Susceptibility of the Anopheles coustani complex to Plasmodium vivax and Plasmodium falciparum infection in Ethiopia

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Abstract

Background

Insecticide-based vector control interventions along with case management with artemisinin-based combination therapy (ACT) reduced malaria incidence and prevalence worldwide. The current control methods focus on the primary malaria vector, *Anopheles gambiae* s.l., however, the role of secondary and suspected vectors has been either sidelined or limited. Determining the susceptibility of secondary and suspected vector species to different parasites in time and space is important in malaria control and elimination programs. Thus, this study aimed at assessing the susceptibility of *Anopheles gambiea s. l., An. coustani* complex and *An. pharoensis* to *Plasmodium vivax* and *P. falciparum* infection in Ethiopia.

Methods

Larvae of *Anopheles* were collected from different breeding sites and reared to adult under controlled conditions. The adult female mosquitoes were identified to species as *An. gambiea s.l, An. coustani* complex and *An. pharoensis*. The three species were allowed to feed in parallel on the same infected blood with gametocytes drawn from *P.vivax* and *P. falciparum* gametocemic patients by Indirect Membrane Feeding Assays (IDMFA). Fed mosquitoes were kept under controlled laboratory conditions. After seven days, all survived mosquitoes were dissected to detect mid-gut oocyst using microscopy and enumeration. Data were analyzed using R statistical software package version 4.2.0.

Results

Out of a total 5,915 female *Anopheles* mosquitoes exposed to gametocyte infected blood, 2,106 (35.60%) mosquitoes fed successfully in the 32 independent infection experiments. There was a significant variation in feeding rates among *An. gambiae* s l, *An. pharoensis* and *An. coustani* complex ($G = 48.43$, $p < 0.0001$). All the three exposed *Anopheles* species were susceptible to *P. vivax* and *P. falciparum* infection. The proportion of infected mosquitoes following engorgement on an infected blood meal was significantly different among species ($G = 6.49$, $p < 0.05$). The median intensity of infection for *An. coustani complex, An. gambiae s.l.* and *An. pharoensis* was 1.16, 2.00 and 1.25, respectively. Even though, the proportion of infected mosquitoes significantly differed in intensity of infection, infection rate and mean oocysts among the species, gametocyte density was highly correlated to infection rate and mean oocyst of infection all tests ($p < 0.001$).

Conclusion

Anopheles coustani complex were susceptible to both *P. vivax* and *P. falciparum* infection. An effective malaria elimination program might include tools that target outdoor vector surveillance and control, which reduce focal malaria transmission.

Background

Malaria continues to be the worldwide public health problem, which resulted in 241 million estimated cases and 627, 000 deaths in 2020, of which 96% were reported from WHO African region [1]. In Ethiopia, despite considerable decline of malaria morbidity in recent years [1–3], the disease remains point of public health concern with 1.8 million malaria cases were been reported in 2020 [1]. In sub-Saharan Africa, *P. falciparum* and *P. vivax* almost consistently coexist and are often equally important epidemiologically, despite the former is the most virulent species [4]. In the last decades, most disease cases were attributed to *P. falciparum*, which is responsible for 90% of malaria related deaths. In recent years, *P. vivax* has been reported to cause malaria complications among children in endemic regions [4–6].

In Ethiopia, there are more than 47 documented species of *Anopheles* mosquitoes[7, 8] of which *Anopheles arabiensis*, *An. pharoensis, An. funestus* and *An. nili* are recognized malaria vectors. The primary malaria vector is *An. arabiensis* while, *An. pharoensis*, *An. funestus* and *An. nili* are secondary vectors occurring with varying densities, limited distribution and vector competency [9–10]. A new invasive Anopheles species, *An. stephensi*, has been documented in the country in recent times[11]. Populations of *Anopheles* mosquitoes such as *An. arabiensis*, *An. amharicus*, *An. pharoensis*, *An. coustani, An. nilli and An. squamouse* usually co-occur in Eastern Wollega western Ethiopia [12–14]. *Anopheles arabiensis*[ ] *and An. coustani* populations occur abundantly during the rainy seasons in western parts of Ethiopia. Whereas the density of *An. coustani* population occurs in relatively higher abundance in dry seasons. However, *An. pharoensis* is abundant both in the rainy and dry season [12].

Environmental modifications and water development projects, might increase the risk of malaria transmission by contributing to the formation of additional breeding habitats, changing micro weather conditions and micro ecological settings [15, 16]. Malaria distribution is largely governed by the spatial and temporal distribution of malaria vectors in different ecological settings. Construction of irrigation schemes creating additional aquatic habitats through shifting ecology and favor mosquito breeding [17]. Such ecological change may also lead to shifts in mosquito fauna, distribution, vector diversity, abundance, and proliferation [12, 13].

