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Original Article

Genetic identity, human blood indices, and sporozoite rates of malaria vectors in Gaa-Bolorunduro, Kwara State, Nigeria

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Abstract

Introduction: To identify the specific *Anopheles* mosquito sibling species responsible for malaria transmission, determine their vectorial potential, and predict suitable control measures, this study investigated genetic identities, human blood feeding, and sporozoite infection rates of endophilic *Anopheles* mosquitoes in Gaa-Bolorunduro, a cattle rearing community in Kwara State, Nigeria.

Methodology: Monthly pyrethrum spray collections of *Anopheles* mosquitoes were conducted for one year in addition to PCR characterization of sibling species and ELISA probing of human blood meal and sporozoite infections. Mean numbers and human blood indices (HBI) of the different *Anopheles* sibling species identified were compared.

Results: The total of 668 PCR-identified mosquitoes comprised 50.8% *An. arabiensis*, 46.7% *An. gambiae,* and 2.5% *An. coluzzii*. Annual mean numbers of *An. arabiensis* was significantly higher ($p = 0.001$) than *An. coluzzii* but not *An. gambiae* ($p = 0.602$). Proportions of *An. arabiensis* found with human blood (0.29) were lower compared to *An. gambiae* (0.72) and *An. coluzzii* (0.75). However, the annual mean HBI of *An. arabiensis* was not significantly higher than *An. gambiae* ($p = 0.195$) and *An. coluzzii* ($p = 0.249$). *Plasmodium falciparum* sporozoite infection rate was 1.6% in *An. gambiae*, 0.9% in *An. arabiensis* and 0% in *An. coluzzii*.

Conclusions: The prevalent *An. arabiensis* and *An. gambiae* mosquitoes found indoors, despite the outdoor cattle population barrier, could be targeted by community-scale utilization of long-lasting insecticide-treated bed nets. Further studies on outdoor mosquito surveillance and bovine blood meal identification are required for the recommendation of suitable complementary vector control measures for the community.

Key words: Malaria; *Anopheles*; *Plasmodium*; sporozoite; transmission.

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Introduction

Global malaria cases and deaths have remained alarming, especially in sub-Saharan Africa (SSA). About 93 to 94% of the reported malaria cases (228 million) and deaths (405,000) in 2018 occurred in the World Health Organization (WHO) African region [1]. The major *Anopheles* mosquito species responsible for malaria transmission exist as species complexes comprising morphologically indistinguishable but genetically distinct sibling species with diverse ecology and behaviors [2-4]. *Anopheles gambiae s.l.* represents a major African malaria vector species complex containing nine sibling species including *An. gambiae*, *An. coluzzii*, *An. arabiensis*, *An. amharicus*, *An. quadriannulatus*, *An. merus*, *An. melas*, *An. bwambae,* and *An. fontenillei* [5]. Over the years, the dissimilar ecology and principal behaviors of some of these sibling species have been established. *An. gambiae* and *An. coluzzii* species are naturally anthropophagic and endophilic [2,3] while *An. arabiensis* could elicit extensive outdoor animal feeding preferences [3,6-8] in addition to persistent but more cautious and short-lived anthropophagic indoor foraging behavior [9,10]. Following this established body of knowledge, the genetic or sibling species identification of the prevailing *Anopheles* mosquitoes in an area could offer intrinsic predictive value for the behavior, and as such, the suitable control measures for such identified species. For instance, after the genetic identification of *An. gambiae* and *An. coluzzii* as the predominant species available indoors within a community, the anthropophagic and endophilic traits of these species can be targeted by indoor control measures such as long-lasting insecticide-treated bednets (LLIN). In

contrast, insecticidal entry point eave tubes [11], exit point eave baffles [12], and indoor residual spraying of insecticides on the walls of houses have been recommended against the repeated but brief house entry behavior of the *An. arabiensis* mosquitoes [13].

