High Prevalence of Asymptomatic Malaria in Bandafassi, South-east of Senegal: Challenge for Malaria Elimination

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Abstract

Background: Malaria control and elimination strategies are based on levels of transmission that are usually determined by data collected from health facilities. In endemic areas, asymptomatic malaria is thought to represent the majority of infections and is therefore not diagnosed nor treated. As a consequence, they are missed when analyzing data due to the lack of visiting the health facilities unless they are sick. Therefore, there might be an underestimation of the malaria prevalence resulting in inadequate control strategies. In addition, these untreated asymptomatic cases maintain transmission making it difficult or impossible to reach malaria elimination goals. Thus, the aim of this study was to determine the prevalence of asymptomatic malaria in southeastern Senegal.

Methods: A cross sectional study was conducted among asymptomatic individuals (N = 122) living in the village of Andiel located in Bandafassi, Kedougou which consisting of about 200 inhabitants during the malaria transmission season in late October 2019. For each individual without malaria symptoms and who consented to participate in the study, a rapid diagnosis test (RDT) was performed in the field. Results were confirmed in the laboratory with nested PCR and photo-induced electron transfer (PET-PCR).

Results: Malaria prevalence was 70.25% with PET-PCR, 41.80% with RDT and 41.32% based on the nested PCR. The majority of the study population; 92.94% was infected with a single species (mono-infection) and 7.06% had two or three species of *Plasmodium*. *P. falciparum* was predominant and represented 90.22% of the infections, while 6.52% were due to *P. ovale* and 3.26% to *P. malariae*. RDT detected more malaria cases than nested PCR among children and in individuals aged fifteen years and older; PET-PCR detected more cases (64.70%) than nested PCR (62%) and RDT (52.94%) in this age group.

Conclusion: Asymptomatic infection is a threat to malaria elimination. In southeastern Senegal, where the transmission is the highest in the country, malaria control strategies should address asymptomatic cases at the community level. This high prevalence of asymptomatic malaria observed suggests that this area is eligible for mass drug administration. Moreover, non-falciparum species could be more common and its prevalence should be determined countrywide.

Introduction

Malaria is an endemic parasitic disease, with an estimated 228 million cases worldwide in 2018 [1], and most cases occurred in Sub-Saharan Africa, where it causes many deaths even if this declined the past ten to fifteen years [1]. In Senegal, malaria is endemic with 354,708 reported cases in 2019 in health facilities and three regions in the South of Senegal recorded 81% of all cases and 39% of the total mortality [2]. Malaria cases are reported in the health facilities among patients seeking care; many of malaria infections are not detected as in endemic areas individuals can carry the *Plasmodium* parasites without any symptoms. Thus, they serve as reservoirs as they do not treat and clear their infection but rather remain infectious for the duration of parasite carriage maintaining the transmission of malaria [3].

There is overwhelming evidence indicating that a significant proportion of the malaria incidence rate is composed of asymptomatic infections [4, 5]. Therefore, asymptomatic malaria is a serious threat to elimination and eradication and should be addressed by the National Malaria Control Program in their policy implementation for malaria elimination.

The mechanism underlying asymptomatic malaria is still not well known, but it has been shown that individuals who have had several previous episodes of symptomatic malaria are more likely to become asymptomatic carriers upon *Plasmodium* sp. infection [6]. In addition, individuals can remain infected for long periods even though asymptomatic subjects can develop symptomatic disease if they have a dysregulated immune response [6].

In endemic areas, the parasite density among asymptomatic subjects are usually low, and generally not detected by classical methods such as rapid diagnostic test (RDT) and light microscopy which have low sensitivity compared to molecular techniques [7]. Thus, the use of molecular methods to diagnose malaria in the community makes it possible to better identify the asymptomatic infection.

In Senegal *P. falciparum* is the main species responsible for malaria but the prevalence of other species of *Plasmodium* remains unknown. However, in the context of malaria elimination the prevalence of other *Plasmodium* species that may cause asymptomatic malaria with low density infection or relapses must be determined. The circulation of non-falciparum species has been previously reported in Senegal, *P.ovale* and *P.malariae* have been found in Dakar and Kedougou [8–10] as well as the circulation of *P.vivax* in South-East of Senegal of this country [11]. However only few parcel prevalence data are available on non-falciparum species and the circulation of *P. vivax* is debated.

The asymptomatic *Plasmodium spp*. infections are usually not diagnosed in the health facilities due to the absence of symptoms. In remote villages of hyper endemic areas, such as in the South-east of Senegal where malaria transmission is the highest in the country, asymptomatic malaria cases could be more prevalent than symptomatic malaria cases detected in the health facilities, but such study has not been conducted in this region. Therefore, the aim of this study was to investigate the prevalence of asymptomatic cases and to discuss its impact on the malaria control strategies deployed in this in the community of the village of Andiel in Kedougou, southeastern Senegal.

