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The Malaria Parasite Species Composition in Clinical and Asymptomatic Infections Among Children Under the Coverage of Seasonal Malaria Chemoprevention in the Health District of Nanoro, Burkina Faso

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Abstract: Introduction: Despite the deployment of different strategies for malaria control, the disease remains a public health problem in sub-Saharan Africa. Most of the studies are focused on *Plasmodium falciparum*, the predominant species in Africa, with a lack of data on other *Plasmodium* species; yet, considering them in the strategy of interventions is crucial for malaria elimination. This study aims to determine the cumulative number of malaria species, the effect of infection status on parasite density, and the infection type in the Nanoro area, Burkina Faso. **Methods:** Data from 2020 to 2023 were collected in asymptomatic children under seasonal malaria chemoprevention (SMC) and clinical cases from health centers. Malaria diagnosis was conducted via microscopy, and statistical models were applied to evaluate infection status, age, gender, and hemoglobin levels. **Results:** A total of 5726 malaria episodes were diagnosed in 1996 children including 1263 asymptomatic and 733 clinical cases. *P. falciparum* was mostly represented regardless of infection type, followed by *P. malariae* and *P. ovale*. Host age significantly affected infection outcome (p = 0.0001). Furthermore, infection type was influenced by infection status, and clinical infections were mostly observed in mono-infected individuals with *P. falciparum,* while the infections tended to be asymptomatic in mixed infection. **Conclusion:** This study confirmed that *P. falciparum* remains the major malaria species in clinical and asymptomatic infections. Children with *P. falciparum* mono-infection exhibited higher parasite densities than in mixed-infected individuals, but this effect varied with infection type. However, further studies are needed to deeply investigate the effect of these mixed infections with different malaria species in human hosts.

Keywords: malaria; *P. falciparum*; *P. malariae*; *P. ovale*; asymptomatic infections

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1. Background

Malaria remains a major public health problem in Africa despite global efforts over the past decades to control the disease [1]. According to the World Health Organization (WHO), the number of malaria cases in 2023 was estimated at 263 million, leading to 597,000 deaths in the world. Africa alone accounts for 94% of global cases, i.e., 246 million cases and 569,000 deaths [2]. Children aged under 5 years and pregnant women pay the heaviest cost in terms of morbidity and mortality [3].

Malaria is seasonal in Burkina Faso with a peak occurring between July and November and varying in length and intensity across the three major geographic zones due to differences in the rainy season [4]. Despite the combined efforts of the local government and its international partners to mitigate the burden of the disease, the annual malaria incidence remains stubbornly high across the country. Routine data from the Ministry of Health (MOH) show 11,656,675 reported malaria cases and 4335 deaths in 2022. The latter was estimated at 2925 in children under 5 years of age [5]. Following global objectives to eliminate malaria by 2030, National Malaria Control Programs (NMCPs) in Africa focus their interventions mainly on prevention and case management strategies [6]. Among these strategies is seasonal malaria chemoprevention (SMC), specifically targeting children aged between 3 and 59 months in endemic areas. SMC has been adopted in Burkina Faso since 2014 and consists of a monthly administration of four full courses of sulfadoxine-pyrimethamine + amodiaquine (SP + AQ), to children aged between 3 and 59 months living in high-seasonal-transmission areas [7]. In addition, the diversity of plasmodial species plays a crucial role in the dynamics of the disease and influences control and prevention strategies.

Human malaria is caused by several plasmodial species: *P. falciparum*, *P. vivax, P. malariae*, *P. ovale spp*, and *P. knowlesi*. In sub-Saharan Africa, *P. falciparum* represents the major species responsible for severe malaria leading to deaths. However, other species known as minor or neglected in Africa are generally considered infrequent among clinical malaria cases. These species include *P. malariae* and *P. ovale* spp. [8]. The latter two species are rare in mono-infection and are mainly observed in mixed infections with *P. falciparum*. In a context where many efforts are being made to control and eliminate malaria, it is important to gain more insight into the epidemiology of these minor species as well as the major malaria species *P. falciparum* and their contribution to the malaria burden. In Burkina Faso, data on these minor species were limited to the estimation of their prevalence in the human population, specifically in children under the age of five [9,10].

