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# Contrasting vector competence of three main East African *Anopheles* malaria vector mosquitoes for *Plasmodium falciparum*

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There are three *Anopheles* mosquito species in East Africa that are responsible for the majority of malaria transmission, posing a significant public health concern. Understanding the vector competence of different mosquito species is crucial for targeted and cost-effective malaria control strategies. This study investigated the vector competence of laboratory reared strains of East African *An. gambiae sensu stricto*, *An. funestus s.s.*, and *An. arabiensis* mosquitoes towards local isolates of *Plasmodium falciparum* infection. Mosquito feeding assays using gametocytaemic blood from local donors revealed significant differences in both prevalence and intensity of oocyst and sporozoite infections among the three vectors. *An. funestus* mosquitoes presented the highest sporozoite prevalence 23.5% (95% confidence interval (CI) 17.5–29.6) and intensity of infection 6-58138 sporozoites. Relative to *An. funestus*, the odds ratio for sporozoites prevalence were 0.46 (95% CI 0.25–0.85) in *An. gambiae* and 0.19 (95% CI 0.07–0.51) in *An. arabiensis*, while the incidence rate ratio for sporozoite intensity was 0.31 (95% CI 0.14–0.69) in *An. gambiae* and 0.66 (95% CI 0.16–2.60) in *An. arabiensis*. Our findings indicate that all three malaria vector species may contribute to malaria transmission in East Africa, with *An. funestus* demonstrating superior vector competence. In conclusion, there is a need for comprehensive malaria control strategies targeting major malaria vector species, an update of malaria transmission models to consider vector competence and evaluation of malaria transmission blocking interventions in assays that include *An. funestus* mosquitoes.

*Anopheles* mosquitoes are the only arthropod vectors that transmit *Plasmodium* parasites that cause malaria in humans. The disease imposes a significant burden of mortality and morbidity, particularly in Sub-Saharan Africa (SSA)[1] where the most efficient mosquito vectors of malaria are found[2]. Human malaria is mediated only by female *Anopheles*, and of the estimated 460 species, only 40 species or species complexes are considered to be important vectors in the wild[3], notably the *Anopheles gambiae* and *Anopheles funestus* species complexes that dominate malaria transmission throughout SSA[4]. In this paper, we focus on the three major East African vectors of human malaria: *An. funestus sensu stricto*, *An. gambiae sensu stricto* and *An. arabiensis*.

The successful transmission of *Plasmodium* parasites between humans requires intricate transformations within the mosquito vector[5], highlighting the key role of both vectorial capacity[6] and vector competence[7] in determining the local intensity of malaria transmission[7].

Vector competence refers to the ability of an arthropod vector to acquire, maintain and transmit a pathogen. This concept encompasses the inherent ability of a pathogen to effectively enter and reproduce within the vector and be released from the vector's salivary glands to initiate infection in another vertebrate host[7,8]. Vectorial capacity describes the potential intensity of transmission by mosquitoes. It is defined as the total number of infectious mosquito bites on humans that will arise from a single infected person on a single day[9]. This is influenced by a number of factors[6,10], most notably the probability of mosquitoes to feed on humans[11], daily vector survival[12], environmental factors that affect the time it takes for parasites to develop in the mosquito host[13], the availability of larval breeding sites[14], presence of vector control tools[10] and vector competence[15].

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The vectoral capacity of a particular vector is strongly influenced by its ecology. The larval stage of mosquitoes takes place in water where biological factors greatly influence the habitat suitability and carrying capacity. These factors influence vector presence[3], fitness[16], longevity[17], which in turn affects the probability that a mosquito can acquire and maintain a parasite for long enough to become infectious[18]. In East Africa, the three major vectors have different regional distribution due to ecology[19] and hydrology[14] and varying contributions to malaria transmission across different seasons[20–23]. *An. funestus* mosquitoes have permanent breeding sites abundant with vegetation, making them likely to transmit malaria all year round[24,25]. *An. arabiensis* and *An. gambiae s.s.* typically dominate in temporary sunlit pools and their presence is strongly dependent on rainfall or irrigation[26,27]. *An. gambiae s.s.* requires high humidity to survive and occurs almost exclusively during humid and rainy periods[28]. *An. arabiensis* and *An. funestus*, are more resistant to desiccation, are commonly found in abundance during the peak of the wet season and continue into the dry season; sustaining malaria transmission for several months after the end of the rains[23,29].