In irrigation and dam-developed areas, malaria transmission during the dry season is supported by primary vectors, even though the abundance of secondary vectors is also relatively high [12]. The contribution of secondary and suspected vectors is not well studied and understood. The abundance of secondary vectors of malaria have been higher and the role of secondary vectors in malaria transmission has significantly increased over the years [18].

Environmental modification creates different larval habitat [19]. The impact of this changed ecological setting in Arjo-Dedessa area due to the introduction of irrigation scheme and sugar plantation against the bionomics of Anopheles mosquitoes and their vectorial competency is not well studied.

In Ethiopia, studies on the role of secondary vectors are limited or missing. This could be due to the extremely low infection rate or no infections were reported mostly from field collected adult mosquitoes [20, 33]. In addition, existing vector surveillance methods mainly focus on indoor resting and indoor biting species, prioritizing the anthropophagic behavior and vectorial capacity of the primary vector [21, 22]. The data on the secondary vector is limited and findings from limited studies indicate that most secondary vectors do have a short lifespan, with natural mortalities estimated to be around 50–60% per gonotrophic cycle (23,24). Experimental vector susceptibility studies that evaluate the vectorial capacity of suspected mosquito species are limited in Ethiopia [32]. A study conducted in Ethiopia and neighboring country Kenya indicates that *An. coustani* is susceptible to *Plasmodium* infection from wild-caught adult mosquitoes [18, 33, 37], However, experimental infection studies on secondary and suspected vectors are scarce both in time and space in Ethiopia. Therefore, this study was aimed at assessing the susceptibility of *Anophele gambiea s.l.*, *An. coustani* complex and *An. pharoensis* to *P. vivax and P. falciparum* infection using indirect membrane feeding assay at Arjo-Didessa sugarcane irrigation scheme, southwestern Ethiopia.

Methods

Study area

The study was conducted in Arjo-Didessa sugarcane plantation irrigation scheme, south-west Ethiopia (8°41'35.5"N 36°25'54.9"E) located 575 km southwest of the capital, Addis Ababa, Ethiopia. The altitude ranges from 1300 to 2280 m.a.s.l. with a mean annual rainfall of 1477 mm, with a short rain between February–April, and long rain between June-September corresponding to low and high peak transmission season, respectively. The soil type in the study area, Arjo-Didessa sugarcane plantation site, is clay and clay loam soil type with low permeability. Due to this slow rate of percolation, rain water can accumulate simply and form swamps in the area [25]. This creates a wide-range of breeding sites for malaria vector mosquitoes. The area is malaria endemic and *P. falciparum* and *P. vivax* are the principal malaria parasite species responsible for the majority of the infections. *Anopheles* mosquitoes such as *An. arabiensis*, *An. amharicus*, *An. pharoensis, An. coustani* complex and *An. Squamosus* occurs in this area [12, 14].

Mosquito collection, rearing and identification

Anopheline larvae and pupae were collected from the natural breeding habitats of the irrigation clusters and surrounding villages, and adult mosquitoes were collected from resting sites from human dwellings and animal sheds using mouth aspirators at dawn. Adult fed and gravid mosquitoes were used to lay eggs. Field collected mosquitoes were only used for oviposition purpose. Larvae and pupae were collected using a standard dipper (350 ml, Bio Quip Products, Inc. California, USA), immediately transported to the International Centre of Excellence for Malaria Research (ICEMR) field laboratory and reared to adults in enamel trays (27×16×6.5 cm). Mosquito larvae were immediately filtered so as to avoid larvae competitors, predators and unwanted debris, transported to the insectary in water taken from the mosquito's natural breeding sites, and reared to adult stages provided with fish food. The three species were sorted and kept separately in different cages with a daily provision of 10% sucrose solution as a source of energy. Fed and fully engorged mosquitoes were soon transferred for oviposition and further steps in the rearing processes were performed following standard procedures [26]. The eggs from filter papers were washed onto enamel trays; the eggs were allowed for emergence. Larvae were fed with finely ground fish food (Tetramin baby). Pupae were transferred to beakers containing water and kept inside cages and allowed to emerge to adults. The emerged adults were fed on soaked cotton with 10% sucrose solution. Rearing was done under standard conditions (25 ± 2°C temperature, 70% ± 10% relative humidity) [27].

