

Performance Evaluation of Standard Health System Microscopy and Rapid Diagnostic Test for the Focused Screening and Treatment of Malaria in South-eastern Tanzania

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Abstract

Background: The Focused screening and treatment (FSAT) has been identified as one of the key approaches for reducing the malaria burden in Tanzania. However, the diagnostic performance of the local standard health system microscopy and rapid diagnostic tests (RDT) for the malaria infections is yet to be established. Thus, we aimed to evaluate the performance of the local standard health system microscopy and RDTs in comparison to that of the gold standard modality through the re-examination of blood slide microscopy findings.

Methods: We used the paired RDTs and standard health system microscopy results from the participants screened in the FSAT during 2015–2016. With the gold standard modality as reference, the results of local standard health system microscopy and RDTs were evaluated according to their sensitivity, specificity and reliability.

Results: A total of 1,497 paired standard health system microscopy and RDTs results were analysed. Of these, 679 (45.4%) samples and 818 (54.6%) samples were from the high transmission areas (HTA) and low transmission areas (LTA) in the Rufiji District of south eastern Tanzania, respectively. With the gold standard as the reference, the sensitivity of RDTs were higher than those of standard health system microscopy (87.10% vs 76.88%) and (86.99% vs 65.04%) in the HTA and LTA, respectively. Further, the false-negative rates of RDTs were lower than those of standard microscopy in both HTA (12.90% vs 13.01%) and LTA (23.12% vs 34.96%).

Conclusions: RDTs have better performance than the standard health system microscopy in terms of sensitivity and specificity for FSAT in study areas in Tanzania.

Background

Malaria remains one of the most serious vector-borne diseases impairing human health worldwide [1, 2]. More than 228 million cases of malaria were reported in 2018, of which 93% were in Africa. In Tanzania, as in many other countries of sub-Saharan Africa, the national malaria prevalence has decreased to 9.3% in 2017 from 18.1% in 2008 [3]. The “China-UK-Tanzania Pilot Project on Malaria Control” (hereinafter “Pilot Project”) was implemented in the South-eastern Tanzania to explore a new model for reducing malaria burden and possibly scaling-out the approach into other malaria-endemic countries, a collaboration among the National Institute of Parasitic Diseases (NIPD), Chinese Center for Disease Control and Prevention, and the Ifakara Health Institute (IHI), was initiated in 2015 in the Rufiji District in South-eastern Tanzania. Baseline epidemiological data were collected, and microscopy and rapid diagnostic tests (RDTs) modalities were used to identify the malaria hotspots through the focused screening and treatment (FSAT) strategy.

Given the heterogeneity of the malaria transmission intensity, prioritizing areas with the highest numbers of symptomatic cases is a rational initial approach. In this context, FSAT has been established as a key strategy for reducing the high malaria burden immediately. For example, during the campaign to contain artemisinin resistance in the Cambodia-Thailand border, 49% of cases were from 3 of the 109 villages, and 77% of cases were from 10 of the 109 villages.

Microscopy is a simple, convenient, and direct diagnostic tool for the parasitological detection, including of *Plasmodium species* (*Plasmodium sp.*), and is thus the gold standard modality for the parasitological confirmation. However, the accuracy of microscopic diagnosis is affected by the skill of the technician. In general, the blood testing can detect only approximately 50–100/μl *Plasmodium sp.*, and it has a low detection rate in asymptomatic *Plasmodium sp.* carriers with low-density infection [4]. Further, for a patient who used anti-malarial drugs with the consequent reduction of *Plasmodium sp.* density and morphological changes, challenges with parasite detection and differentiation increase, leading to further errors in microscopy [5].

RDTs are immunological methods for detecting specific antigens of *Plasmodium spp.* from the peripheral blood. It has the advantages of having high sensitivity, simple operation, and swift obtainment of results. Further, only short-term training is required for the operation of local healthcare staff. Therefore, RDTs are an ideal routine screening tool to detect malaria in the highly endemic countries with limited access to microscopy [6]. Current RDT kits are sensitive to detecting approximately 100–200 *Plasmodium falciparum* per μl blood [7].