*Anopheles* mosquitoes are dependent on vertebrate blood to provide proteins needed for egg development and undergo multiple cycles of feeding and egg development in their lifetime. Therefore, the preference of a vector for human blood has a direct impact on its efficiency as a vector by increasing its probability of acquiring and transmitting onward infection[30]. *An. arabiensis* have an opportunistic feeding behavior, targeting both human and animal hosts for its blood meals, so it may be a more or less important vector dependent on the relative proportion of cattle in an area[31]. *An. gambiae s.s.*[32,33] and *An. funestus*[34,35] are more specialized blood feeders, feeding on humans, although this does depend on host availability. In addition, there is evidence that multiple blood meals increase the likelihood of *Plasmodium* developing in the mosquito[36,37].

As well as environmental factors that affect vectors' susceptibility to infection and the interactions between the vector, pathogen, and host that impact probabilities of onward transmission, vector competence is influenced by a variety of internal factors, including the genetics of both the vector and the pathogen[5,38]. *Plasmodium* takes resources from its definitive host that results in reduced fitness and reproductive output[39]. Therefore, the mosquito innate immune system either modulates or resists infection[40], while the parasite counteracts mosquito defenses through host manipulation[41] and polyclonality[42]. Mosquito species show different levels of susceptibility to *Plasmodium* from refractory in the case of *Anopheles quadriannulatus*[43,44] to high susceptibility in *Anopheles coluzzi*[45,46].

In East Africa, there is evidence of higher proportions of infected *An. funestus*[47,48] relative to *An. arabiensis* and *An. gambiae*. This can be to an extent explained by the fact that *An. funestus* generally feeds almost exclusively on humans and has been shown to live longer than *An. arabiensis*[49]. However, any differences in degree of vector competence among the three primary malaria vectors has not been evaluated. Understanding vector competence is crucial in understanding the risk of malaria transmission, informing effective malaria control strategies[38,50,51] and parameterizing mathematical models, where mosquito-parasite interactions are rarely considered[15,52].

Therefore, this study investigated whether vector competence towards *Plasmodium falciparum* differs between local East African strains of *An. gambiae s.s.*, *An. funestus* and *An. arabiensis* mosquitoes. By experimentally infecting mosquitoes with field gametocytes using Direct Membrane Feeding Assays (DMFAs), we aim to compare the prevalence and intensity of *P. falciparum* infection between these *Anopheles* mosquito species.

## Results

### Prevalence and intensity of *P. falciparum* infection among local strains of *An. gambiae s.s.*, *An. funestus* and *An. arabiensis* mosquitoes

*Oocysts*

The prevalence of oocyst-infected mosquitoes varied among the three *Anopheles* mosquito species, with *An. funestus* presenting the highest oocyst infection rate of 13.5% (95% CI 9.2–17.6), followed by *An. gambiae s.s.* at 10.7% (95% CI 6.9–14.4), and *An. arabiensis* at 5.6% (95% CI 2.5–8.7) (Table 1). The proportion of oocyst-infected *An. arabiensis* mosquitoes was significantly lower than *An. funestus* mosquitoes (OR = 0.40, 95% CI 0.20–0.80, $p = 0.010$), but there was no difference between *An. funestus* and *An. gambiae* (Fig. 1A). Additionally, there was no significant difference in the proportion of oocyst-infected mosquitoes between *An. gambiae s.s.* and *An. arabiensis* mosquitoes (OR = 0.52, 95% CI 0.25–1.06, $p = 0.072$). There was no significant difference in