However, changes in vector behavior and/or sibling species composition have also been reported among members of this *An. gambiae* species complex based on the availability and changes in certain conditions within a study area [13]. Increased insecticidal bed-net coverage and human protection have been reported to either induce a shift from *An. gambiae* prevalence to *An. arabiensis* dominance [14,15] or elicit zoophagic tendencies among previously anthropophagic *An. gambiae* and *An. coluzzii* sibling species [16,17]. Conversely, scarcity of cattle and abundance of unprotected humans led to anthropophagic, endophagic, and postprandial endophilic behaviours among a naturally zoophagic *An. arabiensis* mosquito population [18,19]. After an increase in cattle population, scale-up of bed-net coverage, and the scarcity of unprotected humans, this historically anthropophagic *An. arabiensis* population later demonstrated earlier outdoor biting times [20] and cattle host affinity [8] within the same study area. Indeed, livestock keeping such as domestic cattle rearing could influence vector behavior and/or species composition of the *Anopheles* mosquitoes in an area. This is because, in addition to the anthropophilic *Anopheles* species attracted to the human population, the heat and odors from the cattle population may also attract zoophagic mosquito species to the outdoor animals and surrounding households thereby leading to higher human malaria exposure [13]. Also, the hoof prints of cattle across rain-fed puddles or at cattle watering sites could provide ephemeral and sunlit larval habitats for increased breeding and unusual abundance of zoophagic *An. arabiensis* mosquito species that usually prefer such breeding sites [21,22]. The above-described occurrence of higher human malaria exposure due to increased attraction of mosquitoes to households and enhanced vector larval habitat creation is called zoopotentiation [21,22]. As such, cattle rearing settlements represent important communities to be considered for malaria vector surveillance, research, and control. However, beyond the genetic identification of these *Anopheles* species and the prediction of their behaviour from established knowledge, empirical evidence on their human blood indices and malaria parasite infection rates needs to be established to identify the major species involved in active malaria transmission within the study area. Actual determination of these key attributes will assist in the identification and subsequent evaluation of suitable malaria vector control measures. Gaa-Bolorunduro is a community in Kwara State, Nigeria, inhabited exclusively by Fulani cattle breeders. Higher *An. arabiensis* (65%) species occurrence compared to *An. gambiae* (29%), *and An. coluzzii* (6%) has been reported in a 6-month mosquito species identification study conducted in Gaa-Bolorunduro community in 2013 [23]. According to the study, the brevity of the mosquito collection period did not permit the determination of *Anopheles* species identity over different seasons capable of influencing their presence [23]. Here, we report the results of a year-long study of *Anopheles* mosquito sibling species composition in addition to the first report of human blood indices and *Plasmodium falciparum* infection rates of *Anopheles* mosquitoes collected in the cattle rearing Gaa-Bolorunduro community in Kwara State, Nigeria.

Methodology

Study area

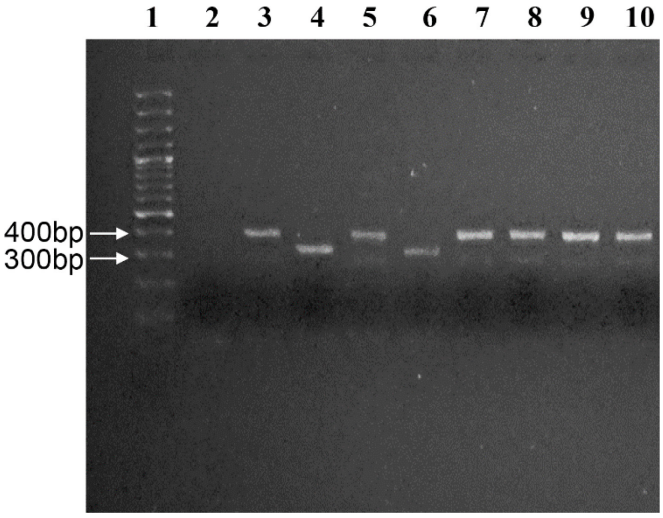
This study was conducted in Gaa-Bolorunduro (08°27' N, 04°38' E), a rural community in the Ilorin South local government area of Kwara State, Nigeria. The village is inhabited exclusively by Fulani residents engaged in semi-settled cattle rearing by grazing the cattle around for some hours during the day and returning the animals to outdoor sheds at various points within the community in the evenings. Houses in the community consist of single-room huts made of mud and straw roofing without ceilings and window nets. All the community residents lacked any form of bed-nets as at the time of this study. The area is under the Guinea savannah zone of Nigeria with mean annual rainfall and temperatures of 1,150 mm and 24-30 °C respectively [24].