The cost and efficiency analyses for malaria detection have been carried out in Africa. Fançonny et al. [8] adopted polymerase chain reaction (PCR) as the gold standard and compared the screening performances of microscopy and RDTs in Angola. They concluded that RDTs have a higher sensitivity for malaria detection than microscopy. Meanwhile, Harchut et al. [9] evaluated the cost efficiency of microscopy and RDTs for malaria diagnosis in the Kilombero Valley, Southern Tanzania and found that RDTs both reduced government expenditure and facilitated rapid diagnosis of malaria. Kahama-Maró et al. [10] reported that the routine malaria microscopic examinations at different health system levels showed poor quality in Dar es Salaam, Tanzania and recommended that microscopy be replaced with RDTs as the first-line diagnostic tool for malaria in all medical institutions.

With the progress of the Pilot Project, it is important to evaluate the performance of both microscopy and RDTs in the Rufiji District. Thus, this study aimed to analyse the performance of standard health system microscopy and RDTs to determine their suitability for malaria diagnosis and optimise their application for local situations. Towards this goal, FSAT blood samples were randomly selected from the pilot area, and standard health system microscopy and RDTs test results were analysed. The malaria diagnosis results of these modalities were then compared against those of the gold standard diagnostic modality (i.e. microscopic re-examination).

Materials And Methods

Study areas

The pilot Project started with workshops and kick-off meetings held by technical personnel from potential partners, with field visits in Tanzania ensuing. The field visits included consultations with central and local government authorities to identify the project study sites. Based on the systematic baseline survey as

well as local logistical convenience, the Rufiji District was selected. The selected study area covered four wards. Of the four wards, two were assigned to the intervention arm and the other two were controls.

Sample selection

FSAT was carried out from 1 November 2015 to 20 November 2016 in the Rufiji District to screen for symptomatic and asymptomatic local residents. In a preliminary investigation on the Rufiji pilot project by Tanzania local slide examiners, 1497 blood slides randomly selected from seven villages in Ikwiriri, a low transmission area (LTA), (n = 679, 45.36%) and from seven villages in Muhoro, a high transmission area (HTA), (n = 818, 54.64%) were tested. All the blood slides were stored at 4°C for the next re-examination to evaluate the microscopy performance of the local slide examiner, to ultimately compare the accuracy of microscopy and RDTs in the study areas (Fig. 1). The production and microscopy of these blood samples were completed by local health facility staff.

Standard health system microscopy

The thin and thick slides were made at the focal points. Peripheral blood slides were created immediately after sample collection on a clean, grease-free microscope slide and allowed to air dry. The films were stained with 10% Giemsa solution for 10 mins. The slides were allowed to the air dry and subsequently examined with light microscopy using an oil immersion objective lens. A slide was declared negative only after 100 microscopic fields with no parasites were observed. For each specimen, the thick films were only examined for malaria parasite detection after the parasite speciation was identified through thin films. The result was recorded as positive when both microscopists recorded a positive result and the same species. Discrepancies were resolved by involving a third microscopist [11].

Rapid diagnostic test

The RDTs used in this study specifically detects *P. falciparum* and *P. vivax* and were purchased from Standard Diagnostics (SD BiolinePf/Pv Biostandard Diagnostics, Gurgaon, Korea). *Plasmodium falciparum* and *P. vivax* were detected using histidine rich protein-2 and vivax-specific lactate dehydrogenase, respectively. A total of 5 µl of whole blood were added to the card pad, and three drops of specific lysis agent were added. The RDT result was read after 15 min according to the manufacturer's instructions and then recorded [11].

Gold standard modality

The re-examination result of microscopy of blood slides was considered the gold standard in this study. The selected slides were re-examined by two NIPD experts with WHO's Level 1 qualification for malaria microscopy following the WHO guidelines for malaria microscopy. Two microscopists from the NIPD independently re-examined each blood sample under double-blind conditions and recorded the microscopy results. Inconsistencies were resolved by involving a third microscopist.