| | Proportion infected | | | Intensity | |
|---|---|---|---|---|---|
| | Number infected (n/N) | Prevalence (95% CI) | OR (95% CI) | Median (Min–Max) | IRR (95% CI) |
| Oocysts | | | | | |
| *An. funestus s.s.* | 35/260 | 13.5 (9.2–17.6) | 1 | 2 (1–12) | 1 |
| *An.gambiae s.s.* | 29/271 | 10.7 (6.9–14.4) | 0.76 (0.44–1.31) * | 1 (1–14) | 1.31 (0.83–2.06) |
| *An. arabiensis* | 12/214 | 5.6 (2.5–8.7) | 0.40 (0.20–0.80) * | 2 (1–3) | 1.04 (0.55–1.95) |
| Sporozoites | | | | | |
| *An. funestus s.s.* | 46/195 | 23.5 (17.5–29.6) | 1 | 3983 (6–58,138) | 1 |
| *An.gambiae s.s.* | 19/166 | 11.4 (6.5–16.3) | 0.46 (0.25–0.85) ** | 2447 (21–7976) | 0.31 (0.14–0.69) ** |
| *An. arabiensis* | 05/102 | 4.9 (0.6–9.1) | 0.19 (0.07–0.51) ** | 714 (20–27,877) | 0.66 (0.16–2.60) |

**Table 1.** Proportion and intensity of oocyst and sporozoite infected laboratory reared mosquitoes with gametocytaemic blood from participants in Bagamoyo, Tanzania. OR were derived from mixed-effect logistic regression while IRR were derived from mixed effect negative binomial regression using study participant as a random effect. *p-value < 0.05, **p-value < 0.01.
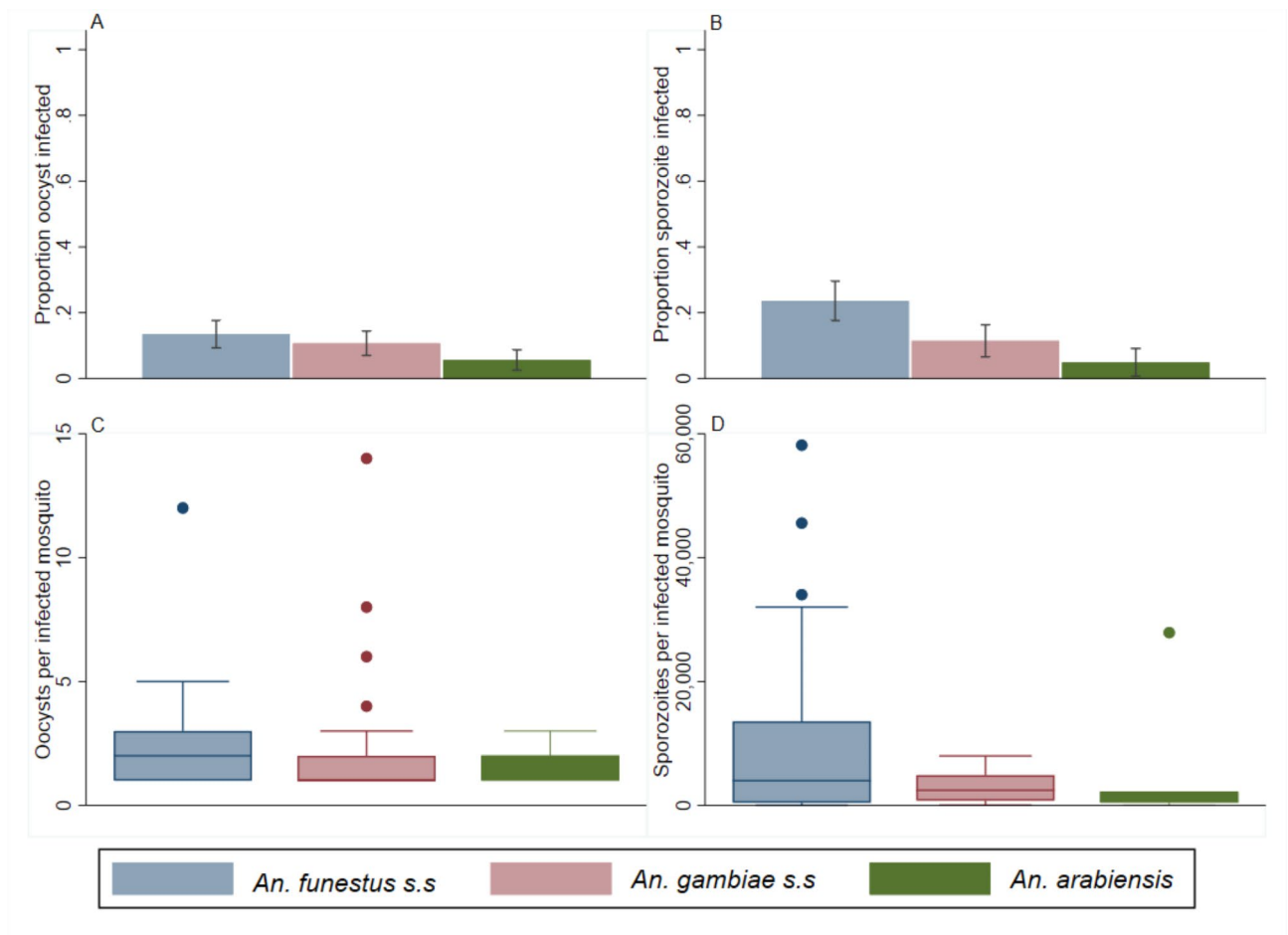
**Fig. 1**. Prevalence and intensity of oocysts and sporozoites in *Plasmodium* infected *Anopheles* mosquitoes. (**A**) Oocyst infected mosquitoes (**B**) Sporozoite infected mosquitoes (**C**) Number of oocysts (**D**) Number of sporozoites.