Material And Methods

Study Site and participants

The region of Kedougou is bordered on the west by the hills of Bassari country and the Mount Assirik, which dominates the Niokolo-Koba National Park. It is one of the wettest region in the country with at least 1300 mm/year. The rainy season lasts about six months, from May to October, with a dry season. The region is subject to the harmattan for seven months (October to April). However, this rainfall is characterized by a great spatio-temporal variability, the months of August and September being the rainiest [12]. Temperatures are generally high with maximums ranging from 34° to 42° and minima from

21° to 25°. The relative humidity is very high during the wet season. It exceeds 97% between August and October.

The study was conducted in the village of Andiel (12°32'37.43"N / 12°22'09.09"O) (12.5435895,-12.3686038), the precinct (or borough) of Bandafassi (12.53965,-12.31061) in the region of Kedougou (12.8856865,-12.286425) in the South-East region of Senegal. The village has around 200 inhabitants composed of the Bedik ethnic who live in the mountain in small communities with a preserved ancestral way of life. Malaria is hyperendemic in the area with the transmission starting in May-June up to December-January. The three Plasmodium species have been reported in the region and *P. falciparum* is the predominant specie [8].

Asymptomatic individuals consisting of adults and children (N = 122) were enrolled during the malaria transmission season at the end of October 2019. The asymptomatic malaria case was defined by the absence of fever or history of fever, headaches, nauseas vomiting, aches and pains. None of the participant reported any of these symptoms. For each participant a rapid diagnostic test (RDT) was performed and blood was collected, spotted on filter paper and dried (DBS) at room temperature. Infected individuals were treated according to the National Malaria Control Program (NMCP) recommendation. The study was approved by the ethical committee of the ministry of health of Senegal.

Rapid diagnosis test (RDT)

The RDT used was the *CareStart*™ Malaria Pf/Pan, Accessbio. It allows the two-band RDT detects the *P. falciparum* histidine rich protein 2 (PfHRP2) specific to *P. falciparum* and the parasite lactate dehydrogenase (p-LDH) of *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*. This allow the RTD to distinguish *P. falciparum* from the other *Plasmodium* species (*Pv*, *Po*, *Pm*) in mono-infections samples or in mix-infections.

Malaria prevalence in the health post of Bandafassi

The health post of Bandafassi has the responsibility of several villages including Andiel. The number of malaria cases recorded in the health post of Bandafassi were collected through the NMCP database. The malaria cases were diagnosed among patients seeking care at the health post and who presented fever during the consultation or a history of fever during the precedent 48 hours. The malaria cases were diagnosed using SD Bioloine Malaria antigen Pf® RDT during the malaria transmission season of 2019.

Nested PCR identification of Plasmodium species

Parasite DNA was extracted from the dried blood spots using the QIAamp DNA Mini kit (Qiagen®) following the manufacturer's instructions.

Plasmodium species were identified by a nested PCR targeting the 18S small sub-unit ribosomal RNA (18S ssrRNA) gene of *Plasmodium spp* as described previously [13–15]. All PCR reactions were performed in a total volume of 25 µl containing: four µl PCR master mix (0.2 U/µl Taq Polymerase, 250 µM deoxyribonucleotide triphosphate), and 0.5 µM of each forward and reverse primer. Positive

(specific for each species) and negative (reagent grade water) controls were systematically incorporated in each PCR run. The nested PCR products were revealed by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized under UV trans-illumination (VersaDoc®, BIORAD, Hercules, USA). The sizes of nested PCR products were estimated using 100 bp DNA ladder (Promega, Madison, USA).

PET-PCR identification of Plasmodium species

Plasmodium species molecular characterization was also performed for all samples using the photo-induced electron transfer (PET)-PCR assay [16] on a Roche LightCycler 96 instrument (Roche Molecular Systems, Inc). Each experimental run included both a negative (no template) and a positive (3D7 *P. falciparum* strain) control. Samples with a cycle threshold (CT) of 40 or less were scored as positive [16, 17]. The specificities of the *P. malariae*, *P. vivax*, and *P. ovale* was performed in a 20 ml reaction containing 2X TaqMan Environmental MasterMix 2.0 (Applied BioSystems), 250 nM each forward and reverse primer, and 5 µl of DNA template. All reactions were performed in a total volume of 20 µl containing: 5 µl of DNA, 10 µl of 2X ABI TaqMan buffer and 250 µM of each forward and reverse primers (Table 1). The reactions were performed under the following cycling parameters: initial hot-start at 95 °C for 15 minutes, followed by 45 cycles of denaturation at 95 °C for 20 seconds and annealing at 60 °C for 40 seconds.

