Plasmodium falciparum* is majority with a percentage of 94% against 5.7% for *Plasmodium malariae*and 1.33% for *Plasmodium ovale.*



Legend: *P f: Plasmodium falciparum, P m: Plasmodium malaria, P o: Plasmodium ovale*

Figure 4: Distribution of subjects by identified *Plasmodium* species

- **Distribution of *Plasmodium* speciesby age group**

The table below - shows the distribution of *Plasmodium* species by age group.From this table it can be seen that the [20-30[year old is most affected by *Plasmodium falciparum* with a percentage of 21.5% while the one most affected by *Plasmodium malariae* is in the age range of [0-10[years and those most affected by *Plasmodium ovale*is in the age range [10-20[

TableI: Distribution of Plasmodium species by age group.

Age (years)	*P. f* (%)	*P. m* (%)	*P/O* (%)	*P. f +P. m* (%)	*P. f +P. o* (%)
[0 ; 10[	35(15,35)	4(1,75)	00(00)	4(1,75)	00(00)
[10 ; 20[	43(18,86)	3(1,32)	2(0,88)	3(1,32)	2(0,88)
[20 ; 30[	49(21,50)	3(1 ,32)	00(00)	3(1,32)	00(00)
[30 ; 40[	48(21,05)	2(0,88)	00(00)	2(0,88)	00(00)
[40-50[	29(12,72)	00(00)	00(00)	00(00)	00(00)
[50-60[	18(7,89)	00(00)	1(0,44)	00(00)	1(0,44)
[60-90]	6(2,89)	1(0,44)	00(00)	1(0,44)	00(00)
TOTAL	228(100)	13(3,64)	3(1)	13(3,64)	3(1)

Legend: *P f: Plasmodium falciparum, P m: Plasmodium malaria, P o: Plasmodium ovale*

- **Distribution of *Plasmodium* species by sex**

Table II below shows the distribution of *Plasmodium* species by sex.This table shows that out of all 228 positive cases, the male sex is the majority at the level of *Plasmodium falciparum* and *Plasmodium malariae*with respectively 116 cases out of 228 representatives 50.87% and 8 cases out of 13 representatives 3,51%, while at the level of *Plasmodium ovale*it is the female sex that dominates with 2 cases out of 3 representatives 0.88%.

TableII: Distribution of Plasmodium species by sex

Sex	*P. f* (%)	*P. m* (%)	*P/O* (%)	*P. f + P. m* (%)	*P.f +P. o* (%)	Total
Masculine	116(50,87)	08 (3,51)	1(0,44)	08 (3,51)	01 (0,44)	125
Feminine	99 (43,42)	05 (2,19)	2(0,88)	05 (2,19)	2(0,88)	106

Legend*: P f: Plasmodium falciparum, P m: Plasmodium malariae, P o: Plasmodium ovale*

- **Different values of parasitic densities (DP) according to *Plasmodium*species**

Table III shows the distribution of PD by *Plasmodium* species. From this table it appears that at the level of*P. falciparum*, parasitic densities vary from 7 to 929578 Parasites/µL of blood with a median of **464796** P/µL. At the *P. malariae* level, parasitic densities range from 5626 to 107658 Parasites/µL of blood, the median being 56652 Parasites/µL and at the *Plasmodium ovale* level, parasitic densities range from 13088 to 60878 Parasites/µL of blood with a median of 36983 Parasites/µL of blood. The mean parasitic density of *P. falciparum* is higher than that of *P. malariae* and *P. ovale.*

TableIII: Degrees of Infestation by *Plasmodium* Species

Plasmodium species	Positive topics	Scope of the RFP	Median
P. falciparum	228	7 to 929578 P/µL	464796P/µL
P. malariae	13	5626 to 107658P/µL	56642P/µL
P. oval	3	13088 to 60878P/ µL	36983P/µL

Caption*: P. falciparum: Plasmodium falciparum, P. malariae: Plasmodium malariae, P. ovale: Plasmodium ovale*

