VIA MEDICA

ORIGINAL ARTICLE

Int Marit Health
2024; 75, 2: 103–108
10.5603/imh.100440
www.intmarhealth.pl

ISSN 1641-9251
eISSN 2081-3252

Prevalence of malaria in Arusha Region in the northern Tanzania

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ABSTRACT

Background: *The World Health Organization (WHO) reported an estimated 249 million malaria cases globally in 2023, of which 94% were reported from Africa. Tanzania, a Sub-Saharan African country, has an exceptionally high malaria prevalence (3.6 million in 2023). The aim of the present study was to assess malaria prevalence rates in the Arusha Region, northern Tanzania. This region is famous for its national parks and wildlife reserves, and it is visited by thousands of tourists from all over the world each year. The assessment of malaria prevalence in the region is important in the context of the necessity to administer antimalarial chemoprophylaxis to international travellers.*

Material and methods: *The study group consisted of 101 people, residents of the Karatu District in the Arusha Region, aged between 1 and 73 years, who volunteered to participate in the screening. Phase I of the study was conducted in July 2022 in the Karatu Lutheran Hospital in Karatu Town (located close to the Ngorongoro Conservation Area and the Serengeti National Park). During this phase a venous blood sample was collected from each patient. The samples were tested for malaria using a rapid diagnostic test (mRDT); the same samples were also used to measure haemoglobin concentration and next they were applied onto the Whatman FTA micro cards for further molecular diagnostics in Poland (phase II).*

Results: *mRDT detected two (2.0%) infections caused by Plasmodium (the etiological factor of malaria), the molecular tests (RT-PCR) confirmed the two positive results by mRDT but also detected infections in six other samples (7.9% in total). The study found that six patients were infected with the Plasmodium falciparum species, while two other subjects had co-infections (P. falciparum + P. ovale, P. falciparum + P. vivax + P. malariae).*

Conclusions: *The study findings confirm the prevalence of malaria in areas located close to national parks in northern Tanzania and support the use of antimalarial chemoprophylaxis in international travellers visiting the area. The present study found co-infections caused by four different species of Plasmodium species which supports the prevalence of different parasitic species in Sub-Saharan Africa and is in line with CDC reports but contrary to WHO reports which estimate that 100% of malaria cases in Sub-Saharan Africa are caused by P. falciparum.*

(Int Marit Health 2024; 75, 2: 103–108)

Keywords: malaria, *Plasmodium*, mRDT, PCR, Tanzania

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Received: 26.04.2024 Accepted: 18.05.2024

INTRODUCTION

Malaria is a parasitic disease which in humans is primarily caused by one of the five species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi*. Thanks to the development of molecular diagnostics two other species of *Plasmodium*, which could potentially be responsible for malaria in humans, have been identified recently: *P. simium* and *P. cynomolgi*. Similarly to *P. knowlesi*, they are enzootic species, but little is yet known about the two newly discovered species. Malaria is transmitted by female *Anopheles* mosquitoes (a vector of infection) through a bite from an infected insect when sporozoites (a stage of *Plasmodium* at which the parasite is invasive to humans) enter the human bloodstream. Malaria can also be transmitted through a blood transfusion or vertically, from an infected mother to a foetus. Malaria infection can be symptomatic or asymptomatic. Symptomatic malaria usually manifests with fever, chills, excessive sweating, nausea and vomiting while asymptomatic patients show no clinical signs of the disease. Severe forms of malaria (usually caused by *Plasmodium falciparum)* manifest with high fever (39–41°C) and are often complicated by severe anaemia, thrombocytopenia, coagulation disorders, metabolic acidosis, multiple organ failure, coma (cerebral malaria), pulmonary complications, acute kidney injury [1, 2]. The World Health Organization (WHO) reported of an estimated 249 million malaria cases globally in 2023, of which 94% were seen in Africa. Tanzania, a country lying in Sub-Saharan Africa, also has a high malaria prevalence (3.6 million in 2023) [3]. According to the WHO reports, *P. falciparum* species is responsible for all malaria cases in Sub-Saharan Africa [3]. In contrast, the reports published by the Centres for Disease Control and Prevention (CDC) point to the fact that although most infections in Sub-Saharan Africa are indeed caused by *P. falciparum*, infections with *P. malariae*, *P. ovale*, and *P. vivax* can also be found in the region [4]. Although the overall epidemiological situation in Tanzania has improved in recent years and the number of confirmed malaria cases has dropped compared to previous years, the disease remains a major public health concern and is a leading cause of morbidity and mortality in the country, particularly in children under 5 years of age; this fact is supported by the statistics released by many hospitals and outpatient clinics [5]. Currently, Tanzania ranks four globally in terms of malaria-related mortality (4% of all malaria deaths are reported from Tanzania), following Nigeria (31%), the Democratic Republic of the Congo (12%) and Niger (6%) [3]. It needs to be pointed out that malaria endemicity and epidemiology in Tanzania is changing due to a change of climate and topography [6]. Malaria transmission was previously observed only in the lowland areas of the country, but it is now also prevalent in the mountainous regions which were formerly considered non-endemic. The Karatu District with its capital Karatu Town (located at 1,420 m above the sea level and with two rainy seasons: a short rainy season from October until December and a long rainy season from March until June) is a perfect example of such a change. Karatu District lies in close proximity to the famous national parks (the Ngorongoro Conservation Area, Serengeti, Lake Manyara, Tarangire, Kilimanjaro) which are visited by hundreds of thousands of tourists from all over the world every year [7].

