Prevalence of *Plasmodium* spp. in symptomatic BaAka Pygmies inhabiting the rural Dzanga Sangha region of the Central African Republic

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D – Writing the article, E – Critical revision of the article, F – Final approval of the article

▌ Abstract
Introduction. Malaria remains a diagnostic and therapeutic challenge in many endemic regions of sub-Saharan Africa. It is one of the most important causes of morbidity and mortality, especially in children <5 years. *Plasmodium falciparum* is responsible for the majority of severe malaria cases in sub-Saharan Africa, but is not the exclusive one.
Objective. The objective of the study was to assess the prevalence of *Plasmodium* spp. in BaAka Pygmies with clinical symptoms of malaria, and define the percentage distribution of infections caused by species other than *P. falciparum* in order to assess the need for diversification of malaria treatment protocols.
Materials and method. The study was conducted during the dry and rainy seasons in 2018 and involved a group of 540 symptomatic BaAka Pygmies, patients of both genders, aged 1–75-years-old. Two diagnostic methods for detecting *Plasmodium* in the bloodstream were used: RDTs targeting HRP2-protein specific for *P. falciparum,* and PCR assays aimed at detecting *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* species.
Results. Only 40.5% of symptomatic patients tested with RDTs for *P. falciparum* infections were positive. Molecular tests (PCR) confirmed *P. falciparum* in 94.8% of the samples and also revealed the genetic material of *P. malariae* (11.1%), *P. ovale* (9.8%), and *P. vivax* (0.7%). BaAka Pygmies aged <5 years of age dominated in patients with positive results; the common clinical symptoms reported by the sick individuals were fever, shivers and fatigue.
Conclusions. The study suggests the need for introducing accurate diagnostic methods for the diagnosis of malaria and the revision of malaria treatment protocols. Assessment of the Pfhrp2/Pfhrp3 deletions is necessary for evaluating malaria epidemiology in Central Africa.

▌ Keywords
PCR, malaria, Pygmies, Central African Republic, *Plasmodium* spp, RDT

▌ Abbreviations
CAR – Central African Republic; **WHO** – World Health Organization; **PCR** – polymerase chain reaction; **RDT** – rapid diagnostic test; **HRP2** – histidine rich protein 2; **ACT** – artemisinin-combined therapy; **NGO** – non–governmental organization; **FUO** – fever of unknown origin

INTRODUCTION

Malaria, a vector-borne parasitic disease, is a major health issue in sub-Saharan Africa. In humans it is caused by five different species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. The disease is transmitted from a bite by a malaria-infected female *Anopheles* mosquito, which introduces the invasive forms of *Plasmodium* (sporozoites) into the bloodstream of the human host. It may also be transmitted through transfusion of blood containing *Plasmodium* trophozoites or schizonts, or by the vertical route, i.e. from the infected mother to the foetus. The *Plasmodium* life cycle is divided into several distinct stages which take place in the red blood cells and the liver [1].

Malaria is endemic in 95 countries and affects approximately three billion people globally. In 2018, the World Health Organization (WHO) reported 228 million cases of malaria, of which 93% were reported in Africa. In the same year, there were an estimated 405,000 deaths from malaria globally, 94% of the deaths in Africa [1, 2]. The most common pathoetiological malaria species responsible for a vast majority of all cases worldwide are *P. falciparum* and *P. vivax* (80–95%). The WHO reports have pointed to the predominance of *P. falciparum* infections in Africa [1]. Therefore, in line with the WHO recommendations, malaria control on this continent is primarily based on the use of rapid diagnostic tests (RDTs) targeting the HRP2–protein found exclusively in *P. falciparum*. The results obtained from RDTs, however, do not reflect the actual number of malaria cases in the region. The exact rates of malaria caused by other than *P. falciparum* species in the Central African Republic (CAR) are unknown or underestimated. An important consequence of this fact is the need for introducing a different

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drug protocol for the treatment of infections other than those caused by *P. falciparum*.