In recent years, several studies have reported a rise in interest in these minor species as well as an increase in their prevalence over time in sub-Saharan Africa [11–13]. In addition, they are commonly found in asymptomatic infections with low parasitemia and are most widely co-infected with *P. falciparum* [14,15]. Asymptomatic infections are known to lead to silent transmission of the parasite because they are neither detected nor treated, yet they continuously maintain the transmission cycle, jeopardizing the effectiveness of strategies implemented to control malaria.

Of particular interest is the fact that in mixed infections, different malaria species share the same environment (the host and its immune response) and use the same resource (the host red blood cells) and, in such a context, the intrinsic growth rate of each species can be limited by the presence of other species, that is, within-host competition occurs [16,17]. Indeed, the driving factors of the predominance of any particular malaria species in the case of mixed infection are poorly assessed. Furthermore, the effect of the co-existence of different malaria species in the symptomatology of the disease is hitherto unknown. In Burkina Faso, besides *P. falciparum*, the predominant species causing severe forms of malaria, *P. malariae* and *P. ovale*, are also observed and commonly in co-infection with *P. falciparum,* yet limited data exploring the effect of these co-infections within the human host are available. Therefore, this study aimed to assess the cumulative number of malaria species, the effect of infection status (mono versus mixed infection) on parasite density, and infection type (clinical versus asymptomatic) in children under SMC coverage in the Nanoro health district in Burkina Faso.

2. Materials and Methods

2.1. Study Area

This study was embedded to two major projects carried out in the health District of Nanoro (Burkina Faso) and aimed to improve the impact of seasonal malaria chemoprevention through simultaneous nutrient supplementation (SMC-NUT, N°: 2020-00639) [18] and through the simultaneous screening and treatment of other household members of children receiving SMC (SMC-RST, N°: 2021-03-059) [19]. The health district of Nanoro (HDN) is located in the Central-western region of Burkina Faso, at approximately 85 km from Ouagadougou, the capital city. This rural area is characterized by an alternance of two distinct seasons: a rainy season from June/July to October/November during which malaria transmission is high, followed by a long dry season from November to May [20].

2.2. Study Design and Data Collection

Data and samples for this study were collected between 2020 and 2023 during the SMC-NUT and SMC-RST studies. Descriptions of the protocols of the two studies have been previously detailed elsewhere [18,19]. Included children were assessed through monthly home visits. In the event of illness, parents were invited to bring their children to the health center, and this was considered an unscheduled visit.

Monthly and unscheduled visit datasets from SMC-NUT and SMC-RST participants were used for the present study. The data from participants who experienced at least one episode of malaria infection over the study period were used. Two groups were defined regarding the infection type (clinical or asymptomatic). The asymptomatic group comprised individuals with positive parasitemia detected only during the monthly home visits without any symptoms and with any malaria-related healthcare attendance throughout the study period. The clinical group was individuals who attended the health facilities at least once (unscheduled visit) to seek care with RDT and/or microscopically confirmed malaria diagnosis. Similarly, participants were classified into two groups regarding *Plasmodium* species infection status as mono-infection or mixed infection according to the number of malaria species detected on the thin film by light microscopy.

2.3. Determination of Species and Estimation of Parasite Density

Malaria slides (thick and thin) were prepared from peripheral blood obtained from a finger prick. Thin smears were fixed using absolute methanol. The thin fixed and thick smears were stained with 3% Giemsa for 30 min. Slides were read by two independent experienced microscopists qualified by the National

Institute for Communicable Diseases (NICD, South Africa) [21], using an Olympus CX21 microscope (Olympus Corporation, Tokyo, Japan).