The aim of the present study was to assess malaria prevalence rates in residents of the Arusha Region, northern Tanzania. The assessment of malaria prevalence in the region is particularly important in the context of the necessity to administer antimalarial chemoprophylaxis to international travellers.

MATERIAL AND METHODS

The study group consisted of 101 people, residents of the Karatu District (which is one of the seven Districts in the Arusha Region), aged between 1 and 73 years who volunteered to participate in the study. The patients were required to complete and sign a patient health questionnaire and an informed consent form (written in Swahili). Parental consent was required for paediatric patients. Phase I of the study was conducted at the Karatu Lutheran Hospital in Karatu Town (located close to the Ngorongoro Conservation Area and the Serengeti National Park) in July 2022. During phase I of the study, a venous blood sample was collected from each patient. The samples were tested for malaria using a rapid diagnostic test (mRDT). The same samples were also used for haemoglobin concentration measurements. Next, the samples were applied onto the Whatman FTA micro cards (200–300 µL) for further studies to be conducted in January 2023 in Poland (phase II consisting of molecular diagnostics).

METHOD DESCRIPTION

Malaria Rapid Diagnostic Test (mRDT, AllTest Malaria Pf/Pv/Pan) was used for the detection of infections with four different *Plasmodium* species: *P. falciparum, P. vivax, P. ovale/P. malariae* (Pan) in whole blood samples. The test works based on the immunochromatographic principle. To perform the test, a drop of venous or capillary blood (5 µL collected from the test tube or directly from a fingertip pricked with a calibrated pipette provided by the manufacturer) was applied into the sample well on the test pad. The blood specimen migrates through the nitrocellulose membrane by capillary action. Next, 3 drops of the buffer assay, which is attached to the test kit, were added into the buffer well. The membrane is pre-coated with anti