Patient recruitment and sample collection

Malaria-suspected persons who were febrile and visited health facilities were enrolled in the study. Samples were collected from febrile patients screened for malaria infection at five health facilities (Arjo Diddessa Sugar Factory clinic, Abote Didessa Health post, Kerka Health post, Command Two Health post, and Command Five Heath post). First, all suspected malaria patients visiting health facilities were screened for malaria by standard finger prick and Giemsa-stained blood smears. Out of the total positive patients, individuals diagnosed with gametocyte blood stage parasite were consented in order to enroll in the study. Patients who failed to give consent and assent, had severe illness and were mentally sick as well as had taken anti-malaria drugs within 2 weeks were excluded from the study. Then all patients agreed to participate in the study were transferred to the ICEMR Laboratory for venous blood sample collection [28]. Blood samples were collected with heparin tubes.

Thus, samples used for membrane feeding assay were double screened (1st for *Plasmodium* infection; 2nd for the presence of gametocyte blood stage presence). Gametocyte and parasite density was determined against 8,000 leukocytes/µl of blood. The sexual and asexual parasite stages were counted against 500 and 200 leukocytes, respectively. The availability of gametocyte carriers' patient was not known, due to this, always mosquito was ready for membrane feeding assay; thus, experimental infection always performed on the same day. All suspected individuals who had fever during physical examination and were positive for malaria parasites through blood film examination were treated immediately as per the national malaria treatment guidelines [29].

Indirect membrane feeding assay (IDMFA)

Three to five days old adult female mosquitoes were starved 8–12 hours prior to membrane feeding; this might increase the blood feeding activity of mosquitoes. Mosquitoes were provided with infected blood from *P. falciparum* and *P. vivax* positive patients for a period of 30 to 60 minutes via artificial membrane feeding apparatus and micro glass feeders were used for experimental mosquito infection. All glass feeders closed with membrane were placed on top of the meshes covered paper cups of size 8.5/11 cm diameter/depth with starved mosquitoes. In the feeding process, temperature was

kept constantly at 37 ± 0.1°C using a high-speed water circulation system-connected to glass feeders, which allowed to fed the three species in parallel and up to 450 mosquitoes at a time [30].

An artificial membrane feeding apparatus with a water pump, temperature maintained at 37°C (Model 8005; Polyscienca, Illinois, USA) was used to pump warm water through glass feeders in parallel. The temperature in each feeder was controlled and found to be at the same temperature of 37 ± 0.1°C among the nine individual glass feeders. The feeders were closed at the bottom using membrane (National Can, Chicago, USA) and filled with 200 µl of freshly drawn blood. The nine micro-feeders were poured with infected blood using a blunt needle. Then, kept starved mosquitoes with paper cups covered with mesh and allowed to feed on the same infected blood for a period of 30–60 min under calm or reduced light conditions. A total of nine paper cups were used in the single experiment; in each paper cup 50 starved mosquitoes with three replicates for each species and the three species were allowed to feed in parallel with the same infected blood source [30]. Feeding success was measured after 30 minutes. Subsequently, the glass feeders were removed and unfed, partially fed mosquitoes were removed, killed and discarded. Fully fed mosquitoes were maintained at 27 ± 1°C and 75 ± 10% relative humidity. Until day eight, infected mosquitoes were provided with cotton balls soaked in a 10% sucrose solution daily.

Mosquito dissection

Eight days after feeding on infected blood, all experimentally infected and survived, mosquitoes were immobilized and dissected and examined for midgut oocyst development and enumeration. Midguts were removed and oocysts counting was performed by examining the wet mount midgut stained with a 5% mercurochrome solution in phosphate–buffered saline (PBS) [30, 31]. Midgut oocysts were examined and counted using a microscope at 40×objective. For each infection assay, the infection rate (IR) and intensity of infection (II) were determined.

Data analysis

R statistical software package version (4.2.0) was employed to analyze data. The rate of infection to *P. vivax* and *P. falciparum* for *An. gambiae* s l, *An. coustani* complex and *An. pharoensis* was assessed by the presence and the number of oocysts in the midguts. Infection rate was calculated as IR= $\frac{oocystposetivemosquitoes}{totalmosquitoesdissectedinindividualfeeding} * 100$. Similarly feeding rate was calculated as FR = $\frac{Fedmosquitoes}{totalexposed} * 100$. G- tests were used to compare the feeding rate and frequency of infection among mosquito species conjointly, as well as pairwise comparisons between each pair of species. The overall infection rate and intensity of infection were compared between populations of *An. gambiae* s l., *An. coustani* complex and *An. pharoensis* using Pearson's Chisquare test and the Kruskal-Wallis test, respectively. Spearman's Rank correlation was used to correlate blood-circulating gametocyte numbers with mean oocyst numbers and infection rate.