Table 1
Social demographic characteristics of the study population. Study populations were presented according to age range and sex. Frequency was presented in percentage.

Age range	Prevalence by age range (%) (N = 122)	Sex	
		Female (%) (N = 67)	Male (%) (N = 55)
0–4 years	9.84	8.95	10.91
5–9 years	14.75	20.90	7.27
10–14 years	17.21	14.93	20
>= 15 years	58.20	55.22	61.82

Results

Social demographics

Overall, 122 subjects were included in this study among them 54.92% (N = 67) were female and 45.08% (N = 55) male. The participants' ages ranged from 11 months to 90 years with the mean of 27.19 years and a median of 19 years. Age group distribution is summarized in Table 2. Children between 0 to 4 years old represented 9.84%, the age group of 5 to 9 years 14.75%; the 10 to 14 years 17.21% and individuals

with 15 years and more represented 58.20%. The male represented 10.91% among the 0 to 4 years 7.27% of the 5 to 9 years; 20% of the 10 to 14 years and 61.82% for the over 15 years (Table 1).

Table 2
malaria prevalence by age range

Age range (N = 122)	RDT positivity (%) (N = 122)	Nested PCR positivity (%) (N = 121)	PET-PCR positivity (%) (N = 121)
0–4 years	5.88	4	5.88
5–9 years	15.69	14	14.12
10–14 years	25.49	20	15.29
>= 15 years	52.94	62	64.70

Malaria prevalence

RDT was performed for all the 122 subjects at the community level in the village and 121 samples were tested for nested PCR and PET-PCR in the laboratory. The nested PCR and the PET-PCR were able to differentiate between *P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*.

The prevalence of malaria was 70.25% with PET-PCR, 41.80% with RDT and 41.32% with nested PCR (Fig. 1). Compared to PET-PCR, RDT failed to detect 28.45% of the infection as well as the Nested-PCR.

Compared to the malaria prevalence diagnosed at the health post of Bandafassi using RDT, the prevalence of malaria was 49.08% during October when the samples were collected for this study. The malaria prevalence in the general population during November was 44.07% and 31.93% in December (Fig. 2).

Low parasite density and detection using molecular techniques

The Ct for the samples that were detected by PET-PCR but negative with Nested-PCR were higher than the Ct for the samples positive for both techniques (Fig. 3). The Ct mean and the median for the samples positive by PET-PCR but negative with nested PCR were respectively 37.53 and 38.12. The samples positive for both nested and PET PCR presented a Ct mean of 33.51 and a Ct median of 33.95.

Malaria detection and age

RDT detected more malaria cases than the nested-PCR among the children (Table 2). PET-PCR detected the same positive results as RDT among children aged 0 to 4 years (5.88%); it identified less case than RDT among the 5–9 years (14.12% vs 15.69%) and 10 to 14 years (15.29 vs 25.49%). In individuals aged

fifteen and more PET-PCR (64.70%) detected more cases than the nested-PCR (62%) and the RDT (52.94%) (Table 2).

Description Age vs PET-PCR

Among positive samples, single infections (caused by one Plasmodium species) represented 94% with nested PCR *versus* 92.94% in PET-PCR; mixed infections (caused by two or more Plasmodium species) were detected at 6% for nested PCR and 7.06% by PET-PCR (Fig. 4).

RDT identified *P. falciparum* in 94.12% (N = 48 samples) of the samples and the P. falciparum and pan bands suggesting the presence of non- falciparum species was detected at 5.88% (N = 3 samples). To confirm the presence of non-falciparum species two PCR techniques were performed for all samples; the PET-PCR and the nested PCR. Among the three samples positive for the pan species with the RDT, two were mixed infection with *P. falciparum* and *P. ovale* by PET-PCR and the other one was a *P. falciparum* single infection by nested PCR.

Both nested PCR and PET-PCR detected *P. falciparum* in the majority of the infections with respectively 88.89% (N = 48) and 90.22% (N = 82). *P. ovale* was identified at 7.41% (N = 4) with nested PCR and 6.52% (N = 6) by PET-PCR; while *P. malariae* was detected at 3.70% (N = 2) by nested PCR and 3.26% with PET-PCR (N = 3) (Table 5). Overall, the PET- PCR detected 34 more *P. falciparum*, 2 more *P. ovale* and 1 more *P. malariae* than the nested PCR (Table 3).