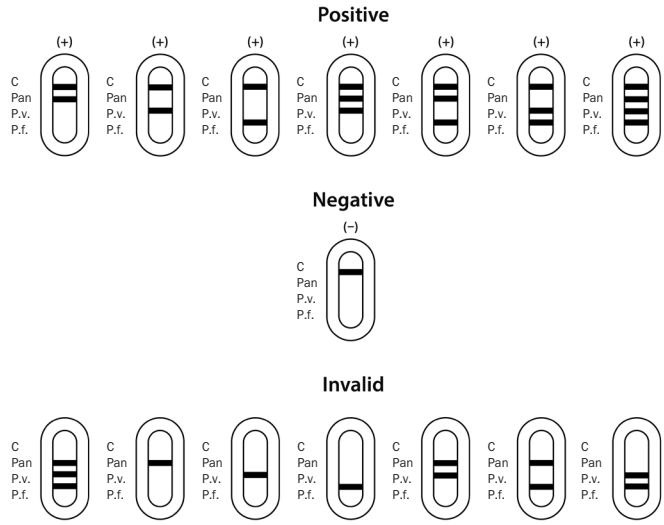

Figure 1. Interpretation of the mRDT (AllTest Malaria Pf/Pv/Pan) test results

P. falciparum anti-HRP2 (histidine rich protein-2) antibodies as well as with anti *P. vivax* and anti-Pan *Plasmodium* LDH (lactate dehydrogenase) antibodies. The blood sample is absorbed by the membrane and if *Plasmodium* parasites are present in the blood sample *P. falciparum* HRP2 antigens bind to the anti-HRP2 antibodies; *P. vivax* LDH antigen binds to anti-LDH antibodies and *P. ovale* and/or *P. malariae* LDH antigens bind to anti-Pan LDH antibodies showing a positive result. The interpretation of the test results depends on the presence or absence of colour bands in the control and test areas (Pf, Pv, Pan) on the test pad. The presence of two bands — in the control area and the test area indicated a positive test result. The presence of a band in the control area only indicated a negative test result. The presence of a band or absence of bands on the test pad indicated that the test was invalid, and the interpretation of the results was not possible (Fig. 1). The results were read after 10–15minutes. The test's sensitivity is assessed to be 98.7% and its specificity — 99.3% [8].

Molecular diagnostics by RT-PCR was carried out during phase II of the study at the Department of Epidemiology and Tropical Medicine of the Military Institute of Medicine — the National Research Institute in Gdynia, Poland. To perform the test, genetic material was extracted from dried blood specimens (200–300 µL of venous blood) collected on the Whatman FTA micro cards [9], stored with a desiccant in tightly sealed foil bags. Four 2-mm-discs were punched out from the dried blood samples (with the Harris Uni-Core punch) and the genetic material was isolated from the discs using the Sherlock AX kit (A&A Biotechnology, Gdańsk, Poland) following the manufacturer's instructions [10]. The extracted DNA was stored at 4 °C if samples were to be analysed without delay or at the temperature of –20 °C if they were to be analysed at a later time. The FTD Malaria differentiation real-time multiplex PCR kit (Fast Track Diagnostics, Sliema, Malta) specifically targeting *P. falciparum*, *P. vivax, P. malariae*, and *P. ovale* were used to detect *Plasmodium* DNA. RT-PCRs were run on an AriaMx Real-time PCR system (Agilent Technologies, Santa Clara, California, USA).

ETHICAL CONSIDERATIONS

Ethical clearance for conducting health research entitled "*The prevalence of malaria in northern Tanzania in the context of the necessity to administer antimalarial chemoprophylaxis to European travellers visiting national parks*" was obtained from the Ministry of Health and the National Institute for Medical Research, Dar es Salaam, the United Republic of Tanzania (Ethical Clearance Certificate for Conducting Medical Research in Tanzania, Ref. No. NIMR/HQ/R.8a/Vol. IX/4040, 05 July 2022).

RESULTS

The study group consisted of 101 patients, including 14 children aged 1–15 years (13.9%) and 87 adults aged 16–73 years (86.1%), both males and females, residents of the Karatu District. Malaria rapid diagnostic tests (mRDT) showed two infections (2.0%) with *Plasmodium* — one *P. falciparum* infection in a child and one co-infection with *P. falciparum* + Pan (*P. ovale/P. malariae*) in an adult. Both results were later verified by light microscopy at the Karatu Lutheran Hospital. The RT-PCR tests which were done in Poland during phase II of the study detected a total of eight

Table 1. *Plasmodium* infections detected with mRDT vs. PCR among patients treated at the Karatu Lutheran Hospital (n = 101)

Sex	Age	Temperature (°C)	Hb [g/dL]	mRDT	PCR
F	73	37.7	15.5	P.f. + Pan	P.f. + P.o.
F	4	39.0	7.8	P.f.	P.f.
M	32	37.7	16.2	Negative (–)	P.f.
M	79	37.7	14.8	Negative (–)	P.f.
F	30	36.7	8.6	Negative (–)	P.f.
F	34	36.4	14.3	Negative (–)	P.f.
F	20	36.0	9.5	Negative (–)	P.f.
F	1	36.4	9.1	Negative (–)	P.f. + P.v. + P.m.