Patients infected with *P. vivax* or *P. ovale* require an extended course of antimalarial treatment. In *P. vivax* and *P. ovale* infections the predominant proportion of merozoites is released into the bloodstream, while a certain number invades other hepatocytes (parenchymal cells in the liver) entering a dormant phase known as the hypnozoite. Hypnozoites can reactivate and undergo erythrocytic schizogony at a later time, resulting in the relapse of malarial symptoms after several weeks or even years from the primary infection; therefore the treatment of *P. vivax* and *P. ovale* requires the use of 8-aminoquinoline (primaquine), targeting the liver stage schizonts and preventing a relapse [1].

The diagnosis of malaria is based on the detection of *Plasmodium* parasites in the bloodstream. Currently, RDTs targeting *Plasmodium* antigen or enzymes are used as the major diagnostic tool, especially for screening purposes. In CAR, RDTs detecting histidine rich protein 2 (HRP2-protein) of *P. falciparum* have been distributed free of charge. However, in cases of low parasitemia or when malaria is caused by other than the *P. falciparum* species, RDTs are likely to give a negative result. The gold standard, unavailable in the most remote areas of sub-Saharan Africa, are light microscopy and molecular biology methods, mostly polymerase chain reaction (PCR).

According to the WHO, the number of malaria cases in CAR, a country inhabited by 4.8 million people, was estimated at 1.8 million and the number of deaths due to malaria at 4,800 (383,000 cases and 3,689 deaths were laboratory-confirmed). All the reported malaria cases and malaria deaths were reported as *P. falciparum* infections, even though the tests for *P. vivax* or *P. ovale* were rarely being conducted [3]. The vast majority of patients in the the CAR received the artemisin-combined therapy (ACT) which requires the administration of six doses of arthemeter-lumefantrine over three consecutive days. The majority of patients in the rural regions of the CAR, including the hunter-gatherer BaAka Pygmies inhabiting the local forests, obtain medical care at the lowest level medical facilities or mobile health units run by non-governmental organizations (NGOs). The most common health problems among BaAka Pygmies are fevers of unknown origin (FUO), which are clinically diagnosed and treated as malaria. About 50% of malaria cases are confirmed with *P. falciparum*-specific RDTs, but the remaining group of patients with a negative result of RDTs do not receive any anti-malarial treatment despite the presence of malarial symptoms: recurring fever, shivers, dehydration, abdominal pain, diarrhea and vomiting. If left untreated, malaria can be fatal, especially in children <5 years old, pregnant women and patients with comorbidities.

OBJECTIVES

The aim of this study was to assess the prevalence of *Plasmodium* spp. in BaAka Pygmies with clinical symptoms of malaria, inhabitants of the rural Dzanga Sangha region in the CAR. Additionally, the research aimed to define the percentage distribution of infections caused by species other than *P. falciparum* in order to assess the need for diversification of malaria treatment protocols in the region of Central Africa.

MATERIALS AND METHOD

Study population. The study was conducted the during the dry and rainy seasons in 2018 and involved a group of 540 BaAka Pygmies with clinical symptoms of malaria, patients of both genders, aged 1–75 years, living in rural areas within 50 km of Monasao village (Sangha-Mbaere prefecture) in the south-western part of the CAR (Fig. 1). The number of patients was limited by the time (4-month period predicted for the study) and number of available microcards for the collection of samples. All patients with malaria symptoms admitted for consultation in the outpatients clinic in Monasao or a mobile clinic visiting the surrounding villages every two weeks or once a month (depending on weather and road conditions) were included in the study.