DISCUSSION

This study, conducted in the laboratory of the Dr Pierre Boni clinic in Cotonou, determined the frequency of *Plasmodium* species identified in malaria patients from June to July 2022. In our study, 731 patients were diagnosed with malaria, 228 of whom were positive, a prevalence of 31.19%. Indeed, this number of patients received is higher than that of Olafa who had received 421 patients of which 150 are positive in three months in the same clinic (Olafa, 2020), on the other hand, the prevalence we obtained is much lower than that of Boko in 2021, that is 82.56%. This difference could be explained by the study period. We observed that men were in the majority with a percentage of 52.80% versus 47.20% and the sex ratio was 1.11 in favour of the male sex. Similar rates to ours were observed by Boko in 2021 with 54.92% male versus 47.67% female, Sall in 2006 with 58.6% male, Niambélé in 1999 with 55% male and Doumbia with 53.6% male in 2012 in the Gabriel Touré CHU pediatric ward (Pierre *et al.*, 2019, Lehmann *et al.*, 2008). In addition, people in the age group [20-30[years are most received for the diagnosis of malaria with a percentage of 21.5%. The minimum age of patients was D1 and the maximum age was 90. In characterizing the positive cases obtained, men were the majority with 50.67% of positive cases compared to 49.33% of women, but no link was found between sex and the positive character of malaria. These results could be explained by a wider distribution of men. However, our results differ from those obtained by Olafa at the Dr Pierre Boni clinic, which shows a clear predominance of the female sex with 56.67%. These results could be explained by the study period and by the sample size.

Three plasmodial species as well as patients with double species were identified in the 228 patients tested positive on microscopic examination of the thick gout and blood smear: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale*and double species *(Plasmodium falciparum* associated with *Plasmodium malariae)* and *(Plasmodium falciparum* associated with *Plasmodium ovale)* with a high prevalence of *95.5% Plasmodium falciparum.* Indeed, these results we obtained are similar to the results obtained by Atchade in 2014 who had worked on the blood bags (2515 blood bags) collected in three Departmental Blood Transfusion Centers (CDTS) in Benin: Atlantic-Littoral, Ouémé-Plateau and Mono-Couffo. He found three species of *Plasmodium: Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale* and mixed infections *(Plasmodium falciparum* associated with *Plasmodium malariae) and (Plasmodium falciparum* associated with *Plasmodium ovale)* with a high prevalence of *Plasmodium falciparum* at 95.0%. Similarly, these results are similar to

the results obtained by Damien and colleagues in 2010. They worked on the samples of asymptomatic children and sick children and also identified three plasmodial species namely: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale*and mixed infections (*Plasmodium falciparum* associated with *Plasmodium malariae), (Plasmodium falciparum* associated with *Plasmodium ovale)* and (*Plasmodium falciparum* associated with *Plasmodium malariae*and *Plasmodium ovale*) only as the annual prevalence rate of *Plasmodium falciparum*infection increased to 21,80% is much lower than ours. This prevalence *ofPlasmodium falciparum*may be explained by the fact that*Plasmodium falciparum*is the most widespread and dangerous species in the world. Persons whose age was in the [20-30[years old made the large number of our study being 21.50% for*Plasmodium falciparum,* people whose age was in the [0-10[years old made the large number of our study being 1.75% for*Plasmodium malariae*, people whose age [10-20[years have made the plurality of our study: 0.88% for *Plasmodium* ovale on a total of 95.50% of species of*Plasmodium falciparum*,2.85% of*Plasmodium malariae*and 1.65%*Plasmodium ovale. Plasmodium malariae* was not identified in individuals aged 50 to 90 years. Ces résultats sont semblables à ceux obtenus par Yman et ses collaborateurs en 2019 en Tanzanie qui ont trouvé 90% d'espèces de *Plasmodium falciparum* et 10% de *Plasmodium malariae*. Indeed, *Plasmodium malariae*differs from the other species in that it has a longer incubation period (15-20 days) and, above all, in its ability to cause very late outbreaks.

CONCLUSION

At the end of this study, we identified three species and then associations of species, *Plasmodium falciparum* and *Plasmodium malariae, Plasmodium ovale, (Plasmodium falciparum* associated with *Plasmodium malariae)* and *(Plasmodium falciparum* associated with *Plasmodium ovale*) with a high prevalence of *Plasmodium falciparum* malaria (95.50) in the patients. The prevalence of malaria in patients was estimated at 31.19%. These results highlight the need to carefully monitor the prevalence of all species of the genus *Plasmodium.* So elimination of malaria requires accurate detection of infection and discrimination of *Plasmodium* species using highly trained microscopists, good quality microscopes and improved tools for effective surveillance of all *Plasmodium* species.

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