Statistical analysis

The chi-square test (χ^2 test) was adopted for pairwise comparison of diagnostic accuracy and coincidence rate among different methods, with $\alpha = 0.05$ taken as the significant level. With the gold standard modality as reference, the results of standard health system microscopy and RDTs were evaluated according to their accuracy, reliability, and applicability [12] [10]. Evaluation indexes included sensitivity, specificity, and kappa value. All statistical analyses were performed using SAS 9.3 software.

Results

A total of 1,497 pairs of standard health system microscopy results and RDTs test results were collected in the Rufiji pilot area. Of these, 679 (40.48%) and 818 (59.52%) of the samples were from HTAs and LTAs, respectively. Of the 1,497 slides, 244 (16.30%), 382 (25.45%), and 309 (20.64%) slides were detected to be positive on standard health system microscopy, RDTs, and the gold standard modality (Table 1). The positive rate of RDTs was higher than that of the gold standard and standard health system microscopy (Table 1).

Table 1
Comparison of the positive rates among three diagnostic modalities

Wards	Diagnostic modality	Number of slides	Number of positive slides	Positive rate (%)
Ikwiriri (LTA)	Standard health system microscopy	818	93	11.37
	RDT (+)		159	19.44
	Gold standard		123	15.04
Muhoro (HTA)	Standard health system microscopy	679	151	22.24
	RDT (+)		222	32.70
	Gold standard		186	27.39
Total	Standard health system microscopy	1497	244	16.30
	RDT (+)		382	25.45
	Gold standard		309	20.64

Performance of standard health system microscopy and rapid diagnostic tests

RDTs had higher sensitivity than the local standard health system microscopy in both the HTAs and LTAs ($\chi^2 = 7.54$, $P = 0.0105$; $\chi^2 = 20.48$, $P < 0.0001$). The difference in sensitivity between RDTs and standard health system microscopy was smaller in the HTA (87.10% [95% CI: 82.26%-91.94%] vs. 76.88% [95% CI: 70.43-82.8%]) than that in the LTA (86.99% [95% CI: 80.49%-92.68%] vs. 65.04% [95% CI: 56.10%-73.17%]). Meanwhile, the standard health system microscopy had higher specificity than RDTs in both the HTAs ($\chi^2 = 41.95$, $P < 0.0001$) and LTAs ($\chi^2 = 21.76$, $P < 0.0001$). The difference in specificity between RDT and standard health system microscopy was significantly higher in HTAs (87.83% vs. 98.38%) than that in LTAs (92.52% vs. 98.13%) ($P < 0.05$). Collectively, these results support that RDTs are more accurate and reliable for screening for malaria cases. However, standard microscopy can better identify non-infected individuals than RDTs (Table 2).

Table 2
Comparison of the performance among three diagnostics modalities

Study area	Sensitivity(%)	95% CI	McNemar's Chi-squared	P	Specificity(%)	95% CI	McNemar's Chi-squared	P	NPV(%)	PPV(%)	Crude agreement rate (%)
Microscopy (both)	72.17	(67.31–77.02)	26.65	0.000	98.23	(97.47–98.91)	70.43	0.000	93.14	91.39	92.85
RDTs (both)	87.06	(83.17–90.61)			90.57	(88.89–92.26)			96.42	70.60	89.85
Microscopy (HTA)	76.88	(70.43–82.8)	7.54	0.006	98.38	(97.16–99.39)	41.95	0.000	91.86	94.70	92.49
RDTs (HTA)	87.10	(82.26–91.94)			87.83	(84.99–90.67)			94.75	72.97	87.63
Microscopy(LTA)	65.04	(56.1–73.17)	20.485	0.000	98.13	(96.98–98.99)	27.26	0.000	94.07	86.02	93.15
RDTs(LTA)	86.99	(80.49–92.68)			92.52	(90.5–94.39)			97.57	67.30	91.69

Evaluation Of Reliability

In the HTAs and LTAs, with respect to consistency, there was a significant difference between the standard health system microscopy and the gold standard in both the HTAs and LTAs ($u = 21.03138$ vs $u = 20.34338$, $p < 0.05$). The difference was also significant between RDTs and the gold standard ($u = 18.56158$ vs $u = 20.54004$, $p < 0.05$) (Table 3).