Identification of *Plasmodium* species was on the thin smear and parasite density was determined in the thick blood smear. Using the following mathematical formula, the density of parasites was determined and is represented as ”parasites per microliter of blood”: The parasite count multiplied by 8000 divided by the white blood cell count equals 200 parasites per microliter. When counting 200 WBCs, the calculation based on 200 WBCs was used if more than 10 parasites per 100 fields were found. When counting 200 WBCs, if <9 parasites/100 fields were found, the count should be repeated until 500 WBCs are reached, and the calculation based on 500 WBCs should be used. When the last field was read and the parasitemia was computed using the earlier method, the count was terminated if more than 500 parasites were found without counting 200 WBCs [22]. The final parasite density was obtained by averaging the results of the two independent readers. In the case of discordant results (a negative result against a positive result; the difference in *Plasmodium* species and if the higher count divided by the lower count was $\geqslant 2$), a third laboratory technician intervened for confirmation. A smear was declared negative when the examination of 100 thick-film fields did not reveal the presence of any asexual form of the parasite.

Measurement of hemoglobin (Hb) level was performed using the analyzer HemoCue® 801+ (SOC-HE121916, Danayer group, Angelholm, Sweden). The Hb level in g/dL was read immediately after a capillary blood sample was obtained from a finger prick and drawn into the microcuvette which was inserted into the analyzer.

2.4. Ethics Approval and Consent to Participate

This study was based on two studies that received the approval of the ethics committee for health research in Burkina Faso: (SMC-NUT) N°: 2020-00639 on 23 January 2020, and (SMC RST) » N°: 2021-03-059 on 10 March 2021. The interview for informed consent with parents/guardians was confidential. An impartial and literate witness (not a member of the study staff) was present in case the parents/guardians were illiterate. The parent(s)/tutor(s) and, where applicable, the witness signed the written informed consent.

2.5. Statistical Analysis

Descriptive statistics were performed using the proportion and geometric mean (95% confidence interval) for qualitative data and parasite density, respectively. For continuous variables, the mean (standard deviation) and median (Q1–Q3) were used for normal and non-normal distributed data, respectively. To this end, hemoglobin was used to define anemia status as follows: no amenia (Hb $\geqslant$ 11 g/dL) and anemia (Hb < 11 g/dL) [23]. We also defined age groups as <2 and $\geqslant$2 years.

The cumulative numbers of *P. malariae*, *P. ovale* spp., and *P. falciparum* infections were calculated by counting the number of episodes per type of species (mono or mixed). Furthermore, binomial generalized linear models were used to assess the effect of the infection type (clinical or asymptomatic), age, gender, and hemoglobin level on the infection status (mono or mixed infection). To this end, we defined the outcome variable as infection status by classifying participants with mono-infection throughout the study period as mono-infection, while those with at least one mixed infection were categorized as mixed infection. We then performed univariate logistic regression, and variables with p-values less than 0.20 were included in multivariable analysis.

Additionally, a linear mixed model with log-transformed parasite density as the outcome was used to assess the effect of the infection type (clinical or asymptomatic) on the parasite density while adjusting for age, gender, time, and the interaction between the time and infection type.

Data were analyzed using R version 4.3 and Stata version 14 (StataCorp, College Station, TX, USA), and a *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics of the Study Population

Over the study period, 1996 children had at least one malaria episode during the follow-up (from inclusion to the last visit), including 1263 children with asymptomatic infections and 733 children who experienced clinical malaria. The median age of all children (asymptomatic and clinical) was 2.99 years (2–4.02). Both genders were almost equally represented in this study: 50.45% (females) vs. 49.55% (males). The median age of asymptomatic children was 3.16 years (2.16–4.13) with a female predominance of 51.31% (648/1263) in the corresponding group.

Concerning hemoglobin levels, 75.06% had an Hb level <11 g/dL, while 24.94% of children had an Hb level ⩾11 g/dL. The baseline characteristics of the study population are summarized in Table 1.

Table 1: General characteristics of the study population.