Sample collection and laboratory procedures. All BaAka patients were admitted for doctor consultation in several villages during the regular work of a mobile clinic, and at the health facility in Monasao, who presented malaria symptoms such as fever, shivers, signs of dehydration, abdominal pain, diarrhea, and vomiting, were included in the study. The data were collected during a medical interview based on the short questionnaire asked in Sango, the local language. All the population of Bantu origin treated at the dispensary were not included in the study. They were diagnosed with RDT and treated the same as the BaAka patients. The standard of care included medical triage when the body temperature, blood pressure, heart rate, weight were measured, RDT performed, and sample for PCR testing taken. The medical interview, physical examination and distribution of proper medications followed. Regardless of the RDTs result, all patients presenting symptoms received malaria treatment. Two diagnostic methods for detecting *Plasmodium* in the bloodstream were used. During the first stage, immunochromatographic RDTs targeting *P. falciparum*-specific HRP2–protein (CareStart Malaria Pf/HRP2/Ag, Access Bio; SD Malaria Ag Pf/05FK50/, Bioline Standard Diagnostics, Inc.) were performed [4]. The tests were carried out immediately after collecting 0.5 ml of whole blood collected by venopuncture from the participants enrolled in the study. The next stage was the application of blood samples onto the FTA Micro Cards (Whatman™); the biological material was then transported to Poland (under an agreement with the Ministry of Science and Resear – CAR). The specimens were analyzed at the Institute of Maritime and Tropical Medicine, Department of Tropical Parasitology of the Medical University of Gdańsk with the PCR method (detecting four species of *Plasmodium*: *P. falciparum, P. vivax, P. ovale, P. malariae)*. On behalf of the paediatric patients, consent to participate in the study was given by their parents/guardians. Exclusion criteria of the study were anti-malarial treatment received in the past 28 days and difficulties in venopuncture procedure.

Statistics. All statistical calculations were performed using the StatSoft statistical package STATISTICA Inc. (2014) (data analysis software system), version 12.0. www.statsoft.com and an Excel spreadsheet. Quantitative variables were characterized by the arithmetic mean, standard deviation, median, minimum and maximum value (range), and 95%CI (confidence interval). The variables of the qualitative type were presented in terms of counts and percentages (percentage). Chi–square tests of independence were used
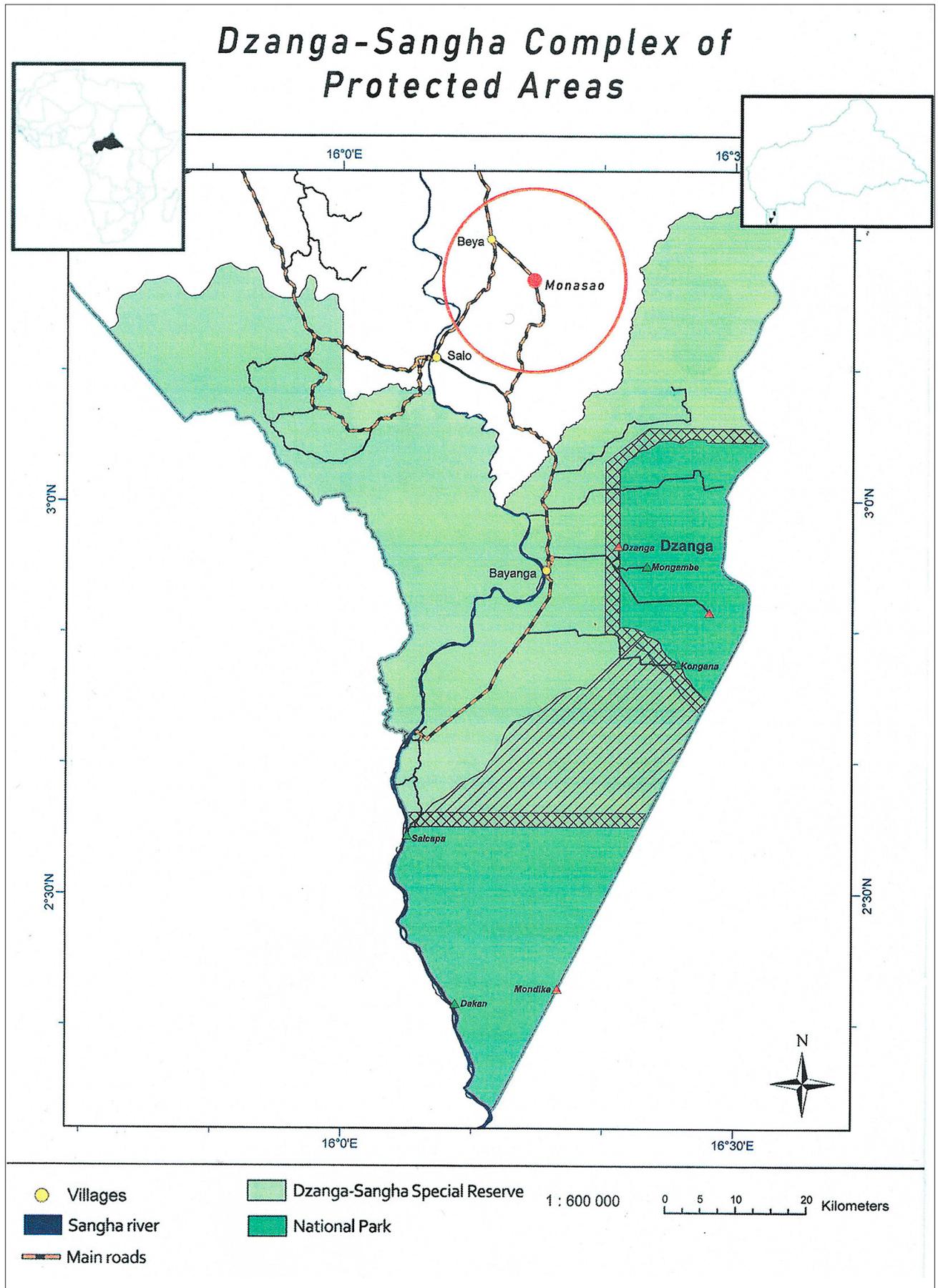
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Figure 1. Dzanga Sangha region, Central African Republic