Ethical consideration

The protocol was reviewed and approved by National Research Ethics Review committee (NRERC) of the Ethiopia (Ref #: 10/31/2018). Permission was also obtained from Buno Bedele and East Wollega Zonal Health Offices, and Arjo-Didessa Sugar factory, Oromia Regional State, Ethiopia. Gametocyte carriers were recruited among febrile patients visiting selected health facilities to seek treatment for malaria. Study participants with *P. vivax* and *P. falciparum* infection and with gametocytemia were informed about the benefits and risks of the study. Both oral and written consent was obtained from participants or legal guardians/ parents in the case of children. During the study period, all patients positive for malaria parasites through blood film examination were treated as per the national malaria treatment guideline for free.

Results

Anopheles mosquito exposure, feeding, survival and infection rate using IDMFA

Overall, 586 febrile patients were screened for malaria during the study period. Ninety-four individuals who were unwilling to participate and 57 who received anti-malarial treatment within the past 48 hours prior to sample collection were excluded. A total of 125 patients were confirmed as malarial positive. Out of the total, 54 (43.2%) were found to be infected with *P. vivax* and 71(56.8%) were *P. falciparum.* Of these, 40 (74%) of *P. vivax* and 19 (26.7%) *P. falciparum* infections were microscopically confirmed as gametocyte carriers.

Even though, all gametocyte carrier individuals fulfilled the inclusion criteria and enrolled in the experimental infection, only 32 parallel successful mosquito feeding assays were considered for analysis. Of these, 32 feeding experiments, 24 (68.57%) were infected with *P. vivax* and 8(31.43%) were infected with *P. falciparum.* The three mosquito species differed in feeding time. *Anopheles gambiae* s l. fed quickly with 35% of individuals Hemo-concentration in 30 minutes while *An. pharoensis* and *An. coustani* complex fed more slowly with less than 35%, and feeding took approximately 40–60 minutes.

Of the total 5,915 female mosquitoes allowed to feed on infected blood, 2,106 (35.60%) fed successfully in 32 independent experimental infections. Feeding rate for *An. gambiae* s l, *An. pharoensis* and *An. coustani* complex was 1025 (39.65%), 487(29.24%) and 594(35.67%), respectively. The percentage of fed mosquitoes after exposure to an infected blood meal was significantly different among species ($G = 48.43$, $df = 2$, $p < 0.0001$) (Table 1). The proportion of infected mosquitoes following engorgement on an infected blood meal was significantly different among species ($G = 6.4955$, $df = 2$, $p = 0.0388$) (Table 1).

Overall, pairwise comparison shows that only significant infection rate was found in between *An. gambiae* s. l. (61.14%) and *An. coustani* complex (56.83%) ($p = 0.011$) (Table 1), Whereas, the rest comparison showed non-significant difference.

Table 1
Anopheles mosquito species by their physiological state and infection rate

Anopheles mosquito species	Parasite species				
		# Mosquitoes exposed	Fed # (%)	# Survived and dissected	Infection rate (%)
An. gambiae s l	*P. vivax*	1775	723(%)	229(%)	188(82%)
	P. falciparum	810	302(%)	108(%)	66(61.11%)
	Total	2585	1025(39.65%)	384(%)	254(66.14%)
An. pharoensis	*P. vivax*	1170	435(%)	244(%)	157(64.34%)
	P. falciparum	495	159(%)	111(%)	60(54%)
	Total	1665	594(35.67%)	355(%)	217(61.12%)
An. coustani complex	*P. vivax*	1170	351(%)	225(%)	136(60.44%)
	P. falciparum	495	136(%)	97(%)	47(71.3%)
	Total	1665	487(29.24%)	322(%)	183(56.83%)

Infection Intensity measurement using IDMFA

Overall, there was significant difference in mean infection intensity among *An. gambiae s l.*, *An. pharoensis* and *An. coustani* complex (χ 2 = 10.709, df = 2, p = 0.00473) (Fig. 1). However, pairwise comparisons of Wilcoxon test indicated that the differences were only between *An. gambiae* s.l. and *An. coustani* complex (p = 0.0043). The median infection intensity for *An. coustani* Complex was 1.16 compared to 2.00 in the *An.gambiea* s l, while for *An. pharoensis* was 1.25.