Variables	Overall	Infection Type	
		Asymptomatic	Clinical
Age (years)			
n	1996	1263	733
Median (Q1–Q3)	2.99 (2–4.02)	3.16 (2.16–4.13)	2.59 (1.66–3.66)
Gender			
n	1996	1263	733
Male	989 (49.55)	615 (48.69)	374 (51.02)
Female	1007 (50.45)	648 (51.31)	359 (48.98)
Hemoglobin			
n	1680	1222	458
<11 g/dL	1261 (75.06)	892 (27.00)	369 (19.43)
⩾11 g/dL	419 (24.94)	330 (73.00)	89 (80.57)

3.2. Cumulative Number of Malaria Parasite Species

During the study, a total of 5726 malaria episodes were recorded in 1996 children. *P. falciparum* was the most represented species regardless of infection type, followed by *P. malariae* and *P. ovale.* However, *P. malariae* co-occurred mostly with *P. falciparum.* The cumulative number of mixed infection of *P. falciparum* with *P. malariae* or *P. ovale* in asymptomatic infection was higher than in clinical infections (71 cases versus 15 cases). Table 2 shows the distribution of the malaria episodes in mono and mixed infections and per infection type (clinical versus asymptomatic).

Table 2: Cumulative number of malaria species per infection type.

Malaria Species	Infection Type	
	Asymptomatic	Clinical
P. falciparum	3774	1834
P. malariae	9	14
P. ovale	7	2
P. falciparum + *P. malariae*	45	10
P. falciparum + *P. ovale*	26	5

3.3. The Effect of Infection Type, Age, Sex, and Hemoglobin Level on Infection Status

Host age had a significant effect on infection status, and clinical infections were mostly observed in children with a single infection. Furthermore, infection type was influenced by infection status (mono and mixed infection) [OR = 2.38; 95%CI: 1.34–4.22] (Table 3). Clinical infections were mostly observed in mono-infected individuals with *P. falciparum,* while the infections tended to be asymptomatic in mixed infection with other malaria species (*P. falciparum* + *P. malariae* or *P. falciparum* + *P. ovale*) (Figure 1).

Table 3: Multivariate analysis of the relationship between infection type (clinical or asymptomatic), age, sex, and hemoglobin level on infection status (mono or mixed infection).

Parameters	Crude OR	95%CI	*p*-Value	Adjusted OR	95%CI	*p*-Value
Age (Years)						
<2	1			1		
⩾2	2.60	1.29–5.25	**0.007**	2.23	1.10–4.53	**0.025**
Gender						
Female	1					
Male	1.28	0.82–2.01	0.271			
Hb level						
<11 g/dL	1.32	0.74–2.35	0.344			
⩾11 g/dL	1					
Infection type						
Asymptomatic	2.63	1.49–4.65	**0.001**	2.38	1.34–4.22	**0.003**
Clinical	1					