Mosquito collection and processing

Ten different single-room huts inhabited by residents willing to allow consecutive monthly mosquito surveys for one year were selected for adult mosquito collection using the pyrethrum spray catch method [25]. The same rooms, selected on the basis of the willingness of the residents to allow unrestricted access, were used for mosquito collections once a month from October 2016 to September 2017. Each *Anopheles* mosquito sample collected was preserved on silica gel in an Eppendorf tube for further analysis at The Molecular Entomology and Vector Control Research Laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. The mosquito

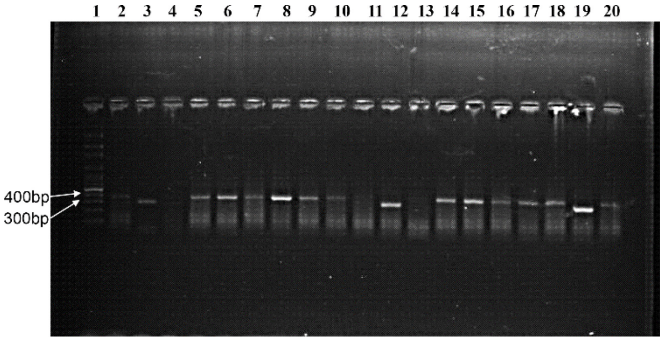
samples identified morphologically [2] as *An. gambiae s.l.* were characterized to sibling species level (Figure 1) using the standard species-specific PCR primers and protocol of Scott *et al*. [26]. The primers were designed from the DNA sequence of the intergenic spacer region of *An. gambiae s.l.* include Universal GTGTGCCCCTTCCTCGATGT, *An. gambiae* s.s CTGGTTTGGTCGGCACGTTT, *An. merus* and *An. melas* TGACCAACCCACTCCCTTGA, *An. arabiensis* AAGTGTCCTTCTCCATCCTA and *An. quadriannulatus* CAGACCAAGATGGTTAGTAT [26]. *Anopheles gambiae s.s.* identified from following the procedure of Scott *et al*. [26] were further characterized as *An. coluzzii* or *An. gambiae* (Figure 2)

Figure 1. PCR Gel Electrogram of some field collected *An. gambiae* s.s and *An. arabiensis*.



Lane 1: DNA ladder/Marker; Lane 2: Negative control; Lane 3: *An. gambiae* s.s positive control; Lane 4: *An. arabiensis* positive control; Lane 5, 7-10: *An. gambiae* s.s; Lane 6: *An. arabiensis*.

Figure 2. PCR–RFLP electrogram of some field collected *An. gambiae* and *An. coluzzii*.



Lane 1: DNA ladder/Marker; Lane 2: positive control *An. coluzzii*; Lane 3: positive control *An. gambiae*; Lane 4: Negative control; Lanes 5-10, 14-18, 20: *An. coluzzii*; Lanes 12, 19: *An. gambiae*.

by digestion of the *An. gambiae s.s.* PCR products using *Heamophilus haemolyticus* (Hha1) restriction enzyme as detailed in the standard PCR-RFLP protocol of Favia *et al*. [27]. The digestion was carried out at 37 °C for 6 hours in a thermal cycler [27]. The standard sporozoite ELISA procedure [28] was used to test for *P. falciparum* sporozoite infections in the heads-thoraces of the female *Anopheles* mosquito samples collected using monoclonal antibodies and positive controls from the Centers for Disease Control and Prevention (Atlanta, USA). Human serum (Rockland immunochemicals, Gilbertsville, USA), and monoclonal antibodies procured (Kikergaard and Perry Laboratories, Gaithersburg, USA) were used to test for the presence of human blood in the abdomens of all blood-fed *Anopheles* mosquito samples collected following the standard ELISA procedure for mosquito blood meal identification [29].