oocyst intensity between the three species, although as with prevalence, oocyst intensity was similar between *An. funestus*, ranging from 1 to 12 and *An. gambiae s.s.*, ranging from 1 to 14; and it was lower in *An. arabiensis*, ranging from 1 to 3 (Fig. 1C). The oocyst infection prevalence from DMFAs for each gametocyte carrier, along with the corresponding gametocyte densities, is displayed in Supplementary Fig. S1.

*Sporozoites*
The burden of sporozoite-infected mosquitoes varied among the three *Anopheles* mosquito species, with *An. funestus* presenting the highest sporozoite infection rate of 23.5% (95% CI 17.5–29.6), followed by *An. gambiae s.s.* at 11.4% (95% CI 6.5–16.3), and *An. arabiensis* at 4.9% (95% CI 0.6–9.1) (Table 1). *An. funestus* had a higher probability of being sporozoite-infected than *An. gambiae s.s.* (OR = 0.46, 95% CI 0.25–0.85, $p = 0.013$) or *An. arabiensis* (OR = 0.19, 95% CI 0.07–0.51, $p = 0.001$), (Fig. 1B). Moreover, there was no statistically significant difference observed in the proportion of sporozoite infection between *An. gambiae s.s.* and *An. arabiensis* mosquitoes (OR = 0.41, 95% CI 0.14–1.17 $P = 0.098$), although there were few infected *An. arabiensis*. Additionally, there were similar trends observed in sporozoite intensity among the three species, with highest intensity observed in *An. funestus*, ranging from 6 to 58,138 followed by *An. gambiae s.s.*, ranging from 21 – 7,976 and *An. arabiensis* ranging from 20 to 27,877. A significant difference was observed in sporozoite intensity between *An. funestus* and *An. gambiae s.s.* (IRR = 0.31, 95% CI 0.14–0.69, $p = 0.004$) (Fig. 1D), although not between *An. funestus* and *An. arabiensis* likely due to low sporozoite prevalence (5%) in *An. arabiensis* leading to uncertainty in the estimates. The sporozoite infection prevalence from DMFAs for each gametocyte carrier, along with the corresponding gametocyte densities, is displayed in Supplementary Fig. S1.

