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Investigation of Malaria Parasitemia among Asymptomatic Individuals in Designated Districts of Central and Muchinga Provinces of Zambia – A Cross Sectional Study

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***Abstract*. The aim** of the study was to investigate asymptomatic malaria in health-conscious population across four selected districts. Malaria is a life-threatening disease caused by *Plasmodium* spp. transmitted through bites of infected female *Anopheles* mosquitoes. Asymptomatic malaria refers to the presence of malaria parasites *in vivo* without symptoms, which usually provides a reservoir for the disease transmission. **Materials and methods.** Blood collected in EDTA underwent testing through RDT (SD Bioline one-step malaria antigen P.f. (HRP-II) rapid test kits), while thin and thick blood smears Giemsa stained were microscopically examined. **Results and discussion.** Out of 385 individuals examined, 84 people (21.8 %) tested positive for malaria using RDT and 101/385 (26.2 %) – through microscopy. Microscopic examination further identified 27 individuals (7.0 %) with gametocytes and 74 (19.2 %) – with trophozoites. Intriguingly, 17 (4.4 %) samples showed negative results in RDT but exhibited trophozoites and gametocytes upon smear examination. District-wise analysis demonstrated the highest malaria positivity rate in Kanchibiya district, with 32 cases (8.3 %) detected by RDT and 35 (8.5 %) – through microscopy. Chitambo district followed closely: with RDT and microscopy values of 25 (6.5 %) and 33 (8.4 %), respectively; while Mpika and Serenje districts had 13 (3.4 %) and 14 (3.6 %) prevalence, respectively, with RDT and microscopy at 12 (3.1 %) in both districts [x^2=16.3, p-value=0.0118]. The study also revealed that 365/385 (95 %) of the participants demonstrated knowledge and positive attitudes toward malaria. Our findings accentuate the presence of asymptomatic malaria, encompassing trophozoites and gametocytes, among seemingly healthy individuals which poses a health risk to the community. Therefore, it is imperative to implement preventive chemotherapy and strengthen vector control efforts against malaria in order to reduce the infection rate.

Key words: asymptomatic malaria, malaria parasitemia, *Plasmodium* species, prevalence.

Conflict of interest: The authors declare no conflict of interest.

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Bioethics: This study was a field community based study, involving direct contact with participants. The human blood samples were used for the study. Confidentiality of participants were maintained by ensuring that no participant's name or identity number appeared on data information sheet; newly generated identification numbers were applied. Permission to carry out the study was obtained from the Provincial Health Directors of Central and Muchinga Provinces. The study was approved by the Chreso University Research Ethics Committee (CUREC), Protocol REF No. 1577-12-2022 and National Health Research Ethics Board, NHRA REF No. B005/12/06/2023.

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Исследование малярийной паразитемии среди бессимптомных лиц в ряде округов провинций Центральная и Мучинга Замбии – перекрестное исследование

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Цель исследования – изучение бессимптомной малярии среди населения, заботящегося о своем здоровье, в четырех районах Замбии. Бессимптомная малярия означает наличие малярийных паразитов *in vivo* без симптомов и является резервуаром для передачи заболевания. **Материалы и методы.** Кровь, собранную в пробирки с ЭДТА, тестировали с помощью ИХА (наборы для одноэтапного экспресс-тестирования на антиген P.f. [HRP-II] SD Bioline [Южная Корея]), а окрашенные по Гимза тонкие и толстые мазки крови подвергались микроскопическому исследованию. **Результаты и обсуждение.** Из 385 обследованных лиц 84 (21,8 %) дали положительный результат на малярию с помощью ИХА и 101 (26,2 %) – с помощью метода микроскопии. При микроскопическом исследовании у 27 (7,0 %) идентифицированы гаметоциты и у 74 (19,2 %) – трофозоиты. Показали отрицательные результаты при ИХА 17 (4,4 %) образцов, но при исследовании мазка обнаружили трофозоиты и гаметоциты. Анализ по районам показал, что в районе Канчибия наблюдается самый высокий показатель положительных результатов по малярии: 32 (8,3 %) выявлены с помощью экспресс-теста и 35 (8,5 %) – с помощью микроскопии. В провинции Читамбо показатели ИХА и микроскопии были 25 (6,5 %) и 33 (8,4 %) соответственно, в то время как результаты ИХА в округе Мпика и Серенье были 13 (3,4 %) и 14 (3,6 %), а при использовании микроскопии – 12 (3,1 %) в обоих округах [x^2=16,3, p-значение=0,0118]. Продемонстрировали знания и серьезное отношение к малярии 365 из 385 (95 %) участников. Наши результаты подтверждают возможность бессимптомного течения болезни, вызванной возбудителями малярии, включая трофозоиты и гаметоциты, среди внешне здоровых людей, которые представляют риск для здоровья общества. Поэтому крайне важно проводить профилактическую химиотерапию и активизировать усилия по борьбе с переносчиками инфекции, чтобы снизить уровень инфицирования.