Data Analysis

Human blood index (HBI) was determined as the proportion of individual mosquito species with human blood [25]. The number of people who slept in the rooms overnight was taken as a reflection of human availability for mosquito bites because the persons in the village were not sleeping under bed-nets. The data obtained were transformed ($\sqrt{n + 0.5}$) to normal distribution [30] and analyzed using SPSS 16.0. Significant differences ($p = 0.05$) in the numbers of the 3 different *Anopheles* mosquito sibling species collected were determined using ANOVA while the numbers of each mosquito species collected in the dry and wet seasons were compared using student *t*-test. Human blood indices of the different *Anopheles* species were compared using logistic regression. Sporozoite infection rates were taken as a percentage of mosquitoes with *Plasmodium falciparum* sporozoite infection.

Results

The numbers of *Anopheles* mosquito species collected are presented in Table 1. A total of 668 PCR-identified *Anopheles* samples comprised 339 (50.8%) *An. arabiensis*, 312 (46.7%) *An. gambiae* and 17 (2.5%) *An. coluzzii* species. A comparison of the numbers of the 3 *Anopheles* mosquito species collected showed that the annual mean number of *An. gambiae* (4.91 ± 1.62) was significantly higher ($F = 3.65$, $p < 0.001$) than *An. coluzzii* (1.25 ± 0.68) but not *An. arabiensis* ($F = 0.44$, $p = 0.602$) (Table 1). Also, there was significantly higher ($F = 3.21$, $p = 0.001$) number of *An. arabiensis* (4.47 ± 3.09) compared to *An. coluzzii* (1.25 ± 0.68) mosquitoes.

Seasonal mean numbers of each *Anopheles* species collected were derived from the dataset shown in Supplementary Table 1. The derived results of the numbers of each mosquito species collected in the dry (October - March) and wet (April - September) seasons are shown in Figure 3. Only the number of *An. arabiensis* species was significantly higher ($t = 5.43$, $p = 0.003$) in the dry season (7.07 ± 1.79) compared to the wet season (1.86 ± 1.28). Numbers of *An. gambiae* ($t = 1.38$, $p = 0.227$) and *An. coluzzii* ($t = 0.305$, $p = 0.773$) mosquito species in the wet season were not significantly different from the numbers of the same species collected in the dry season (Figure 3).

In the wet season, significantly higher ($t = 4.63$, $p = 0.006$) number of *An. gambiae* mosquitoes (4.26 ± 1.34) were found compared to *An. arabiensis* (1.86 ± 1.28). However, in the dry season, the number of *An. arabiensis* (7.07 ± 1.79) mosquitoes was significantly higher ($t = -3.89$, $p = 0.011$) than *An. gambiae* (5.56 ± 1.73) species. In both dry ($t = 4.99$, $p = 0.004$) and wet ($t = 4.59$, $p = 0.006$) seasons, the numbers of *An. gambiae* collected were significantly higher than *An. coluzzii* (Figure 3). Similarly, there was significantly higher ($t = 7.12$, $p = 0.001$) number of *An. arabiensis* (7.07 ± 1.79) compared to *An. coluzzii* (1.29 ± 0.90) in the dry season. However, the number of *An. arabiensis* (1.86 ± 1.28) was not significantly higher ($t = 1.11$, $p = 0.32$) than *An. coluzzii* (1.13 ± 0.51) in the wet season (Figure 3). Gel electrophoresis images showing samples of the different field-collected mosquito sibling species are shown in Figures 1 and 2.