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for qualitative variables (respectively using Yates correction for cell counts below 10, checking Cochran conditions and Fisher's exact test). Cohen's kappa and Scott's pi coefficients inte-rater reliability were used to assess the compliance of RDT and PCR methods. In all calculations, the level of significance was set at p=0.05.

RESULTS

The study involved 540 BaAka Pygmies (328 females and 212 males) presenting with clinical signs of malaria; *P. falciparum*-specific RDTs were positive in 40.5% of the subjects. PCR tests performed on the same blood samples confirmed *P. falciparum* infections in 94.8% of the study group; in addition, PCR tests revealed infections with species other than *P. falciparum (Pf)*, i.e. *P. malariae (Pm), P. ovale (Po)* and *P. vivax (Pv)*, which were not detected by RDTs targeting solely HRP2-protein specific for *P. falciparum* (Tab. 1).

Table 1. Detection rates of *Plasmodium* species by RDTs and PCR in the group of BaAka Pygmies with clinical symptoms of malaria (n=540)

Method *Plasmodium* species	CareStart Malaria Pf /HRP2/Ag test	Molecular biology (PCR)
P. falciparum	40.5%	94.8%
P. vivax	0%	0.7%
P. ovale	0%	9.8%
P. malariae	0%	11.1%

The test results were not consistent between the methods used (RDTs and PCR). Cohen's kappa coefficient inter-rater reliability (0.03/0.02) was close to zero, i.e. at the random level. In turn, Scott's pi coefficient inter-rater reliability (-0.34/-0.28) took a negative value, i.e. lower than at the random level (Tab. 2).

Table 2. Comparison of RDTs and PCR results in BaAka Pygmies with clinical symptoms of malaria (n=540)

Coefficient inter-rater reliability	Females (n=328)	Males (n=212)	Total (n=540)
Cohen's kappa	0.03	0.02	0.03
Scott's pi	-0.34	-0.28	-0.31

Table 3 presents the demographic data (age, gender, body weight) of the BaAka Pygmies tested with RDTs, including their body temperature and of the time between symptoms onset and medical consultation. The majority of subjects with positive tests results were individuals <5 years old, with body temperature <38.0 °C, and symptoms lasting for up to seven days.

Patients with positive results of the RDTs were more likely to present with fever, fatigue, and shivers (Tab. 4).

The majority of patients with positive results of the PCR tests (as in the case of positive RDTs) were aged <5 years old, with body temperature <38.0ºC, and symptoms lasting for up to seven days (Tab. 5).

BaAka Pygmies with positive PCR test results were more likely to present with fever, shivers, and fatigue (Tab. 6).