Table 3
The Kappa test among three modalities

Group	Result	u	P value
Results based on standard health system microscopy and gold standard in HTA	high_reader	21.03138	p < 0.05
Results based on RDTs and gold standard in HTA	high_RDTs	18.56158	p < 0.05
Results based on standard health system microscopy and gold standard in LTA	low_reader	20.34338	p < 0.05
Results based on RDTs and gold standard in LTA	low_RDTs	20.54004	p < 0.05

Discussion

The diagnostic performance of RDTs and local standard health system microscopy for malaria in comparison to the gold standard modality is yet to be clarified. This study showed that RDTs performed better than microscopy in the local settings in Tanzania. RDTs were more sensitive, although microscopy results were more specific.

In general, RDTs are more suitable for FSAT because microscopy training and equipment are often challenging in the low-income countries. Moreover, there are difficulties in maintaining the local staff capacity for diagnosing malaria [11]. RDTs are widely distributed in Tanzania with reliable quality control and are easy to use and interpret. In this study, RDTs positively identified *P. falciparum* in 25.45% of the slides, whereas the positive rate of the local standard health system microscopy was only 16.30%. These results were consistent with the findings of Shakeley *et al.* [13] who reported the accuracy and usefulness of RDTs for case detection in Zanzibar. Further, our findings showed that RDTs have higher sensitivity than the local standard health system microscopy in both HTAs and LTAs, indicating that RDTs are more reliable than local standard health system for case detection.

In addition, the difference in sensitivity was more significant in LTAs than that in HTAs. The false negative rates of RDTs were also lower than those of standard microscopy in both HTAs and LTAs, indicating that RDTs more accurately identified individuals infected with malaria. For specificity, RDTs showed lower rates than standard health system microscopy in HTAs and LTAs. This indicated that compared with RDTs, the local standard health system microscopy could more correctly identify individuals without malaria. However, it should be noted that at 7-days posttreatment, the microscopy results can be negative, while RDTs may remain positive due to delayed clearance of the HRP2 antigen. In our study, all positive RDT results became negative 14 days after treatment, suggesting that the effective detection time of RDTs is longer. In a previous case report, a positive RDT result was obtained, while a negative microscopic result was not a false positive case, as there might be some residual antigens in the patient [14].

In general, the blood detection of *Plasmodium sp.* requires at least 50–100/μl of the pathogen in the blood, and it is difficult to detect the asymptomatic *Plasmodium sp.* carriers with low-density infection. Compared with patients in HTAs, the protozoa density is normally lower in patients in LTAs [4]. In this study, the area under the curve of RDTs was significantly higher than that of microscopy in LTAs ($P < 0.01$), whereas there was no significant difference between them in HTAs ($P > 0.05$). These results demonstrated that RDTs had similar diagnostic accuracy to the gold standard modality in HTAs. In LTAs, RDTs addressed the limitations of microscopic examination for malaria diagnosis [15][16]. RDTs can be useful as an alternative to standard microscopy, especially in the HTAs. However, the medical institutions with microscopy capabilities (such as those at the district level) should continue to reinforce training on microscopy, external evaluation, and quality control. Improvements in FSAT may lead to the need for various detection methods, particularly those that are more sensitive screening tools (e.g. PCR), to improve the accuracy of detection.

This study has some limitations. The samples in this study were not subjected to PCR evaluation. Hence, some patients with low-density parasitemia, particularly those with asymptomatic infections, may have been missed.

Conclusions

RDTs are more accurate than the local health system standard microscopy for malaria case screening in the pilot district of Rufiji in Tanzania. RDTs are appropriate for the case identification. The combination of RDTs and microscopy can effectively improve the detection rate of *Plasmodium sp.*, lowering the false positive rate.