Table 3
Plasmodium species by PCR techniques.

Techniques	*P. falciparum*	*P. malariae*	*P. ovale*	*P.f + Pm + P.o*	*P.f + P.m*	*P.f + P.o*
Nested	45	1	1	1	0	2
PET-PCR	77	1	1	1	1	4

The table presents the numbers of samples in which the Plasmodium species were identified using nested PCR and PET-PCR. For each technique, the number for each Plasmodium species was counted and presented as absolute values.

Three species were identified; *P. falciparum*, *P. ovale* and *P. malariae*. *P. vivax* was not detected with the techniques used in this study. Only two non-falciparum infections were single infection with *P. ovale* and *P. malariae* and both were detected with the nested and the PET-PCR. A mixed infection with *P. falciparum* and *P. malariae* were detected by the PET-PCR. An infection with *P. falciparum*, *P. ovale* and *P. malariae* was detected by both techniques. A mixed infection with *P. falciparum* and *P. ovale* was detected in two subjects by the nested PCR and in four individuals PET-PCR detected two mixed infections. The two mixed infections with *P. falciparum* and *P. ovale* were identified by the PET-PCR.

Discussion

In southeastern Senegal, the incidence of malaria is the highest in the country. Under such conditions of transmission, it is hypothesized that asymptomatic malaria is not uncommon. Thus, this study was conducted with the objective of determining the prevalence of asymptomatic malaria in Kédougou during the 2019 transmission season.

The results of this study showed a malaria prevalence of 70.25% with the PET-PCR, which is the most sensitive technique among those used during this study, while at the same period a malaria prevalence of 49.08% was recorded passively at the health post of Bandafassi using PfHRP2 RDT. This high asymptomatic malaria prevalence in this study conducted in the village of Andiel could be explained by the malaria endemicity particularly the transmission intensity with an entomological inoculation rate higher than 100 infective bites per person and per year [2]. Therefore, individuals are frequently exposed to mosquito bites and *Plasmodium spp*. As known, these frequent infections allow to acquired immunity in moderate and high transmission areas and mostly malaria is associated with asymptomatic cases [18, 19]. Asymptomatic infections do not present any clinical manifestation, thus remain undetected and untreated.

The main characteristic of these asymptomatic infections is the low parasite density, which from a diagnostic point of view requires sensitive means. Indeed, it has been shown in endemic areas that low parasite density is a factor explaining the absence of symptoms [7]. During asymptomatic malaria, the parasite density may fluctuate [20–22]. These fluctuations could be due to several factors such as immunity [23], premunition or the genetics of the parasite. These factors could then determine the clinical outcome of malaria; indeed, multiplicity of infection (MOI) has been associated with host age, clinical severity, and intensity of transmission [24].

Three techniques were used for malaria diagnosis in this study. Among these techniques, PET-PCR detected most of the malaria cases with a prevalence of 70.25%, RDT showed a prevalence of 41.80% and 41.32% with nested PCR. These results suggest that nested PCR and RDT do not seem sensitive to detect asymptomatic malaria cases, which could be due to the low parasite density that usually characterizes these infections. Indeed, with the PET-PCR, Ct values were higher for samples that were not detected with nested PCR, suggesting that their low DNA concentration and that neither the RDT nor nested PCR were sufficiently sensitive to detect the low parasite densities that are generally associated with asymptomatic malaria. Indeed, a previous study showed a high sensitivity of PET-PCR compared to nested PCR [25] for symptomatic malaria cases during which parasite densities are higher compared to asymptomatic malaria. Moreover, it has been shown that parasite density is correlated with the onset of symptoms [25], with infections with low densities generally showing no clinical signs in subjects living in endemic areas.

The results showed that RDT was good at detecting infections in children, whereas in adults PET-PCR identified more malaria cases. This suggests that the low parasite densities that characterize asymptomatic infections were probably the cause, as in hyper endemic areas the immunity is able to

keep the parasitemia low, which are usually not detectable using the RDTs due to their low sensitivity [26–32].

The detection of low parasite densities requires the use of sensitive techniques such as real-time PCR to ensure better diagnosis of asymptomatic infections. During outbreak investigation in low transmission settings in Senegal a surveillance system is set up by the NMCP. However, during these surveys, RDT is used as the diagnostic method, which may not reveal asymptomatic infections due to their lack of sensitivity in case of low parasite density. In addition, this surveillance should be extended to the detection of asymptomatic malaria at the community level in areas of the country where transmission is higher such as the south.