## Discussion
This study represents the first attempt to measure whether the degree of vector competence towards *P. falciparum* infection varies between East African *An. funestus s.s.*, *An. arabiensis*, and *An. gambiae s.s.* mosquitoes. Through experimental feeding of mosquitoes with gametocytaemic blood from donors in Tanzania, we observed significant differences in both prevalence and intensity of oocyst and sporozoite infections among *An. gambiae s.s.*, *An. funestus s.s.*, and *An. arabiensis* mosquitoes. *An. funestus s.s. mosquitoes* presented with the highest

prevalence and intensity of infection indicating that they are more competent than either *An. gambiae s.s.* or *An. arabiensis*. This is the first time, to our knowledge that the vector competence of *An. funestus s.s.* has been evaluated. The findings of this study largely agreed with the findings of a study in Burkina Faso, that found no difference in genetic susceptibility to *P. falciparum* measured by oocyst infection between three sympatric population groups of the *An. gambiae s.l.* complex including *An. coluzzi, An. gambiae* s.s. and *An. arabiensis* that had been reared from wild-caught larvae[46].

*Anopheles* mosquitoes are reported to have varying levels of vector competence, influenced by genetic factors, as well as environmental conditions, and host-parasite interactions[5,38,53]. *An. gambiae s.s.* has long been recognized as a highly polymorphic and efficient malaria vector, possibly due to the strong co-adaptation of *P. falciparum* to this specific mosquito species[54]. However, our study reveals that *An. funestus* were more competent. This finding is consistent with reports of increasing malaria cases caused by *An. funestus* in Tanzania and other parts of SSA[55–59]. The high prevalence and intensity of infection observed in *An. funestus* mosquitoes suggest its potential as a predominant malaria vector, particularly in areas with suitable breeding sites abundant in vegetation. Our results are consistent with the observation that the massive introgression event that lead to the evolution of *An. funestus* 13,000 years ago that facilitated its adaptation to new environments resulting in its subsequent dramatic geographic range expansion across most of tropical Africa also enhanced vectorial capacity in *Anopheles funestus* mosquitoes[60].

Although *An. arabiensis* mosquitoes play a crucial role in malaria transmission, particularly in arid regions in the Horn of Africa[47], our findings show lower prevalence and intensity of oocyst and sporozoite infections when compared to *An. funestus* and *An. gambiae s.s.* mosquito infections. While *An. arabiensis* is often abundant, and is widely discussed as a vector of residual and outdoor malaria, the more competent and endophilic malaria vectors *An. funestus s.s.* and *An. gambiae s.s.* should be targeted for control.

While several studies have shown that insecticide resistant vectors are more competent to *Plasmodium*[61,62], our study also found that *An. funestus* mosquitoes which are resistant to pyrethroids showed increased susceptibility to *Plasmodium* infection. The susceptibility of mosquitoes to *Plasmodium* infection may result from either their increased survival and longevity[63] compared to susceptible mosquitoes, which are killed by insecticide[64], or could be due to reduced immunity to parasites[65]. Therefore, we cannot rule out that the difference in insecticide susceptibility affected the results through a change in mosquito immunity to parasites. There is strong evidence suggesting that insecticide resistance mutations increase the vector competence of *An. gambiae* for *Plasmodium*, potentially sustaining malaria transmission[62]. However, the *An. arabiensis* used in the study were also pyrethroid resistant and were still relatively less susceptible to infection.

An additional study has shown that insecticide resistance mechanisms have an effect on the activation of the mosquito immune system and its physiology, resulting in differences in parasite development and survival[66]. Variations in parasite burden may not significantly affect parasite transmission, however, the intensity of infection does influence the activation of the vector immune system[67]. Nonetheless, in our study, all three species showed susceptibility to *Plasmodium* infection, with *An. funestus* presenting a higher susceptibility compared to *An. gambiae s.s.* and *An. arabiensis*. It was suggested that the susceptibility of *An. gambiae* to *Plasmodium* infection is due to persistent immune suppression to prevent excessive activation of the immune response following blood meal ingestion[68]. Moreover, research has shown that genetic diversity within mosquito populations can also significantly influence their susceptibility to *Plasmodium* infection[69].