Figure 1. Box plot showing mean intensity of Plasmodium-infection following indirect membrane feeding assay in populations *An. gambiae* s l., *An. coustani complex and An. pharoensis* Species

Relationship between gametocyte abundance and Oocyst density

There was significant correlation between gametocytes and mean oocyst (p < 0.001). Correlation comparison between gametocyte density and mean oocyst density showed positive linear relationship. Although, there was variability in the association among species *An. gambiae* s l. (r = 0.65, p = 0.000026), *An. pharoensis* (r = 0.66, p = 0.000016) and *An. coustani* complex (r = 0.76, p = 0.00000013) (Fig. 2).

In general, a positive correlation between the gametocytes/500 leukocytes and the infection rate of the mosquitoes was observed. However, the association varied among species *An. gambiae* s.l. (r = 0.46, p = 0.0064), *An. pharoensis* (r = 0.64, p = 0.000043) and *An. coustani* complex (r = 0.44, p = 0.0088) (Fig. 3).

Discussion

In the current study, *An. coustani* complex showed susceptibility for the development of *P. falciparum* and *P. vivax* parasites in experimental infection. However, *An. gambiae* s.l. and *An. pharoensis* had a significantly higher infection rate than *An. coustani* complex which is considered as a suspected malaria vector in Ethiopia and all three species showed high mean oocysts density, with the highest oocyst density found in *An. gambiae* s.l., which is one of the main malaria vector species in sub-Saharan Africa. Studies in some countries under natural conditions indicate that *An. coustani* complex supports the development of both *P. falciparum* and *P. vivax* [33, 34]. In contrast to what we are reporting here, similar experimental studies conducted in Jimma town of southwest Ethiopia indicated that *An. coustani* complex is not susceptible to *P. vivax* development [32]. This discrepancy might be due to larval breading site variability and the presence of different member species of *An. coustani* complex i.e., there might be different sibling species present in different parts of the country.

Determining the susceptibility of Anopheline mosquito' species to malaria parasites in time and space is important in vector control programs [31–33]. In this study, it is documented that there is a difference in susceptibility among the three species. The rate of mosquito infection is affected by various factors such as environmental, biological as well as behavioral [19, 35, 36]. *An. coustani* complex was known as suspected vector in Ethiopia [33, 34]. Immunological studies using wild catch mosquitoes of *An. coustani* in different countries in sub-Sharan Africa have shown its susceptibility to *P. falciparum* and *P. vivax* infection [33–40].

The susceptibility of *An. gambiae* s.l., and *An. pharoensis* to malaria parasites *P. vivax* and *P. falciparum* infection has been long established [41] and growing evidence including this study confirm the susceptibility of *An. coustani* complex to both *P. vivax* and *P. falciparum* from Ethiopia, Kenya, Zambia, DRC, and Cameroon [18, 33, 36, 38, 42], field populations were susceptible, 33, 34, 43].

Vector competency varies from species to species and usually there is high proportion of uninfected mosquitoes when the infection process is controlled. This is confirmed in studies conducted in Ethiopia and elsewhere in world [32, 44, 45]. On the other hand, infection rates were much higher in experimental studies than those reported in natural infection for the three species examined. As determined by ELISA technique, based on the use of species-specific

anti-sporozoite monoclonal antibodies, mosquito populations had different infection rates in sub-Saharan countries. For instance, the infection rate of *An. gambiae* s.l ranged from 0.3 to 9.3%; An. *pharoensis* from 0.4 to 5.2%; and *An. coustani* complex from 0.3 to 1.81% [18, 33, 46].

In this study, gametocyte density was found to be significantly positively correlated with infection rates. Irrespective of the differences in mosquito and parasite species, in our findings also, *Plasmodium* gametocyte density was significantly correlated to infection rates and mean oocysts. This is also true in other studies conducted in Manaus, in the western Brazilian Amazon and Bengbu, Anhui Province, central China [44, 45].

In this study, infected blood with equivalent gametocyte density was provided to mosquitoes' species, but variable susceptibility was observed. This discrepancy in infection rate might be due to different factors, such as, gametocyte maturity, gametocyte sex ratio, different *Plasmodium* genotypes, rhythms in the density and infectivity of transmission forms (gametocytes), immune factors in patient sera, mosquitoes' innate immunity, all of which could alter gametocyte infectivity [47–50]. In addition, recent studies indicate that sub-microscopic gametocyte density is capable of infecting mosquitoes; this shows that rather than gametocyte density, the above contributing factors play a role in the variability of infection rate and mean oocyst. Studies from different countries show that sub-microscopic infections might be the major contributor to malaria transmission [51–54]. This shows that gametocyte density is not the only factor in mosquitoes' infection.