Table 3. Demographic and clinical data of BaAka Pygmies tested with RDTs (n=540)

Demographic and clinical data	RDT (–) negative (n=321)	RDT (+) positive (n=219)	Total (n=540)	*P*-value
gender				0.0070[1]
female	210 (65.4%)	118 (53.9%)	328 (60.7%)	
male	111 (34.6%)	101 (46.1%)	212 (39.3%)	
age (years)				0.0001[2]
<5	81 (25.2%)	142 (64.8%)	223 (41.3%)	
5–18	58 (18.1%)	33 (15.1%)	91 (16.9%)	
>18	182 (56.7%)	44 (20.1%)	226 (41.9%)	
body weight (kg)				0.0001[2]
<10	58 (18.1%)	96 (43.8%)	154 (28.5%)	
10–40	154 (48.0%)	98 (44.7%)	252 (46.7%)	
>40	109 (34.0%)	25 (11.4%)	134 (24.8%)	
body temperature (ºC)				0.0001[2]
<38.0	209 (65.1%)	110 (50,2%)	319 (59.1%)	
38.0–39.0	84 (26.2%)	52 (23.7%)	136 (25.2%)	
>39.0	28 (8.7%)	57 (26.0%)	85 (15.7%)	
duration symptoms (days)				0.0001[2]
1–7	244 (76.0%)	198 (90.4%)	442 (81.9%)	
8–28	35 (10.9%)	8 (3.7%)	43 (8.0%)	
>28	42 (13.1%)	13 (5.9%)	55 (10.2%)	

[1] Chi–square, [2] U Mann–Whitney

Table 4. BaAka Pygmies with clinical symptoms of malaria tested with RDTs (n=540)

Symptoms of malaria	RDT (–) negative (n=321)	RDT (+) positive (n=219)	Total (n=540)	*P*-value
fever	231 (72.0%)	182 (83.1%)	413 (76.5%)	0.0027[1]
shivers	228 (71.0%)	96 (43.8%)	324 (60.0%)	0.0001[1]
headache	141 (43.9%)	41 (18.7%)	182 (33.7%)	0.0001[1]
dizziness	50 (15.6%)	16 (7.3%)	66 (12.2%)	0.0040[1]
generalized pain	172 (53.6%)	60 (27.4%)	232 (43.0%)	0.0001[1]
arthralgia and myalgia	174 (54.2%)	47 (21.5%)	221 (40.9%)	0.0001[1]
weakness	154 (48.0%)	113 (51.6%)	267 (49.4%)	0.4083[1]
vomiting	72 (22.4%)	77 (35.2%)	149 (27.6%)	0.0012[1]
diarrhea	78 (24.3%)	87 (39.7%)	165 (30.6%)	0.0001[1]
abdominal pain	87 (27.1%)	41 (18.7%)	128 (23.7%)	0.0245[1]
loss of appetite	87 (27.1%)	70 (32.0%)	157 (29.1%)	0.2220[1]

[1] Chi–square

DISCUSSION

According to the WHO data, *Plasmodium falciparum* is responsible for 99.7% of all malaria cases in sub-Saharan Africa [1] and as much as 100% of malaria cases in the CAR [3]. The results of the present research suggest that role of infections with *Plasmodium* other than *falciparum* should not be neglected. In 2018, the WHO reported 1,367,986 suspected malaria cases in the CAR, of which 972,119 (71.1%) were confirmed as *Plasmodium falciparum* malaria. Infections caused by *Plasmodium* other than *falciparum* or mixed infections were not found [1]. The WHO reports

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Table 6. BaAka Pygmies with clinical symptoms of malaria tested with PCR (n=540)

Symptoms of malaria	PCR *Pf/Pv/Po/Pm* (−) negative (n=24)	PCR *Pf/Pv/Po/Pm* (+) positive (n=516)	*P*-value
fever	17 (70.8%)	396 (76.7%)	0.5045[1]
shivers	16 (66.7%)	308 (59.7%)	0.4952[1]
headache	8 (33.3%)	174 (33.7%)	0.9687[1]
dizziness	2 (8.3%)	64 (12.4%)	0.5518[1]
generalized pain	10 (41.7%)	222 (43.0%)	0.8956[1]
arthralgia and myalgia	11 (45.8%)	210 (40.7%)	0.6169[1]
weakness	13 (54.2%)	254 (49.2%)	0.6360[1]
vomiting	3 (12.5%)	146 (28.3%)	0.0906[1]
diarrhea	7 (29.2%)	158 (30.6%)	0.8799[1]
abdominal pain	5 (20.8%)	123 (23.8%)	0.7352[1]
loss of appetite	12 (50.0%)	145 (28.1%)	0.0209[1]