To overcome the undiagnostic malaria cases in remote areas, a home management system (Prise en charge à domicile; PECADOM) was initiated by NMCP in 2008. This program was first set up in remote areas that do not have easy access to health facilities and then deployed in several areas. This approach allows the home-based care provider (Dispensateur de soin à domicile; DSDOM) to promote the health of the population by implementing different strategies such as early diagnosis and treatment of uncomplicated malaria cases. The DSDOM diagnoses malaria with the PfHRP2 RDT and treats positive patients with Artemisinin-based combination therapy (ACTs). However, in this home-based management program, asymptomatic infections are not diagnosed because only people with malaria symptoms such as fever are tested and treated. In addition to not being able to diagnose low parasite densities, the PfHRP2 RDT only detects *P. falciparum*.

Although *P. falciparum* is the most common species in Senegal, the mixed infections with the other Plasmodium species have been reported in several regions of the country [8–11, 32]. Likewise, in this study, most of the non-falciparum malaria were identified as mixed infection.

Infections due to species other than *P. falciparum* are generally characterized by low parasite density and are usually asymptomatic. To diagnose non-falciparum malaria infection and determine its prevalence, it would be necessary to conduct sampling at the community level and use methods that are more sensitive than RDTs and capable of species differentiation. Indeed, the Pan-RDT used in this study as a diagnostic method is capable of identifying *P. falciparum* and detecting the presence of other species (*P. ovale*, *P. malariae* and *P. vivax*) without being able to differentiate them. This Pan-RDT detected only three cases of malaria due to species other than *P. falciparum*. This could be due to the low parasite density that is characteristic of these Plasmodium species. Nested PCR detected fewer non-falciparum species than PET-PCR, which is more sensitive suggesting that the low parasite density of these infections could explain the non-detection by the pan-RDT and the nested PCR.

In this study, the majority of non-falciparum species identified were mixed infections with *P. falciparum*, which is not unusual according to the natural characteristics of the region. Indeed, in tropical areas such as southern Senegal, *P. ovale* and *P. malariae* were identified mostly as mixed infections with *P. falciparum* [34]. In addition to this, geographical characteristics could determine the relationships between human malaria species, which certainly involve the bioclimatic variations and the genetic

differences in humans and vector populations. [35, 36] Furthermore, the prevalence of *P. ovale* and *P. malariae* species become often higher when the prevalence of *P. falciparum* decreases such in a context of malaria elimination [37].

However, *P. vivax* was not identified in this study suggesting the no circulation of this specie in this area. Indeed, some studies in Senegal have reported its presence in some parts of the country [8, 11]. In addition, a study has found serological markers (PvMSP1-19) of its circulation in the north and the center of the country, but not in the South where this study have been conducted [38]. More investigations are needed across the country to provide solid evidence of *P. vivax* circulation.

Conclusion

The high prevalence of asymptomatic malaria observed in this study suggests that malaria control strategies should address asymptomatic cases at the community level. Therefore, it becomes necessary to put in place a surveillance strategy for these infections that can serve as a reservoir for malaria transmission in a country such as Senegal, which tends towards malaria elimination. In Senegal, malaria surveillance become an intervention in low transmission settings, however it should be extended to the intermediate and high transmission areas such as Kédougou. Although the prevalence of non-falciparum species should be assessed country wide as while the malaria caused by *P. falciparum* is decreasing it could be replaced by the other species, which might threaten the goal of elimination.

Abbreviations

ACT
Artemisinin-based combination therapy
CT
Cycle Threshold
DBS
Dried Blood spot
DSDOM
Dispensateur de soin à domicile
NMCP
National Malaria Control Program
PECADOM
Prise en charge à domicile
PET-PCR
Photo-induced Electron Transfer PCR
PfHRP2
P. falciparum histidine rich protein 2
p-LDH
parasite lactate dehydrogenase

RDT
Rapid Diagnosis Test

Declarations

Ethics approval and consent to participate

The study was approved by the National Ethics Committee for Health Research of Senegal. Before patients were enrolled in the studies sites, benefits and any perceived risks were explained to all participants in French or local languages. Informed consent for adults or guardians consent for children less than 18 years old were obtained before participant recruitment and sample collection.

Consent for publication

The participants in this study are consent for publication

Availability of data and materials

The data supporting the findings of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BAS, NT, TAB and ND conceived and designed the study. BAS, NT and TAB carried out the experiments and collected data. BAS, NT, TAB, DAB, DMA, SMC, DK, MNG, NM and ND contributed to writing the manuscript. All authors read and approved the final manuscript.