Ключевые слова: малярия, бессимптомное течение, малярийная паразитемия, виды *Plasmodium*, распространенность.

Конфликт интересов. Авторы подтверждают отсутствие конфликта финансовых/нефинансовых интересов, связанных с написанием статьи.

Финансирование. Авторы заявляют об отсутствии дополнительного финансирования при проведении данного исследования.

Биоэтика. Исследование являлось полевым исследованием на уровне общины и включало прямой контакт с участниками. Для этого исследования были использованы образцы человеческой крови. Конфиденциальность участников сохранялась за счет того, что имя или идентификационный номер участника не появлялись в информационном листе, а использовались заново сгенерированные идентификационные номера. Разрешение на проведение исследования получено от руководителей здравоохранения провинций Центральная и Мучинга. Исследование одобрено Комитетом по этике исследований Университета Чресо (CUREC), протокол REF № 1577-12-2022 и Национальным советом по этике исследований в области здравоохранения, NHRA REF № B005/12/06/2023.

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Asymptomatic malaria refers to the presence of malaria parasites in the blood without symptoms, serving as a reservoir for transmission and preceding the onset of symptomatic malaria [1]. Gametocytes play a direct role in preserving *Plasmodium falciparum*, the most lethal human parasite. Malaria, a life-threatening disease transmitted through bites of infected female *Anopheles* mosquitoes, which is preventable and curable. The disease presents symptoms of fever, headache and chills, though these symptoms may be mild and difficult to recognize. Among the five parasite species causing malaria in humans, *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest threat. Despite extensive interventions for malaria elimination, it remains a serious global health challenge.

Globally, 3.2 billion people are at risk of malaria, with an estimated 229 million cases and 409,000 deaths in 2019. Africa bore the brunt with an estimated 215 million cases, constituting 94 % of global malaria cases in 2019. The concerted efforts of vector control, preventive

chemotherapy, and improved diagnosis and treatment contributed to a 60 % decline in malaria deaths in many regions worldwide [2]. Despite these interventions and a reduction in mortality and morbidity, malaria remains a persistent cause of death in Africa. Malaria affects all age groups, with pregnant mothers and children under-five being the most vulnerable.

Malaria persists as a prominent public health challenge and the primary cause of morbidity and mortality in Zambia [3]. Despite a reduction in cases between 2000 and 2015, malaria is still burdensome in Zambian communities. The presence of gametocytes in asymptomatic populations may act as a reservoir, which is crucial for completing the malaria life cycle and transmission [4]. Particularly noteworthy is the role of gametocytaemia in asymptomatic individuals as an infective stage aiding continuous malaria transmission [5].

In Zambia, malaria burden is influenced by climatic factors. At the community level, Zambia faces challenges, especially in highly endemic areas, with provinces like Luapula, Northern, Central, Muchinga, and North Western having a prevalence ranging 11–30 % in 2020. The overall malaria prevalence in Zambia is reported at 78.6 % [6]. Despite ongoing malaria programs, cases persist, with limited information on parasitemia profiles in asymptomatic individuals. Zambia continues to strive for malaria elimination, emphasizing the need for comprehensive identification, treatment, and eradication of all *Plasmodium* species [7]. Detection of the disease is accomplished by using diagnostic tools such as RDT and microscopy examination. Microscopic examination is considered as the gold standard for investigation of malaria parasites. The blood smears are stained with Giemsa and examined for either the gametocytes, schizonts or the trophozoites. While some of the RDT kit may detect the antigen of *P. falcipurm* in the blood and the kit mostly rely on the detection of specific malaria antigens produced by the malaria parasites. The most common target antigen is the histidine-rich protein II (HRP-II) produced by *P. falciparum*, the most deadly malaria parasite. Asymptomatic infections pose a unique challenge, acting as a substantial reservoir for parasites and contributing to malaria transmission [8]. This study holds great importance in generating information about the gametocytaemia profile in asymptomatic individuals, offering insights into parameters involved in malaria transmission. Policymakers and stakeholders can utilize this information to revise national malaria elimination guidelines, formulate effective interventions, and address the challenge of undiagnosed infection reservoirs. **The aim** of this study is to ascertain the prevalence of parasitemia and identify the most common *Plasmodium* species among asymptomatic individuals while assessing their knowledge levels about causes, transmission, treatment, and prevention of malaria. The findings will strengthen effective strategies for reducing malaria cases in Zambia, not only diminishing morbidity and mortality, but also contributing to the development and enhancement of malaria elimination strategies.