This study highlighted that, while *An. gambiae* mosquitoes are commonly used in DMFAs to assess different malaria transmission-blocking interventions[70–72], the observed shift towards *An. funestus* as a major contributor to transmission in SSA[58], with the highest infection burden, suggests the importance of incorporating *An. funestus* mosquitoes into assays for testing malaria transmission-blocking activity. Our study has several limitations. First, it exclusively used laboratory-reared mosquito strains rather than field-collected mosquitoes. Second, the *An. funestus* (FUMOZ) mosquitoes used in the study were originally collected and colonized in Mozambique, reflecting the challenges associated with colonizing *An. funestus* in insectaries. Thirdly, we did not record mosquito survival from day 1 to day 16 post-infection, although only live mosquitoes were dissected so prevalence and incidence is among those mosquitoes that survived the extrinsic incubation period. Lastly, the study was limited by the relatively small number of gametocytemic individuals, attributable to reduced rainfall during the year 2023, which led to few gametocyte-infected individuals. This aligns with observations from other studies in the same region, which also reported comparably low gametocyte counts[37,73,74]. To address this limitation, future studies should focus on recruiting a larger number of gametocytemic participants.

In conclusion, we confirmed that between the three mosquito species, *An. funestus* was the most permissive to *P. falciparum* infection, which is coherent with consistently high sporozoite rates observed in this species across SSA, whereas *An. arabiensis* shows the greatest resistance coherent with its lower observed sporozoites rates. Nonetheless, our findings suggest that all the three vector species play an active role in malaria transmission. The observed differences in vector competence among the three *Anopheles* species highlight the complexity of malaria transmission dynamics and the need for comprehensive malaria control strategies that target key malaria vector species. Furthermore, malaria transmission models should be revised to account for vector competence, and efforts to support malaria transmission blocking interventions tested on multiple malaria vectors are essential for making sustainable progress towards malaria elimination.

## Methods
### Mosquito rearing
*An. gambiae s.s.* mosquitoes were originally collected from the southern region of Tanzania (Njage-Mngeta villages, Ifakara district, Morogoro region) in 1996 and have been maintained at the Ifakara Health Institute (IHI) insectaries, Tanzania. This strain of *Anopheles gambiae s.s.* is susceptible to all classes of insecticides. Field-collected *An. arabiensis* mosquitoes were obtained from the southern region of Tanzania (Sakamaganga village,

Ifakara district) in 2005 and have been maintained in IHI insectaries. This strain is resistant to pyrethroids at a 1× discriminating concentration and is susceptible to other classes. The *An. funestus* colony at the IHI insectaries was established in 2018 and originates from a founder colony initially established in 2000 in Matola province[75], southern Mozambique, where pyrethroid resistance had been documented in the wild population. The eggs used to establish the IHI colony were obtained from the National Institute for Communicable Diseases (NICD) in South Africa. This strain is resistant to pyrethroids at a 1× discriminating concentration and is susceptible to other classes. Mosquito larvae were maintained at a density of 200 larvae per litre of water and fed 0.3 g per larva on Tetramin fish food (Tetra Ltd., UK). For colony maintenance, the adult mosquitoes are provided with cow blood between 3 and 6 days after emergence for egg development using a Hemotek* membrane feeder (SP-6 System, Hemotek Ltd., Blackburn BB6 7FD, UK). Mosquitoes were provided with autoclaved 10% sucrose solution ad libitum. Temperature and humidity within the insectary are maintained between 27 ± 2 °C and 60–85% relative humidity following the MR4 guidance[76].

### Recruitment of asymptomatic gametocytaemic carriers

Gametocytaemic carriers were selected by screening thick blood smears from participants aged 6–40 years located in the village of Wami-Mkoko, in Bagamoyo district located in the coastal region of Tanzania, between June 2023 and August 2023. Participants meeting the inclusion criteria (asymptomatic individuals aged 6–40 who consented and had microscopically detectable gametocytes) were enrolled for blood collection at IHI transmission facilities in Bagamoyo, Tanzania.