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Figures

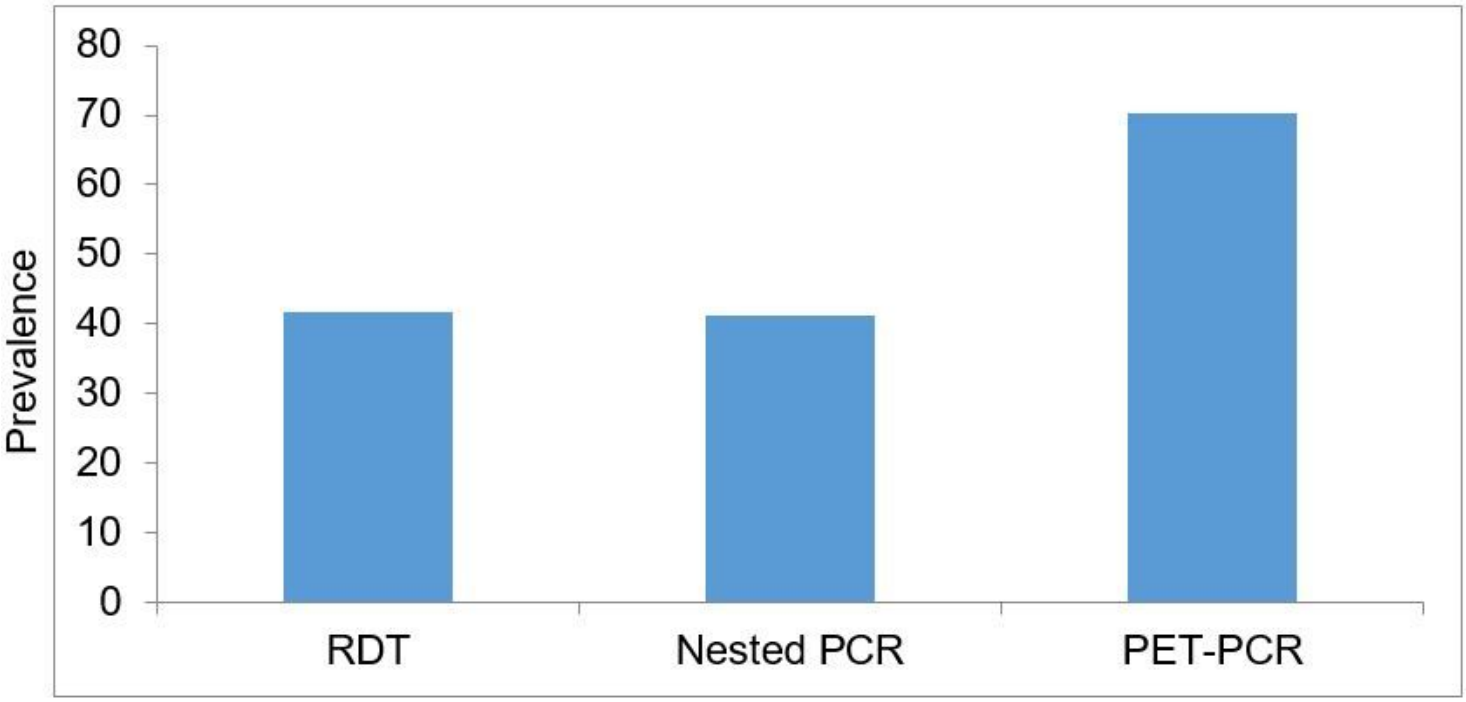

Figure 1

Prevalence of malaria according to the technique (RDT, Nested-PCR, PET-PCR). Each bar presents the percentage of positivity for each of the techniques.