Abbreviations

FSAT, focused screening and treatment

HTA, high transmission area

IHI, Ifakara Health Institute

LTA, low transmission area

NIPD, National Institute of Parasitic Diseases, China CDC

PCR, polymerase chain reaction

RDT, rapid diagnostic test

Declarations

- **Ethics approval and consent to participate**

Ethics approval was obtained from the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (World Health Organization Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases) ethical committee as well as the Clearance Certification for Conducting Medical Research from National Health Research Ethics Committee (NatHREC) in Tanzania.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

Data are available from the Supporting Information files.

- **Competing interests**

The authors have declared that no competing interests exist.

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- **Authors' contributions**

XZ, NX and DW designed the study. KL, WJ, HY, MM and PC provided the data of the patients. XM and SL participated in the data entry. FA, KK and MH checked the data. FL, JF, TE and MH and RG were responsible for the data analysis. KL wrote the draft, and DW, XZ, NX and PC critically revised the manuscript. All authors have read and approved the manuscript.

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References

1. Wang D, Chaki P, Mlacha Y, Gavana T, Michael MG, Khatibu R, et al. Application of community-based and integrated strategy to reduce malaria disease burden in southern Tanzania: the study protocol of China-UK-Tanzania pilot project on malaria control. *Infect Dis Poverty*. 2019;8(1):4.
2. Mekonnen SK, Aseffa A, Medhin G, Berhe N, Velavan TP. Re-evaluation of microscopy confirmed *Plasmodium falciparum* and *Plasmodium vivax* malaria by nested PCR detection in southern Ethiopia. *Malar J*. 2014;13:48.
3. World Health Organization. *World Malaria Report 2018*. Geneva: World Health Organization; 2018.
4. Xiao HH, Zhou SS, Xia ZG, Zhang SS. Methods of detecting asymptomatic plasmodium parasites carriers. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing ZaZhi*. 2014;32(6):474–7.
5. Shi YX, Huang JC, Su JK, Hong Y, Li XB, Zheng K, et al. Nested PCR for malaria detection and *Plasmodium* species identification. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing ZaZhi*. 2011;29(4):263–6.
6. Zheng JF, Lin ZR, Zhao XTJCTM. Application of immune reagents in rapid diagnosis of malaria in China. 2014.
7. PJ G. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development.. *The Lancet infectious diseases*. 2002;9(2).

8. Fancony C, Sebastiao YV, Pires JE, Gamboa D, Nery SV. Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. *Malar J.* 2013;12:284.
9. Harchut K, Standley C, Dobson A, Klaassen B, Rambaud-Althaus C, Althaus F, et al. Over-diagnosis of malaria by microscopy in the Kilombero Valley, Southern Tanzania: an evaluation of the utility and cost-effectiveness of rapid diagnostic tests. *Malar J.* 2013;12:159.
10. Lengeler C. Low quality of routine microscopy for malaria at different levels of the health system in Dar es Salaam. *Malar J.* 2011;10(1):1–10.
11. Osman MM, Nour BY, Sedig MF, De Bes L, Babikir AM, Mohamedani AA, et al. Informed decision-making before changing to RDT: a comparison of microscopy, rapid diagnostic test and molecular techniques for the diagnosis and identification of malaria parasites in Kassala, eastern Sudan. *Trop Med Int Health.* 2010;15(12):1442–8.
12. Wang JH. *Epidemiology.* 7th ed. Beijing: People's Medical Publishing House; 2008.
13. Shakely D, Elfving K, Aydin-Schmidt B, Msellem MI, Morris U, Omar R, et al. The usefulness of rapid diagnostic tests in the new context of low malaria transmission in Zanzibar. *PLoS One.* 2013;8(9):e72912.
14. Tropica LKBJA. Field evaluation of rapid diagnostic tests for malaria in Yaounde. Cameroon. 2013;125(2):214–9.
15. Berzosa P, de Lucio A, Romay-Barja M, Herrador Z, Gonzalez V, Garcia L, et al. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. *Malar J.* 2018;17(1):333.
16. Montiel J, Zuluaga LM, Aguirre DC, Segura C, Tobon-Castano A, Vasquez AM. Microscopic and submicroscopic Plasmodium infections in indigenous and non-indigenous communities in Colombia. *Malar J.* 2020;19(1):157.