Proportions of *An. gambiae* 0.72 (220/304) and *An. coluzzii* 0.75 (12/16) mosquitoes that fed on human blood were 2.4 times higher than that of *An. arabiensis* 0.29 (94/320) (Table 2). However, there were no significant differences between the annual mean HBI of *An. gambiae* 1.11 ± 0.08 and *An. arabiensis* 0.89 ± 0.14 ($F = 1.93$, $p = 0.195$), *An. gambiae* and *An. coluzzii* 0.89 ± 0.24 ($F = 1.49$, $p = 0.249$) and between *An. arabiensis* and *An. coluzzii* ($F = 1.73$, $p = 0.218$) mosquitoes (Table 2). None of the *An. coluzzii* mosquitoes collected were found with sporozoite infection. Sporozoite infection rates of *An. gambiae* (5/312) and *An. arabiensis* (3/339) were 1.6% and 0.9%, respectively.

Discussion

This study investigated sibling species composition, human blood indices, and sporozoite infection rates of endophilic *Anopheles* mosquitoes in Gaa-Bolorunduro, a community inhabited exclusively by cattle breeders.

Figure 3. Mean ± standard error (SE) numbers of female Anopheles mosquitoes collected during dry and wet seasons in the study community. Means with different letters within the same species are significantly different (Student's t-test, $p < 0.05$).

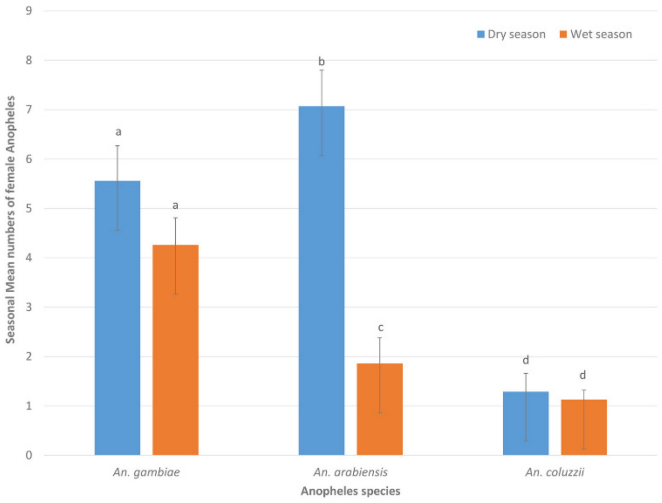


Table 1. Numbers of *Anopheles* mosquito species collected from the community.

Month	Actual (Transformed) No. of *An. gambiae*	Actual (Transformed) No. of *An. arabiensis*	Actual (Transformed) No. of *An. coluzzii*
Oct	24 (4.95)	30 (5.52)	0 (0.71)
Nov	28 (5.34)	38 (6.20)	5 (2.35)
Dec	81 (9.03)	104 (10.22)	0 (0.71)
Jan	24 (4.95)	62 (7.91)	6 (2.55)
Feb	20 (4.53)	48 (6.96)	0 (0.71)
Mar	20 (4.53)	31 (5.61)	0 (0.71)
Apr	40 (6.36)	16 (4.06)	0 (0.71)
May	10 (3.24)	5 (2.35)	3 (1.87)
Jun	16 (4.06)	4 (2.12)	1 (1.22)
Jul	10 (3.24)	1 (1.22)	2 (1.58)
Aug	10 (3.24)	0 (0.71)	0 (0.71)
Sep	29 (5.43)	0 (0.71)	0 (0.71)
Total actual numbers (Mean ± S.D of transformed values)	312 (4.91 ± 1.62[a])	339 (4.47 ± 3.09[a])	17 (1.25 ± 0.68[b])

Data were transformed to accommodate zero values and attain normal distribution. Data transformation formula $X1 = \sqrt{X + 0.5}$, where X1 is the transformed value and X is the actual value. Means with different letter superscripts are significantly different ($p < 0.05$).