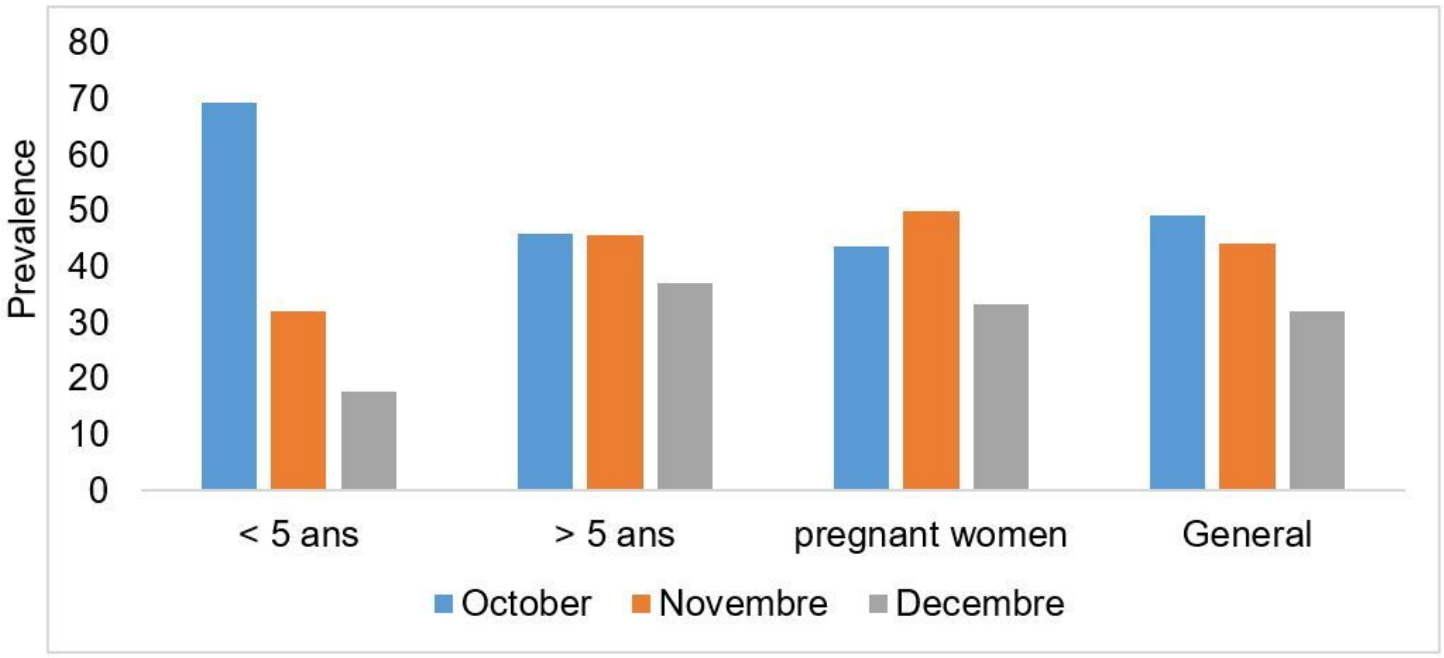

Figure 2

Malaria cases at the health post of Bandafassi during the pic of the malaria transmission season. The malaria prevalence among children under five, up to five, the pregnant women and in the general population at the health post of Bandafassi during the months of October, November and December during which the transmission is the highest in the region are presented here.