Gametocytes were quantified by counting against 500 white blood cells in thick smears, and their density was calculated based on an estimated leukocyte density of 8000/µL of whole blood. Five milliliters of blood were obtained from microscopically confirmed gametocyte carriers with gametocytes density exceeding three gametocytes/500 red blood cells, equivalent to 48 gametocytes/ µL of whole blood. Seven gametocytaemic individuals were recruited to donate blood to feed all three mosquito species during each DMFA. Autologous serum was replaced with pre-warmed malaria-naïve AB serum European donors.

### Experimental infection of *P. falciparum* in *Anopheles* mosquitoes through DMFAs

Infectious gametocytaemic blood was administered to mosquitoes through water-jacketed glass feeders (14 mm Ø, Chemglass, New Jersey, USA) covered with parafilm*, connected to a circulating water bath (39,°C, ELMI, Switzerland) via plastic tubing. On average, 200 mosquitoes from each mosquito strain were fed a blood meal from each participant for a duration of 15 min. After blood feeding, the cups containing mosquitoes were then transferred to Bugdorm plastic cages (30 cm x 30 cm x 30 cm, Megaview Science Co., LTD, Taiwan) and placed in a climatic chamber (S600PLH, AraLab, Lisbon, Portugal) maintained at 75 ± 2% humidity and 27 ± 1 °C at 12:12 h dark: light cycle. Mosquitoes were deprived of sugar for 48 h to allow unfed mosquitoes to die, so only fed mosquitoes were used. Dead mosquitoes were aspirated out after 48 h and then cotton soaked with autoclaved 10% sucrose solution was provided and replaced daily.

### Oocyst and sporozoite scoring

Eight days' post infection (dpi), one-third of mosquitoes from each mosquito strain was dissected and their midguts were stained with a 1% mercurochrome solution before examination for presence of oocysts microscopically. The remaining mosquitoes were kept up to day 16 dpi and the mosquito`s DNA was extracted using DNAzol* reagent[77] for molecular analysis and quantification of *P. falciparum* infection in mosquito stages from the mosquito heads and thoraces (sporozoites stages). Using quantitative reverse transcription polymerase chain reaction (RT-qPCR)[78], absolute quantification of all sporozoites positive samples was performed using the standard curves generated on *P. falciparum*-specific 18 S rDNA plasmid (GenBank: AF145334) from *P. falciparum* (BEI Resources, NIAID, MRA-177). Plasmid copy numbers per µl were calculated as described elsewhere[79]. The standard curves were generated on serial dilutions over eight magnitudes assuming an average of six copies of the 18 S rDNA gene sequence per parasite genome[80]. Each concentration from the serial dilution was run in triplicates to determine qPCR efficiency, limit of detection, slope and y-intercept.

### Statistical analysis

Data cleaning and analysis were conducted using STATA 17 software (StataCorp LLC, College Station TX, USA). Descriptive statistics were employed for data summarization, presenting the proportion of infected mosquitoes with a 95% confidence interval. For parasite intensity (oocysts or sporozoites), the median along with minimum and maximum values were reported.

To evaluate vector competence towards *P. falciparum* infection among the three mosquito strains, mixed-effect regression was used, with mosquito strain as a fixed categorical effect and study participants included as a random effect. For oocyst and sporozoite prevalence logistic distribution was used. For oocyst and sporozoite intensity, negative binomial distribution was used and only infected mosquitoes were included in the intensity analysis.

### Data availability

Data is provided within the supplementary information files.

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## Declarations

### Ethics approval and consent to participate
All adult participants provided written informed consent, while for children under 18 years old, consent was obtained from their parent or guardian, with the children's assent also sought for their participation. All study volunteers received artemisinin-lumefantrine treatment within 24 h of diagnosis, following the Tanzania Guidelines for Diagnosis and Treatment of Malaria[81], administered by a qualified nurse. No adverse effects were reported among the participants during the study period. The study activities were reviewed and approved by the Institutional Review Board of IHI (IHI/IRB/No: 44–2020) and the National Institute for Medical Research Tanzania (NIMR/HQ/R.8a/Vol.IX/3595). All research was performed in accordance to relevant guidelines and regulations.

### Competing interests
The authors declare no competing interests.

### Additional information
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