Figures

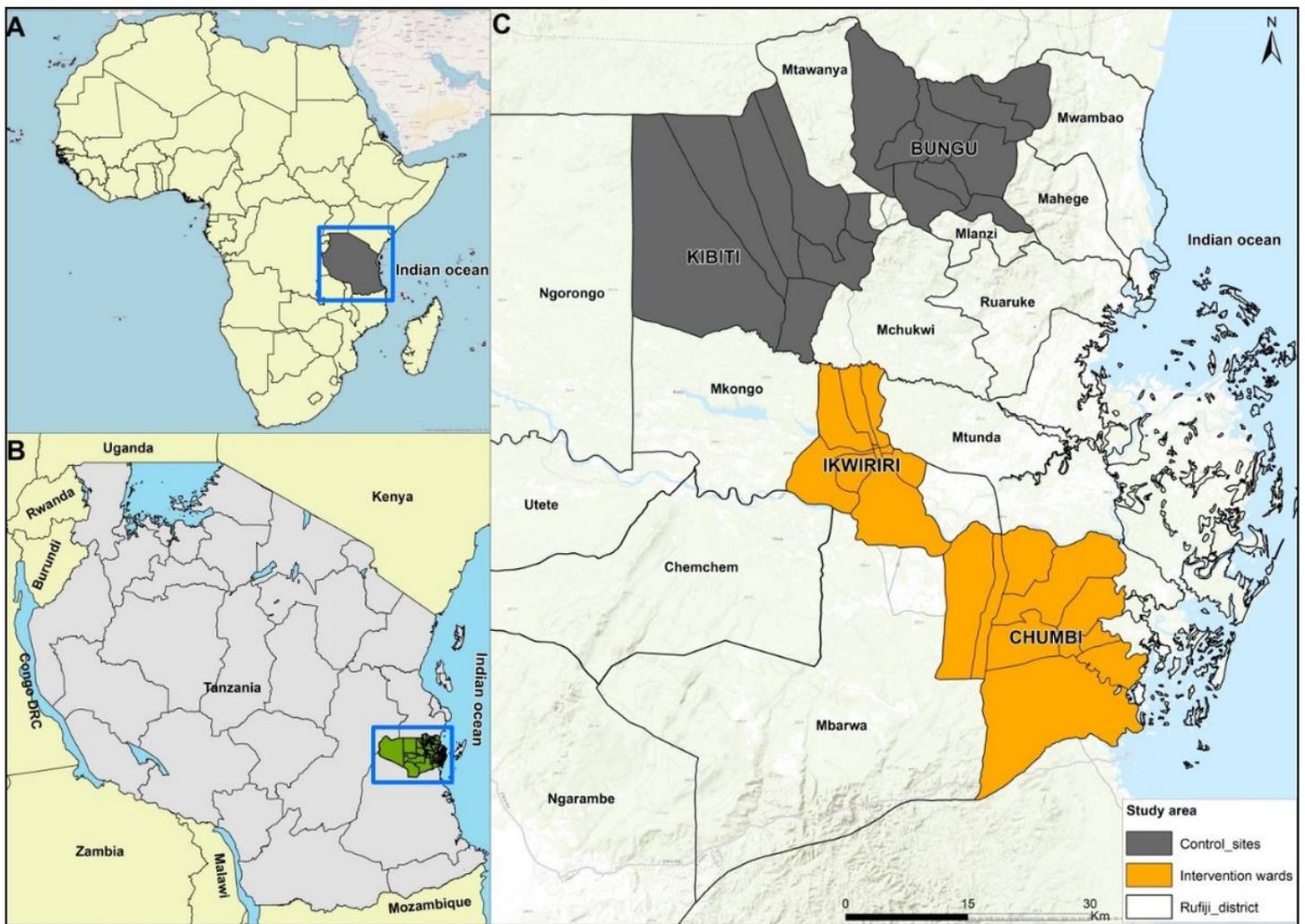


Figure 1
 Location of the study area in Rufiji District, Tanzania Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.