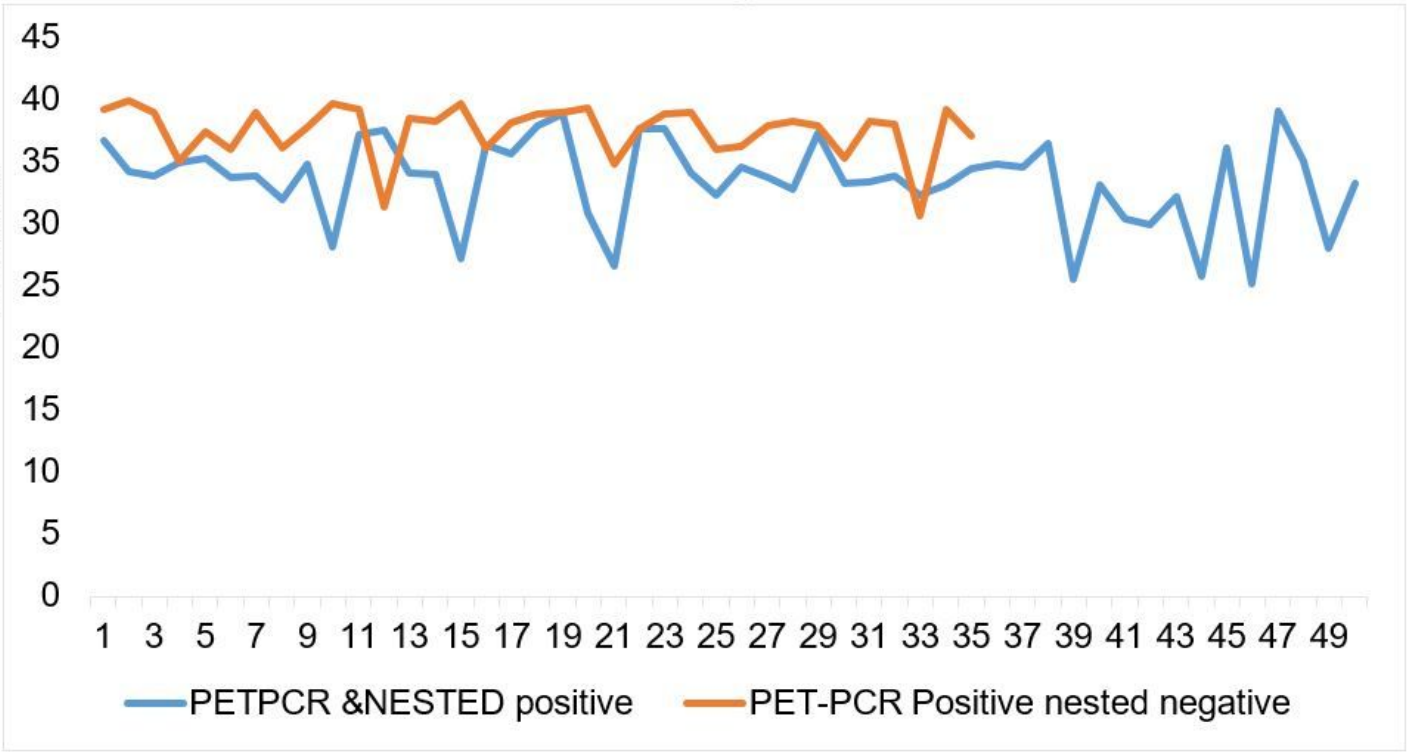

Figure 3

Ct comparison of the samples that were positive with PET-PCR and negative with Nested PCR

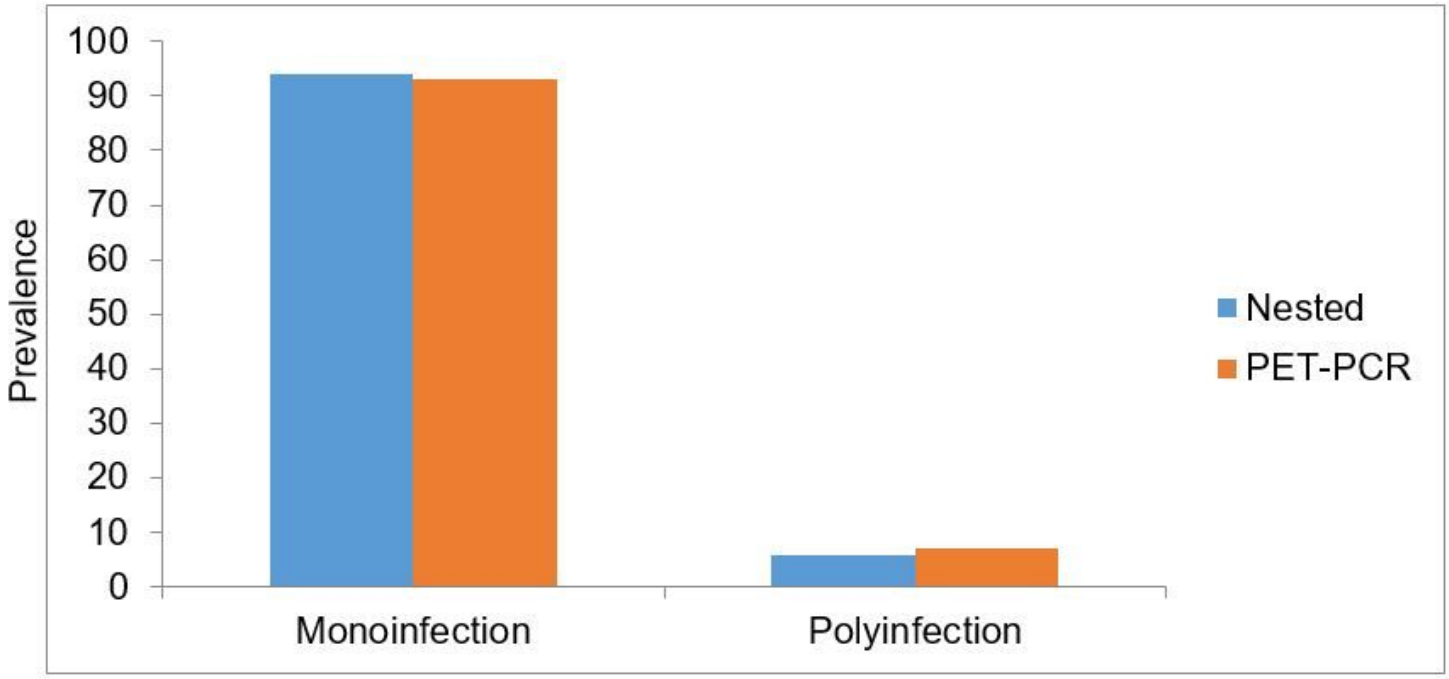

Figure 4

Distribution of single and mixed infections by both nested and PET PCR.