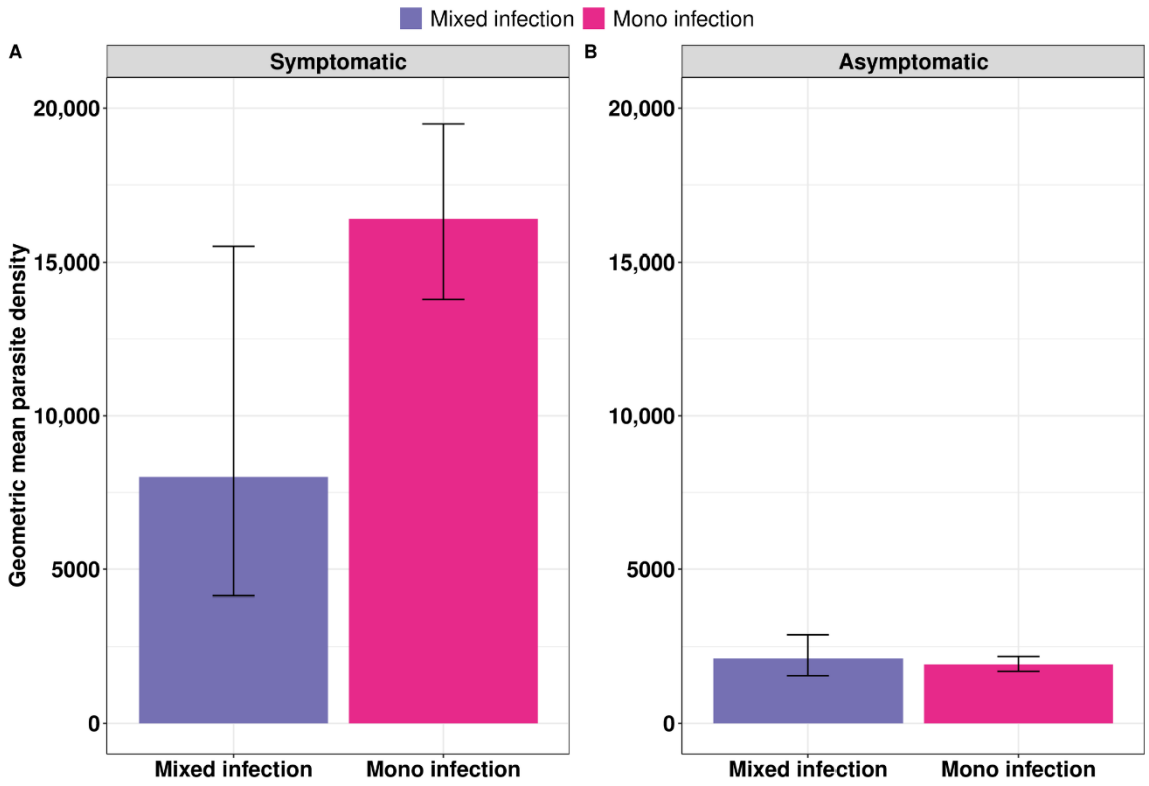

Figure 1: Relationship between parasite density (PD), infection status, and infection type. This figure shows the geometric mean for overall parasitemia in clinical and asymptomatic infections according to the infection status. Two panels showing histogram with CI representing PD in mono-infection with *P. falciparum* versus PD of mixed infection. (**A**) Symptomatic and (**B**) asymptomatic.

3.4. Relationship Between Parasite Density and Infection Type (Clinical or Asymptomatic)

Parasite density is an important determinant of infection type. The higher the parasitemia is, the more the infections tend to be clinical (Figure 2). This relationship is also highlighted in Table 4. As shown in this table, there was an 8.4% increase in the geometric mean of parasite density in clinical infections compared to asymptomatic infections [exp (0.081) = 1.084; 95%CI: 0.010–0.151] (Table 4).