Furthermore, secondary and suspected vectors such as *An. pharoensis* and *An. coustani* complex have relatively higher abundance during the dry season [12]. This might have importance in local malaria transmission, as they may help to increase or prolong the malaria transmission period [55]. Mostly secondary vectors are often outdoor biting and outdoor resting [46, 56]. The role of outdoor-resting anopheles' mosquitoes in malaria transmission is important, secondary vectors are vectors which contribute to malaria transmission in Africa, and their role in transmission is not negligible [56, 57]. Most secondary vectors have a short survival rate with 50–60% natural mortality rates per gonotrophic cycle [23, 24]. This might explain the reason, why population density of the secondary vector has never been high in many settings.

Most anopheles vectors found in nature have only a few oocysts and oocysts have little importance in malaria epidemiology; rather, it is the outdoor biting and resting behavior in nature that contribute to residual malaria transmission in many parts of sub-Saharan Africa and pose new challenges as they cannot be reliably monitored or controlled using conventional tools and outdoor biting proportion increased by 10% [58–60]. In more recent studies in sub-Saharan Africa, *An. pharoensis* and *An. coustani* complex likely became primary role in malaria transmission; this might be as a result of existing interventions targeting primary vectors to achieve complete malaria control [61].

Conclusion

In this study it is confirmed that *Anopheles coustani* complex became receptive for the development of *P. falciparum* and *P. vivax* in this experimental infection using IDMFA. Even though, *An. coustani* complex previously considered as non-vector or suspected malaria vector in Ethiopia. For effective malaria elimination, there should be a focus on *An. coustani* complex to avoid focal malaria transmission or limit residual malaria transmission. In addition, it is highly recommended to re-consider the current vector surveillance and control tools to targeting outdoor transmission, and further investigation of immatures ecology and dynamics can help understand the bionomics of these mosquitoes' permissiveness to *Plasmodium* development.

Abbreviations

IDMFA
Indirect membrane feeding assay
TIDRC
Infectious Diseases Research Center
ICEMR
International Center Excellence for Malaria Research
IR
Infection rate
II
Infection intensity
LLIN
Long-lasting insecticidal net
IRS
Indoor residual spraying

Declarations

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Authors' contributions

AT conceived the study, drafted the manuscript, analyzed and interpreted the data; AT, AD, DH, KH and HG were involved in field data collection and patient sample collection. AT, HG and KH performed mosquito dissection and oocyst detection and quantifications. AA, YG and DH critically reviewed the manuscript for significant intellectual content. The final version of the manuscript was read and approved by all authors.

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Availability of data and materials

The datasets used in this study are available upon reasonable request from the corresponding author.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