Materials and methods

Study site. The study was carried out in Central and Muchinga provinces of Zambia, selected purposefully because other than lying in epidemiological zone of malaria transmission and its ecological nature that provided a geographical gradient, it is also a rural area that experienced various aspects of social developmental inequalities. The study area included: Chitambo, Kanchibiya, Mpika and Serenje districts.

Research design. This was a cross-sectional study involving malaria asymptomatic individuals. The qualitative method of this study was antecedent to quantitative method. In the four selected districts, at least a minimum of 96 samples were collected and four Focus Groups discussion were conducted in each district. Focus groups are facilitated discussions, held with a small group of people who have specialist knowledge or interest in a particular topic. The individuals were selected by random systematic technique in communities/villages. In each selected village 10 % of households, was sampled, the houses were counted and the first house was randomly selected and the rest were systematically selected. The household head was interviewed, and eligible members were sampled for the study.

Eligibility criteria. All asymptomatic individuals of 18 years and above were included in the study.

Exclusion criteria. Symptomatic individuals, described by CDC 2023 and those on treatment for malaria within 3 weeks were not included in the study.

Sampling frame. The random systematic technique was used and a minimum of 96 participants were selected in each district. The participants were drawn from areas after considering district, constituency and wards. The three clusters were selected using random systematic methods, and Focus Group Discussion (FGDs) were also conducted.

Each participant had an interview by administering a questionnaire which helped in data capturing and blood sample was collected for RDT and microscopy examination. The FGDs of 10–12 members consist of different professionals in the community, i.e. farmers, teachers, business people and agriculture officers. The community health workers (CHWs) helped in community mobilization and introducing the data collector to the targeted area and clarify the stories that are associated with blood collection like Satanism and other myths.

Sample analysis. Blood samples were collected in EDTA tubes and examined for malaria infections using rapid diagnostic test known as SD Bioline one-step malaria antigen P.f. (HRP-II) rapid test kits) (RDT) and smear light microscopy (SLM). The RDT was used to detect malaria for fast linkage of participant to care as the outcome for blood smear microscopy took a bit longer [9].

A. *Specimen collection for Malaria examination using Giemsa staining.* The blood smear were prepared from EDTA sample on the grease free frosted microscopic slide and stained using 10 % Giemsa stain. The

microscopic examination of blood smears were done by Qualified Biomedical personnel to detect malaria parasites and identify the most common *Plasmodium* species with reference to Paraistology manual [10].

B. *Malaria Rapid Diagnostic Testing (RDT)*. The malaria rapid diagnostic tests were performed using cassettes of SD Bioline one-step malaria antigen P.f. (HRP-II) rapid test kits following the manufacturer's instructions, after running quality control on the kit daily in the morning and when a new kit was opened.

Knowledge Attitude and Practices (KAP) data. Both qualitative and quantitative data collection was done using the questionnaire and FGD to gather data on knowledge, attitudes and practices of malaria.

Data analysis. Data was entered in excel and analysed using SPSS version 24. Age characteristics, malaria stages, gametocytaemia and species were analysed using descriptive statistics and presented as means, frequencies and percentages. Data was checked for normality using the Shapiro Wilk Test and log transformed so that it is normally distributed. A p-value of ≤0.05 was considered statistically significant.

Results and discussion

A total of 385 individuals were involved in the four districts. Our study showed that 84 out of 385 (21.8 %) were positive for malaria using RDT kit, while 101 out of 385 (26.2 %) exhibited Malaria parasites [gametocytes 27 out of 385 (7.0 %) and trophozoites 74 out of 385 (19.2 %)] on microscopic examination. Additionally, 16 out of 385 (4.2 %) samples were negative under RDT but trophozoites and gametocytes were detected upon smear examination (three *P. falciparum*, ten *P. ovale*, three *P. malariae*).

The results also revealed that Kanchibiya district had the highest number of malaria positivity on both RDT (32 out of 385, 8.3 %) and Microscopic examination (35 out of 385, 8.5 %). This was followed by Chitambo district with RDT positivity at 25 out of 385 (6.5 %) and microscopy examinations at 33 out of 385 (8.4 %). Meanwhile, the districts of Mpika and Serenje exhibited a prevalence of 14 out of 385 (3.6 %) and 13 out of 385 (3.4 %) in RDT, respectively, and had equal results for microscopy, 12 out of 385 (3.1 %).