F — female; M — male; Hb — haemoglobin; mRDT — malaria rapid diagnostic test; PCR — polymerase chain reaction; P.f. — *Plasmodium falciparum*; Pan — *Plasmodium ovale/Plasmodium malariae*; P.o. — *Plasmodium ovale*; P.v. — *Plasmodium vivax*; P.m. — *Plasmodium malariae*

infections with *Plasmodium* (7.9%). The molecular methods confirmed the two positive mRDT results but also detected infections in six other samples which tested negative on mRDTs. Of the eight infected patients, six were infected with the *Plasmodium falciparum* species, while two other subjects had co-infections (*P. falciparum* + *P. ovale*, *P. falciparum* + *P. vivax* + *P. malariae*); four patients had normal body temperature (< 37.0°C) and four had temperature > 37.5°C. Similarly, four infected patients had normal haemoglobin (Hb) concentration and the remaining four patients had Hb concentration ranging between 7.8 and 9.5 g/dL (Table 1). In the group of the remaining 93 patients the results of both RT-PCR and mRDTs tests were negative. The patients who tested negative for *Plasmodium* infection had body temperature ranging between 36.0–38.7°C; six of those patients (6.4%) had body temperature > 37.5°C (fever was caused by illnesses other than malaria). Haemoglobin concentration among negative subjects ranged between 6.4 and 18.1 g/dL (77 patients with a negative malaria test had Hb concentration > 12.0 g/dL, 16 subjects — < 12.0 g/dL).

DISCUSSION

Tanzania is a Sub-Saharan African country with high malaria morbidity and mortality. The country is a popular tourist destination for international travellers from non-endemic countries. They often come to Tanzania to visit national parks and wildlife reserves in the north of the country. The top most often visited national parks in Tanzania include Serengeti (472,000 tourists in 2019), before Tarangire (288,000), Lake Manyara (213,000) and Kilimanjaro (75,000) [7]. Many tourists arriving from non-endemic countries in Europe, North America or Asia fail to have a pre-travel health consultation before visiting Tanzania, and a lot of tourists abstain from antimalarial chemoprophylaxis. In Europe, more than 8,000 cases of imported malaria are diagnosed each year, with most of these cases being imported from Africa. Approximately 50% of malaria cases in Europe are seen in two countries: in France and the UK [11]. In Poland, there are no more than a hundred malaria cases reported each year (26 cases in 2022, 43 cases in 2023) [12], but the number of imported cases is likely to be underestimated due to limited diagnostic capabilities of the healthcare facilities in Poland and diagnostic difficulties.

According to the WHO reports, *P. falciparum* is responsible for 99.7% of all malaria cases in Africa and 100% of malaria cases in Sub-Saharan Africa [13]. Therefore, most quick immunochromatographic assays (rapid diagnostic tests, RDTs), which are used for malaria confirmation in Sub-Saharan Africa can only detect infections caused by *P. falciparum* [14]. The authors' own study carried out in the Central African Republic between 2019 and 2021 showed greater diversity of *Plasmodium* species in the region (*P. vivax*, *P. malariae*, *P. ovale*) [15–17]. The present study, which involved 101 people living in north Tanzania and was conducted in 2022 also found infections caused by different *Plasmodium* species. The estimates released by WHO, according to which *P. falciparum* is responsible for all malaria cases in Sub-Saharan Africa, have their consequences in terms of the management of malaria in the region. Currently, WHO recommends ACT (*artemisinin-based combination therapy*) as the first-line treatment of malaria in Africa. ACT is effective for the treatment of uncomplicated *P. falciparum* malaria, but infections caused by *P. vivax* and *P. ovale* require the use of combination therapy. This is associated with some differences in the lifecycle of *P. vivax* and *P.* ovale; in *P. vivax* and *P. ovale* infections most merozoites are released into the bloodstream of the host, but some penetrate liver cells and form hypnozoites, i.e. a dormant form which can activate and develop into erythrocytic schizonts after several weeks, months or even years after the primary infection thus causing a recurrence of malaria at a later time. The differences between the lifecycle of *P. vivax/P. ovale* and that of P. *falciparum* mean that successful treatment of infections caused by *P. vivax/P. ovale* require combination treatment with quinoline derivatives (primaquine, tafenoquine) as only these drugs can eliminate hepatic schizonts and prevent malaria recurrence [18, 19]. The use of the WHO-recommended malaria treatment protocol, which is limited to the management of cases caused by only one *Plasmodium* species, may contribute to a rise in the number of malaria