[1] Chi–square

that samples were examined by light microscopy or RDTs. A total of 117,267 malaria cases were not confirmed by any diagnostic method [1]. If, however, the molecular biology PCR methods had been used, the results could have been surprising. The *Plasmodium* species identification has great significance for the public health sector. Malaria, as one of the most common and devastating disease in the CAR, needs to be treated according to algorithms based on research showing the participation of other than *P. falciparum* disease etiology. This is crucial for the proper diagnostics and treatment that may lead in the future to the eradication of malaria. This research sheds new light on malaria etiology in the CAR, causes of treatment fail, and growing drug resistance. Study in this field should be continued in the nearest future.

Data on the prevalence of different species of malaria in the CAR is insufficient to define the epidemiological situation in the country. Decades of conflicts and political resulted in the disruption of malaria research, the retrieval of medical data from the rural regions, and worsened access to health facilities. The CAR is considered a country with a high malaria-transmission rate, estimated at >1 case per 1,000 citizens. The malaria epidemiology presented in the national strategy against malaria in 2016 [7] indicated that malaria was the cause of 59% of all consultations in general population in 2013, of which 52% were children under the age of 5 years. Of all deaths recorded in in-patient departments, 34% were attributed to malaria.

The results of studies conducted in Africa have also indicated that the rates of infections with other *Plasmodium* species are higher than those suggested by the WHO. In the absence of data from CAR, a more accurate assessment of malaria epidemiology in the country could be obtained by analyzing the data gathered in neighbouring countries with similar climate conditions, in Cameroon and the Republic of Congo. The overall malaria prevalence in Cameroon is 29% [8]. In the group of children aged six months up to 5-years-old, malaria was reported in 30% in 2011, but there were important differences within urban and rural zones with malaria prevalence at the level 20.6% and 37.1%, respectively [9]. The eastern region of Cameroon, i.e. the area with the largest CAR refugee population (due to recurrent ethnic and political conflicts) is considered the main cause of high malaria transmission in Cameroon [10], suggesting a higher

malaria prevalence rate in the much poorer CAR. Studies conducted in Cameroon using molecular biology methods confirmed the presence of four species of *Plasmodium*: *P. falciparum, P. vivax, P. ovale* and *P. malariae* [10–13], with a definite predominance of *P. falciparum*, responsible for 95% of infections [10]. *P. vivax* was found in 5.6% patients (38.6% of all PCR positive samples) [12], *P. ovale* and *P. malariae* were found predominantly in a very low percentage as a co-infection [14]. These results were consistent with results obtained by in the current study. Another study on the prevalence of malaria in the neighbouring region was carried out by Tsumori et al. [15] in the Republic of Congo, adjoining to the north with the CAR. The study demonstrated that malaria infection rates were found differ between the methods used. Microscopic examination revealed that 37% of the subjects living in urban areas were infected with malaria, whereas PCR tests were positive in 42% of the sample. In the group from rural areas, RDTs were positive in 59% cases and PCR tests in 72% of the subjects [16]. Studies conducted in the Republic of Congo indicate that *P. falciparum* was responsible for the vast majority of malaria cases in the region [15,17,18], although cases caused by *P. vivax* [20], *P. ovale* and *P. malariae* were also reported and should not be neglected [15].

According to the results of the present study conducted in Dzanga Sangha, *P. falciparum* was found in 94.8% of all malaria cases; however, the percentage distribution of other *Plasmodium* species was higher (*P. malariae* 11.1%, *P. ovale* 9.8%, *P. vivax* 0.7%) than the 0.3% reported in the 2019 WHO report. This fact is important in the context of the treatment of malaria patients and highlights the necessity to introduce more accurate diagnostic method, and to revise malaria treatment protocols (introduction of a drug regimen targeting the latent forms the CAR, PCR does not play a role, but in the context of the epidemiological studies its role is crucial. The PCR was essential for establishing the presence of the different *Plasmodium* species, and was a comparative method for RDTs false-negative sensitivity, and for attempting to discoover the factors responsible for it.