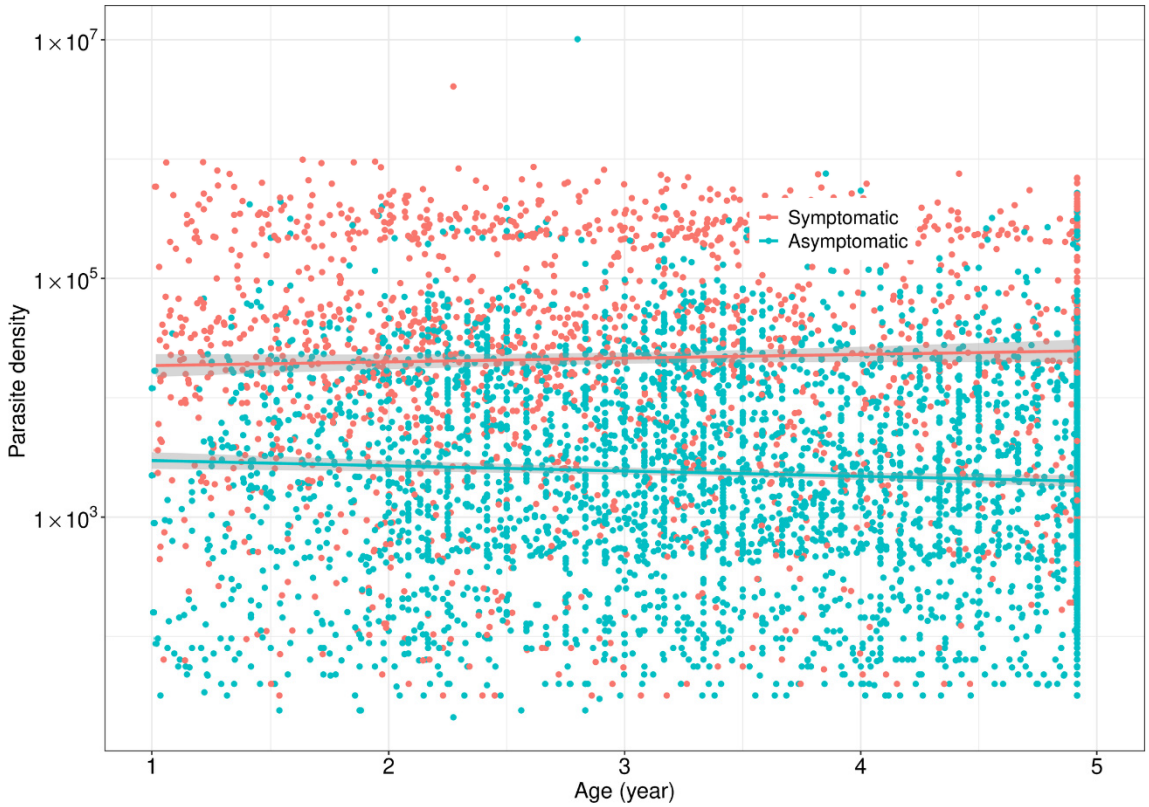

Figure 2: Relationship between parasite density, age, and infection type (the figure shows the relation between parasite density, age, and infection outcome). Age is shown on the x-axis, while parasite density is shown on the y-axis. Each point represents a PD fitted with the corresponding age with 2 curves representing each infection type (clinical versus asymptomatic).

Table 4: Effect of infection type (clinical or asymptomatic) on age, gender, and time using linear mixed model.

Characteristic	Risk Factor	Coefficient	95%CI	*p*-Value
Age (Years)	<2			
	⩾2	0.224	0.059–0.389	0.0078
Gender	Male	−0.052	−0.182–0.078	0.432
	Female	Ref		
Infection type	Asymptomatic	Ref		
	Clinical	2.043	1.829–2.258	<0.0001
Time		0.022	−0.019–0.064	0.303
Time*infection type	Asymptomatic	Ref		
	Clinical	0.078	0.005–0.146	0.034

Children harboring mixed infection exhibited lower parasite densities than children with *P. falciparum* in mono-infection, but this effect varied according to infection type (Figure 1). In clinical infections, parasite

densities in children with *P. falciparum* in mono-infection were significantly higher (16,393 parasites/μL; 95%CI: 13,791–19,487) than in mixed infection (8004 parasites/μL; 95%CI: 4135–15,496), while this difference was not significant in asymptomatic infections (Table 5).

Table 5: Relationship between geometric mean of parasite density, infection status, and infection type.

Infection Type	Infection Status	Geometric Mean	95%CI
Clinical	Mono infection	16,393	13,791–19,487
	Mixed infection	8004	4135–15,496
Asymptomatic	Mono infection	1925	1697–2184
	Mixed infection	2119	1555–2889

4. Discussion

In this study, the plasmodial species encountered in clinical infections were the same as those encountered in asymptomatic infections but their incidence differed according to infection type. Asymptomatic infections were highly frequent in this rural area of Burkina Faso. This population represents a hidden reservoir as asymptomatic individuals are unlikely to receive treatment. The detection of these asymptomatic carriers within the community remains challenging and they are usually missed. This represents a significant threat to malaria control and elimination as they contribute to the sustainability of the transmission. In such a context, community-based interventions such as SMC would help reduce the prevalence of these asymptomatic carriers within the community. However, this intervention is limited to children while older individuals are the most concerned by this asymptomatic carriage, justifying the need for interventions targeting parents and elder siblings not covered by the SMC intervention [19].