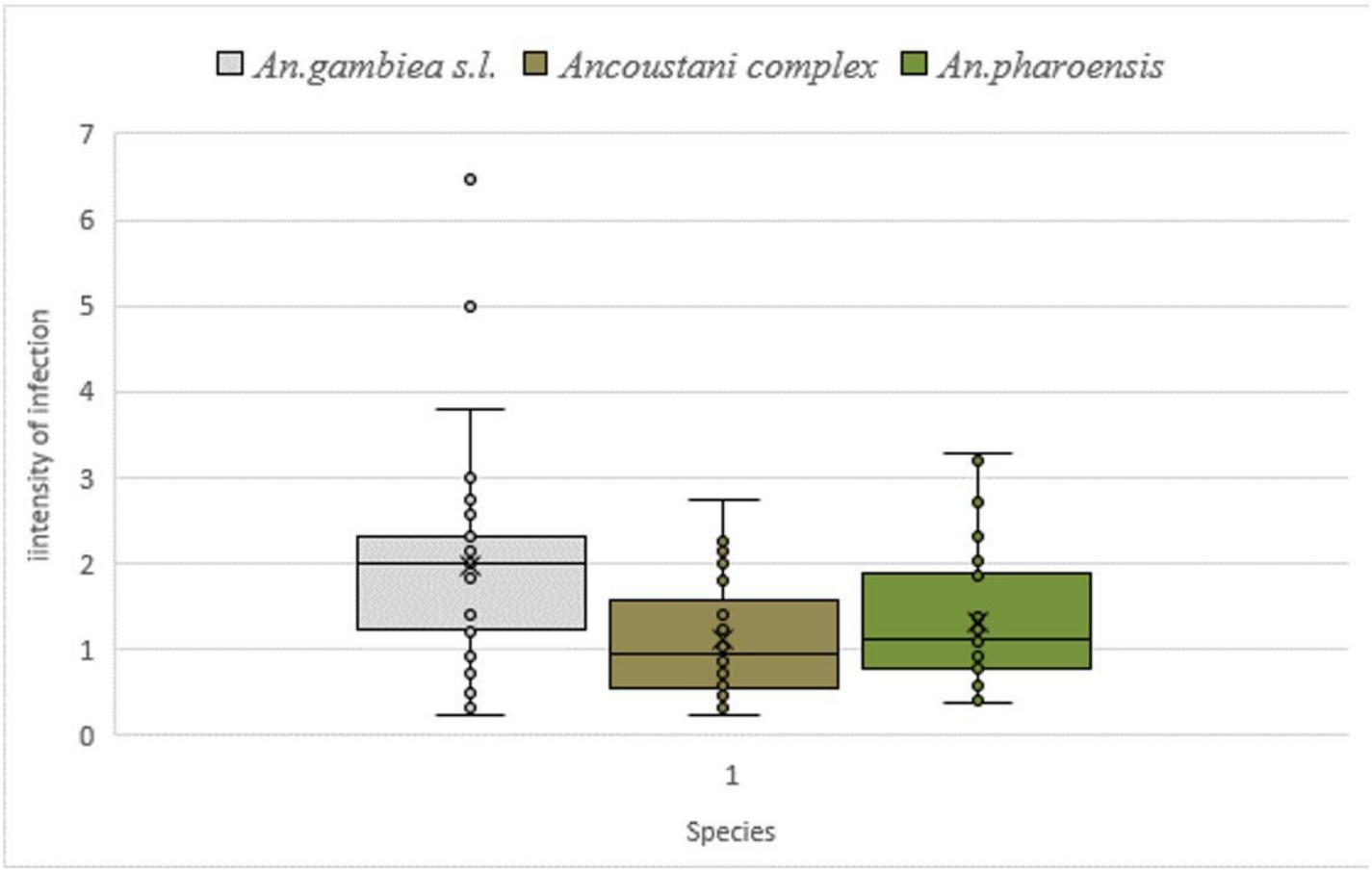

Figure 1

Box plot showing mean intensity of Plasmodium-infection following indirect membrane feeding assay in populations *An. gambiae* s l*., An. coustani complex and An. pharoensis* Species