Until 2010, patients with suspected malaria were administered the recommended treatment but were rarely offered diagnostic tests, which was largely due to limited testing capabilities in the region. Since 2010, however, owing to the WHO suppor, and its recommendations for pre-treatment diagnosis of malaria in every symptomatic patient, RDTs and microscopic examinations have become more common [21]. RDTs in particular are now widely used in the region, and globally their sales increased from 46 million in 2008 to 320 million in 2013 [4].

In the rural areas of Africa, such as Dzanga Sangha in the south-western parts of the CAR, RDTs are often the only available diagnostic method for malaria detection. Unfortunately, the number of RDTs distributed by non-governmental organizations (NGOs), as part of the global malaria programme is often insufficient. The author's (EB-S) observations made during the study period have led to the conclusion that malaria diagnosis and treatment protocols applicable in the CAR leave much to be desired. As an example, a vast majority of medical interventions in the Dzanga Sangha region are performed by people without a medical background, and the use of expired medications and RDTs or random drug use is common. Additionally, medications and RDTs are rarely stored properly and a large

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number of patients purchase medications at local street markets.

The results of the current study in the group of BaAka Pygmies have demonstrated significant differences between the results of RDTs and PCR tests. Therefore, the question should be raised on the accuracy and usefulness of RDTs in a situation in which the initiation of anti-malaria treatment depends entirely on the interpretation of the RDTs results, regardless of the patient's condition or the severity of clinical signs and symptoms. On the other hand, the decision to administer anti-malarial treatment on the basis of non-specific symptoms, which may be the manifestation of many other diseases, e.g. bacterial infections of the respiratory and urinary tract or cosmopolitan and tropical diseases of viral etiology, is not always justified, and may lead to ove-diagnosis of malaria [22, 23]. Primary healthcare providers in sub-Saharan Africa often prescribe anti-malarial treatment in combination with other antibiotics to treat all kinds of health problems in patients, without performing the basic diagnostic tests [22, 24]. There are three main possible causes of false-negativity of the RDTs. Lower sensitivity of RDTs (estimated at 88–90% by their manufacturer) compared to PCR tests is attributable to several factors. One of the limitations of an RDT is its detection limit which has been estimated at between 200 – 2,000–5,000 parasites/µl, which corresponds to 200 infected erythrocytes per microliter [4]. In cases of very low parasitemia, RDT is likely to give a false negative result. This conclusion is also supported by the work of Djallé et al. conducted in the CAR where the sensitivity of three different RDTs was proved to correlate with an intensity of the parasitemia in the blood sample. The sensitivity was 95% in a high parasitemia level (>500 parasites/ul), but in low parasitemia (100 parasites/ul) the sensitivity was less than 70% [25]. The important limitation of this study was that the parasitemia wa not assessed by a quantitative method.

In this study, after comparison of patients with a positive vs. patients with a negative RDT result, it was concluded that patients aged <5, weighing up to 10 kg, were more likely to have a positive RDT result (64.8% vs. 25.2%). This conclusion is consistent with the WHO data [1], according to which malaria is most prevalent in children <5-years old. The results were quite the opposite in the group of adults over the age of 18 (20.1% vs. 56.7%). This prescribing a malaria treatment on the basis of clinical symptoms without any laboratory diagnostics. In the group of patients with an RDT-positive result, there were significantly more patients with body temperature of >39 °C (26.0% vs 8.7%), and it can be assumed that these patients had a higher parasitemia with *P. falciparum* and therefore the HRP2-specific RDT was positive in these cases [25, 26], or the elevated body temperature in the youngest subjects could have been a result of their hyper-reaction to infection with *P. falciparum,* given their lack of immunity. People living in endemic areas, with age, naturally acquire immunity to *P. falciparum* malaria [22, 27–29]. Premunition, or otherwise a partial immunity observed in the asymptomatic or poor-symptomatic individuals with a detectable presence of malaria parasites in the blood, inhabiting endemic areas, is gained with repeated contacts/infections with *Plasmodium spp.,* which is considered a protective factor against severe manifestation of the disease [30]. The second cause of false-negative RDTs might be deletion of the Pfhrp2 gene which results in a lack of the antigen HRP2-protein and which is a target for