Three sympatric *Plasmodium* species have been observed, with *P. falciparum* being the most represented regardless of the infection type, followed by *P. malariae* and *P. ovale* as previously reported in studies conducted in Burkina Faso [9,10,24]. The majority of the non-*falciparum* species (*P. malariae* and *P. ovale* spp.) detected occurred in mixed infections with *P. falciparum*. The non-*falciparum* species and the mixed infections with *P. falciparum* were more common in asymptomatic infections than in clinical infections, pointing out the virulence of *P. falciparum* over the other species in inducing clinical episodes [25]. This is related to its ability to multiply in mono-infection, producing higher parasite density. Indeed, our findings show that parasite density was higher in clinical infections compared to asymptomatic infections. This means that the intrinsic growth of *P. falciparum* is impaired by the presence of non-*P. falciparum* species, suggesting a within-host competition [26]. In such a context, mixed infections would be associated with lower parasite densities and asymptomatic infections rather than clinical infections as observed in the present study. Some authors suggested that mixed infection with non-*P. falciparum* species may have a protective effect, reducing clinical outcomes compared with mono-species *P. falciparum* infections [27–29]. Indeed, previous studies reported a higher prevalence of non-*falciparum* species in asymptomatic infections than in clinical infections [12,25,30]. Similarly, the correlation between clinical episodes and higher parasitemia was previously reported [31,32].

P. malariae was the dominant species after *P. falciparum* and, most frequently, mixed infection with *P. falciparum,* suggesting that it could become the main species to target after the elimination of *P. falciparum*. However, interestingly, its presence in mixed infection with *P. falciparum* tends to mitigate the virulence of *P. falciparum,* resulting in low parasitemia and asymptomatic infection as stated above. It has also been suggested that infection with *P. malariae* can reduce the peak parasitemia from *P. falciparum,* resulting in up to 50% due to competition within the host [12,33]. A cross-protection modulated by host immunity in which the antibodies produced against one species may be functionally protective against another was suggested [34]. Therefore, this malaria species is worth particular attention including the exploration of new paths using *P. malariae* genes to control *P. falciparum,* for instance, in vaccine development.

Host age significantly affected infection type, and clinical infections were observed mainly in younger children with mono-infection in this study. This effect is attributable to the age-related acquisition

of parasite-tolerant immunity. Young children (under 5 years) whose immune systems are still in development are more susceptible to uncomplicated and severe clinical malaria. As aged individuals are repeatedly exposed to the parasite in endemic settings, they often develop partial immunity, which can lead to asymptomatic infections or less severe symptoms [35]. The clinical form of the disease is mainly limited to young children, who are likely to harbor high parasite densities that can rapidly evolve into clinical and severe malaria. Adolescents and adults, on the other hand, are partially immune and suffer far less frequently from clinical episodes, although they often still present low parasite densities [36]. Similar findings regarding the influence of host age and malaria infection were previously reported [13,15,37].

A limitation of this study was the lack of a highly sensitive diagnosis tool for the detection of species. Indeed, we relied on microscopy as a diagnosis tool for species detection as well as the estimation of parasite density, and this could underestimate the prevalence of the different species, especially in asymptomatic infections with low parasite load. A PCR-based diagnosis would have been more appropriate to detect sub-patent infections.

5. Conclusions

This study confirmed that *P. falciparum* remains the major malaria species in clinical and asymptomatic infections in Burkina Faso. Children with *P. falciparum* in mono-infection exhibited higher parasite densities than in mixed-infected individuals (*P. falciparum* + *P. malariae* or *P. falciparum* + *P. ovale*), but this effect varied with infection type. However, further studies are needed to deeply investigate the effect of these mixed infections with different malaria species in human hosts.

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Conflicts of Interest: The authors declare that they have no competing interests.

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