cases in Africa, which can potentially have negative consequences for the global malaria epidemiology in the future. Tourism industry specialists estimated that international tourism will soon return to pre-pandemic levels and that Africa will continue to be a popular tourist destination for international visitors, mainly from Europe. Given the fact that there are many popular tourist destinations in Sub-Saharan Africa, e.g. the national parks in northern Tanzania, the knowledge of current malaria prevalence in Africa is not only important for local healthcare providers, but also for medical practitioners from Europe specializing in the prevention, diagnosis and treatment of febrile tropical diseases in returning travellers, of which malaria has been the most common. Definite diagnosis of malaria is based on the detection of *Plasmodium* parasites in a blood sample, while quick immunochromatographic tests (RDTs), which detect *Plasmodium* antigens, are generally used for screening purposes. Low parasitaemia or an infection by a parasite other than *P. falciparum* may result in a false negative result and eventually a misdiagnosis. For this reason, it is strongly recommended to use mRDTs, which are capable of detecting multiple *Plasmodium* species. It is also recommended that mRDT results be verified (confirmed or ruled out) using diagnostic methods with higher specificity, such the thick or thin smear procedures. The thick smear procedure (where a drop of peripheral blood is examined under a microscope) is used for quantitative diagnosis of malaria and makes it possible to determine the level of parasitaemia starting from parasite density as low as 30–50/μL. A thin smear procedure, on the other hand, is used for qualitative diagnosis, i.e. it is used to identify the species of *Plasmodium* responsible for an infection (this procedure is based on the examination of differences in the morphology and the number of erythrocytic forms of the parasite as well as the observation of changes in the shape of the infected erythrocytes). The biggest disadvantage of light microscopy methods is the shortage of qualified specialists in parasitology. For this reason, molecular biology methods (mainly PCR) have become increasingly common for diagnosing malaria in many countries [1, 20]. The results of the present study show that many different *Plasmodium* species are present in Sub-Saharan Africa; these finding are supported by the authors' previous studies and studies by other authors as well [21–23]. The authors recommend using malaria tests which are capable of detecting infections by various *Plasmodium* species (*P. vivax, P. ovale, P. malariae*) both locally and among travellers returning from Africa to non-endemic countries. This is particularly important since the number of imported malaria cases in Poland is likely to be significantly underestimated. Travellers who experience fever after travelling to a tropical country should be routinely tested for malaria, but it is rarely the case in Poland. Instead, returning travellers presenting with fever and other symptoms which might be indicative of a *Plasmodium* infection are commonly diagnosed as having influenza or a viral infection caused by cosmopolitan pathogens. In view of the fact that millions of Polish people travel internationally each year (in 2019, i.e. before the COVID-19 pandemic, it was around 12.7 million) with tens of thousands travelling to Africa, it is crucial to conduct malaria surveillance and monitoring in the most popular tourist destinations around the world. This is particularly important in the context of the risk of importing malaria to non-endemic countries and the necessity to administer antimalarial chemoprophylaxis to international travellers.

CONCLUSIONS

The present study confirms the prevalence of malaria in northern Tanzania and supports the necessity to administer antimalarial chemoprophylaxis to international travellers visiting this part of Africa. The study found co-infections caused by four different *Plasmodium* species in the local population, thus demonstrating that different parasitic species are present in Sub-Saharan Africa. These findings are in line with the CDC reports, but contrary to the WHO reports, according to which 100% of malaria cases in Tanzania are caused by *P. falciparum*.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article.

Ethics statement: Ethical clearance for conducting health research entitled "*The prevalence of malaria in the northern Tanzania in the context of the necessity to administer anti-malarial chemoprophylaxis to European travellers visiting national parks*" was obtained from the Ministry of Health and the National Institute for Medical Research, Dar es Salaam, the United Republic of Tanzania (Ethical Clearance Certificate for Conducting Medical Research in Tanzania, Ref. No. NIMR/HQ/R.8a/Vol.IX/4040, 05 July 2022).

Author contributions: Daria Kołodziej — diagnostics, preparation of the project and typescript; Heriel Zacharia Ammi — diagnostics, logistics; Wanesa Richert — diagnostics, selection of the data; Małgorzata Marchelek-Myśliwiec — selection of the data; Krzysztof Korzeniewski — diagnostics, preparation of the final version, diagnostics.

Funding: This research was funded by The Ministry of Science and Education in Poland, grant number 611/WIM, and the APC was funded by the Military Institute of Medicine, Waszawa, Poland. The funders had no role in study design, data collection and analyses, decision to publish, or preparation of the manuscript.

Acknowledgements: No acknowledgements.

Conflict of interest statement: Authors declare no conflict of interest in relation to this article.
Supplementary material: No supplementary material.

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