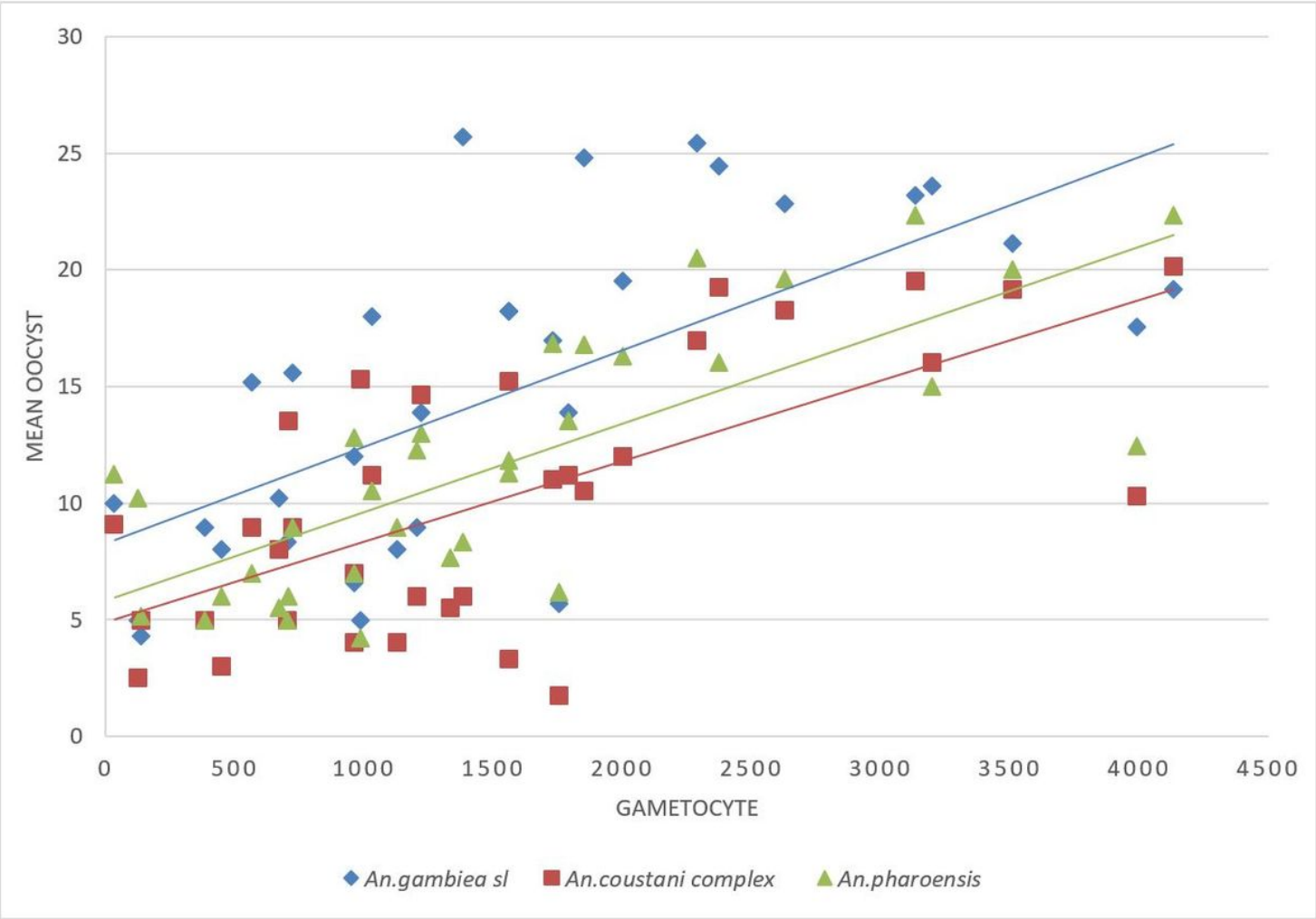

Figure 2

Correlation between mean gametocyte and oocyst (density/µl) in populations of *An. gambiae sl.*, *An. coustani* complex and *An. pharoensis*.

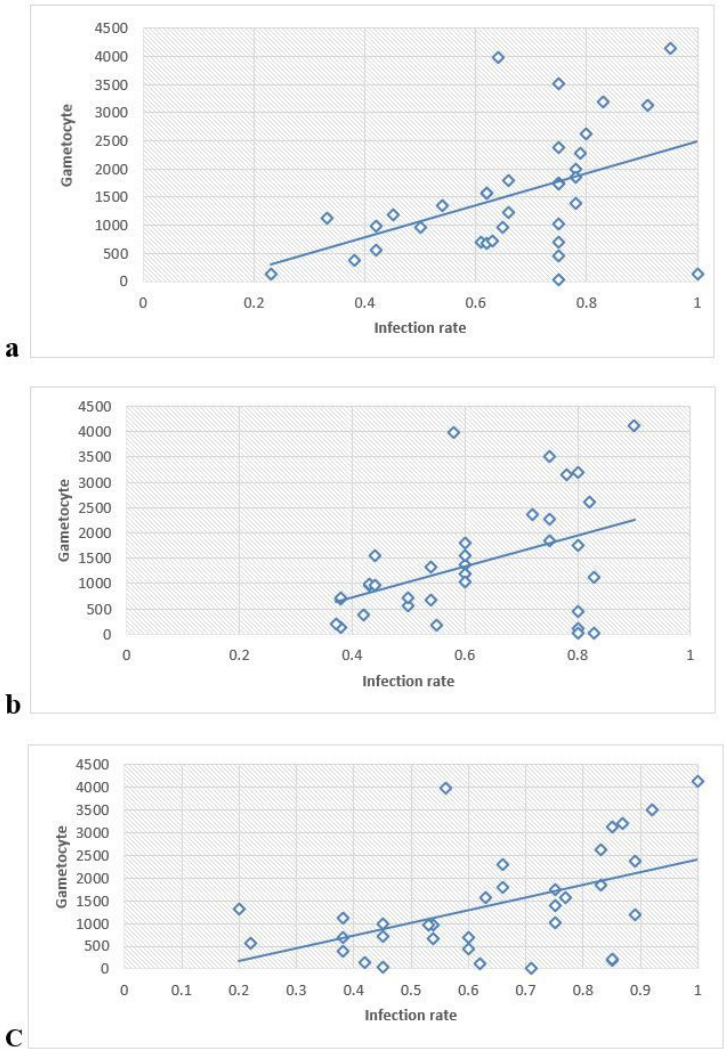

Figure 3

Correlation between infection rate and gametocyte density of *P.vivax* and *P. falciparum*. IDMFA performed for Individual feeds are presented parallel with *An. gambiae s.l.* (a), *An. pharoensis* (b) and *An. coustani* complex (c).