the RDTs HRP2. A high percentage of infections caused by *P. falciparum* parasites with a Pfhrp2 gene deletion has been reported from sub-Saharan Africa, in the Indian sub-continent, and in Peru, South America. The Pfhrp2 gene is responsible for the production of the HRP2 protein, which is an antigen detected by the *P. falciparum*-specific RDTs. The rate of such infections has been estimated at approximately 5% in sub-Saharan Africa [31–33], up to 40% in Peru [34, 35], and even over 80% in Eritrea, depending on the region [36]. As suggested by the WHO, the use of RDTs targeting the HRP2 protein for the diagnosis of malaria is questionable when the percentage of *P. falciparum* species with a Pfhrp2 deletion is over 5% in a given area [37, 38]. There are no data available on the prevalence of species with Pfhrp2 deletion in the Central African Republic; however, in the neighbouring Democratic Republic of Congo, the rates of *P. falciparum* species with the Pfhrp2 gene deletion have been estimated at 6.4% [39], results which may be similar in other countries of the region, including the CAR. The current study did not analyse the exon 2 for the assessment of the Pfhrp2/3 deletion. Further studies to evaluate the prevalence of the Pfhrp2/3 gene deletion seem necessary in the context of determining the usefulness of RDTs targeting HRP2 protein in Central Africa. Additionally, the existence of anti-HRP2 antibodies forming the immune-complexes with HRP2 antigen in *P. falciparum* infected individuals may also result in false-negative RDTs results [40].

In order to reduce the number of false negative RDT results, in countries with a high prevalence of *P. falciparum* species with a Pfhrp2 deletion or regions with high rates of malaria parasites other than *P. falciparum*, RDTs detecting pLDH are additionally used [26]. However, they are less sensitive in cases of low parasitemia ≥200–1000 parasites/µl than RDTs directed against HRP2 protein [22] or aldolase. Both enzymes are produced by all malaria species found in humans [37] and have a short half-life of 2–4 days. Therefore, they can be found in the blood of individuals with an active malaria infection [41]. Nevertheless, the WHO recommends restricting the use of RDTs that detect both HRP2 and pLDH proteins to regions where infections by one species of malaria are expected [42], presumably for a cost-effective reasons. In the current study, RDTs other than those targeting HRP2 specific for *P. falciparum,* were not available in the Dzanga Sangha region. In the CAR the gold standard for malaria diagnostics is microscopic examination [7], but in the remote and poor areas like Dzanga Sangha, RDTs remain the only available alternative.

There are some important limitations of the presenteds study, including lack of quantitative designation of parasitemia in the PCR method that could precisely indicate cases of active malaria. Light microscopy for evaluation of thick and thin blood smears would allow better comparison of the effectiveness of all three methods – light microscopy, RDT and PCR. It would be best to choose RDTs from one manufacturer, ideally RDTs that differentiate *Plasmodium* species. These limitations are open to future research to improve and deepen this subject.

CONCLUSIONS

Malaria diagnosis is an important element of effective treatment, and thus limiting the transmission and ultimate

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elimination of the disease worldwide. In developing countries, such as those in sub-Saharan Africa, RDTs provide a good, albeit imperfect, alternative to clinical symptoms-based diagnosis. The presented study of BaAka Pygmies, showing a significant disproportion in RDTs and PCR results, requires that the clinical manifestation should be an important component of the final diagnosis. It is worth considering whether, due to the presence of over 5% of infections with *Plasmodium* species other than *P. falciparum*, the use of RDTs detecting pLDH and/or aldolase would not contribute to greater diagnostic efficiency. In the future, the prevalence of Pfhrp2/Pfhrp3 gene deletions in Central Africa might provide valuable data on the utility of RDTs based on the detection of the *P. falciparum* HRP2 antigen.

Aknowledgements

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