Plasmodium specie. *P. falciparum* is the most common species of malaria parasite, accounting for 86 out of 101 (85.1 %), followed by *P. ovale* at 12 out of 101 (10.9 %), *P. malariae* at 3 out of 101 (2.9 %), and there was no *P. vivax*.

KAP about Malaria. 365/385 (95 %) of the participants demonstrated that they have knowledge and a positive attitude toward malaria disease. Only 5 % of the participants exhibited a poor attitude toward malaria prevention and control measures. Income, religion, occupational status, and educational status of the participants were associated with poor knowledge, attitude, and practices toward malaria prevention and control measures.

A significant overall prevalence of 26.2 % and 21.8 % was observed, using microscopy examination and RDT techniques, respectively, in asymptomatic healthy individuals across four selected districts in the Central and Muchinga provinces of Zambia. While this figure is higher, it remains comparable to the asymptomatic malaria prevalence (6.7 %) reported in studies conducted in Ethiopia [11]. Notably, other studies in Ethiopia still demonstrated a lower prevalence of asymptomatic malaria infection at 21.7 % [12] compared to our findings. Our study adopts the definition of asymptomatic malaria as infections in individuals who exhibit no signs or symptoms and have not undergone treatment within the past 21 days.

The data shows that both gametocytes (7.0 %) and trophozoites (19.6 %) were detected in healthy individuals, with the Kanchibiya district exhibiting the highest prevalence. This suggests that preventive measures may not have been effectively implemented. The presence of gametocytes in blood smears holds the importance in the transition of malaria infections from asexual to sexual forms within a human host, contributing to the transmission of malaria from one host to another. This observation aligns with studies conducted in Afghanistan, India, and Tanzania and suggests revising the surveillance, monitoring, and evaluation system of malaria elimination programs.

The results also revealed that microscopic examination detected more malaria parasites than RDT techniques, suggesting that microscopy technique is more sensitive and serves as the gold standard for detecting malaria parasites compared to RDT, which may probably only detected one species of malaria, the *P. falciprum*. There could be some false-negatives in RDT as suggested by C.O. Falade *et al*. who reported that high parasitemia could be due to other infection or result from a prozone effect [13]. The authors reported that there were 49 cases of RDT false-negative results with a parasite density range of 40–54 059/μL. Our findings are consistent with those reported in Nigeria by B.I. Garba *et al.*, where RDT kit detected 8.6 %, while microscopy – 46.7 % [14]. M.C. Mwenda *et al*. reported high diagnostic accuracy of microscopic examination as compared to RDT: (91.3 % vs 84.6 % respectively) false-positive in RDT [15]. Such results are significant as clinicians often consider RDT negative results as accurate reflections of the patient's condition, which may be rectified through microscopy examination as recommended by other researchers.

Asymptomatic malaria infections were found in healthy individuals, a pattern observed in Afghanistan, India, Ethiopia, and Tanzania. The study also reveals that *P. falciparum* was the most common species (85.1 %), followed by *P. ovale* (11.9 %), and *P. malariae* (2.97 %), with no detection of *P. vivax*. While inconsistent with Afghanistan, these findings align with those in Ethiopia and Tanzania [16]. The study underscores the limitation of *P. falciparum*-specific RDTs, as they missed detecting species other than *P. falciparum*, which were only

identified through microscopic examination. This has significant implications for recognizing the risk of undiagnosed infection reservoirs in the context of malaria parasite elimination, especially given the current reliance on *P. falciparum*-specific RDTs in most healthcare settings.

The level of knowledge, attitude, and practices (KAP) towards malaria was generally good (95 %). The results suggest that the majority of individuals interviewed possessed knowledge about the control and treatment of malaria disease.

In conclusion, asymptomatic malaria infections were identified in the study areas, the results indicates both trophozoites and gametocytes in healthy individuals. Notably, the RDT kit exhibited limitations in detecting certain infections, while microscopy revealed parasites in the same samples. *P. falciparum* was the most common species, second – *P. ovale*, third – *P. malariae* and there was no *P. vivax*. KAP towards malaria prevention and control measures were generally good in the study areas. Recommendations: strengthen health education on the management of malaria disease, introduce RDT Kits capable of detecting all species of *Plasmodium* in health centres lacking laboratory facilities for malaria detection. The utilization of RDT kit (specifically, SD Bioline one-step malaria antigen PF-HRP-II rapid test kits) is inadequate for detecting all types of malaria. There is a need for preventive chemotherapy against malaria and intensify vector control especially at the beginning of the rainy season in order to reduce the infection rate in the area.

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