Higher *An. arabiensis* (50.8%) occurrence was found compared to other species. This result is similar to the 64% *An. arabiensis* prevalence reported earlier in a 6-month mosquito surveillance study conducted in Gaa-Bolorunduro in 2013 [23]. The predominance of *An. arabiensis* mosquito sibling species have been reported in other studies conducted in cattle rearing communities in Africa [6,8,31,32]. The high indoor *An. arabiensis* occurrence despite outdoor alternative cattle host population in Gaa-Bolorunduro is not surprising. Tirados *et al*. [32] had also reported that a significantly higher number of *An. arabiensis* flew through a ring of cattle to a human-baited trap than to a cattle-baited trap. Outdoor cattle inability to significantly divert *An. arabiensis* mosquitoes from entering houses could be attributed to zoopotentiation and/or partial intrinsic endophilic nature of the mosquitoes. Nevertheless, the indoor mosquito collection carried out in our study may still have under-estimated *An. arabiensis* abundance in Gaa-Bolorunduro community considering that this species can also exhibit outdoor foraging and resting behaviors [31]. Higher occurrence of *An. arabiensis* species observed in our study in Gaa-Bolorunduro is in contrast with the results of low *An. arabiensis* (0.6-10%) incidences in other communities in Kwara State [23,33,34] where cattle were not available. Cattle hoof prints have been identified to provide suitable ephemeral, sunlit water pools preferred by *An. arabiensis* species for egg-laying and pre-adult development [35]. The large cattle population in Gaa-Bolorunduro could have created several of these suitable breeding sites leading to the *An. arabiensis* prevalence (50.8%) in the community compared to the low incidences reported in communities without cattle. Besides, *Anopheles arabiensis* is known to exhibit both endophilic and exophilic behaviors depending on prevailing conditions within the study area [13]. At the time of this study, indoor mosquito control measures (such as LLIN and IRS) that could discourage the endophilic tendencies of the *An. arabiensis* species were not found in the Gaa-Bolorunduro community. The result of 46.7% *An. gambiae* occurrence in the present study is higher than the 29% *An. gambiae* incidence was found earlier in the Gaa-Bolorunduro community in 2013 [23]. The 2013 study in Gaa-Bolorunduro was done for 6 months (November 2013 to May 2014) compared to our present one-year study. Higher *An. gambiae* occurrence (46.7%) observed in our study could therefore be as a result of year-long surveillance which accommodated the whole of the rainy season period during which the species is known to find suitable rain-fed breeding sites for larval development [36]. Accordingly, our study showed significantly higher numbers of *An. gambiae* compared to *An. arabiensis* during the wet season.

Despite the overall higher occurrence of *An. arabiensis* (50.8%) compared to *An. gambiae* (46.7%) and *An. coluzzii* (2.5%), the mean number of *An. arabiensis* mosquitoes were significantly lower than *An. gambiae* in the wet season. Significantly higher numbers of *An. arabiensis* only in the dry season attest to the affinity of *An. arabiensis* species for dry conditions and environments [2,37,38] when large rain-fed water bodies would have receded to form the

Table 2. Human blood indices of different *Anopheles* mosquito species in the community.

Month	*An. gambiae*			*An. arabiensis*			*An. coluzzii*		
	No of *An. gambiae* with human blood	Total no of *An. gambiae* with blood	Actual (Transformed) HBI	No of *An. arabiensis* with human blood	Total no of *An. arabiensis* with blood	Actual (Transformed) HBI	No of *An. coluzzii* with human blood	Total no of *An. coluzzii* with blood	Actual (Transformed) HBI
Oct	20	24	0.83 (1.15)	6	28	0.21 (0.84)	0	0	0.00 (0.71)
Nov	23	28	0.82 (1.15)	7	36	0.19 (0.83)	4	5	0.80 (1.14)
Dec	54	80	0.68 (1.09)	26	92	0.28 (0.88)	0	0	0.00 (0.71)
Jan	10	20	0.50 (1.00)	10	51	0.19 (0.83)	3	6	0.50 (1.00)
Feb	17	20	0.85 (1.16)	24	48	0.50 (1.00)	0	0	0.00 (0.71)
Mar	10	20	0.50 (1.00)	10	31	0.32 (0.91)	0	0	0.00 (0.71)
Apr	29	40	0.73 (1.11)	7	16	0.44 (0.97)	0	0	0.00 (0.71)
May	5	10	0.50 (1.00)	2	5	0.40 (0.95)	2	2	1.00 (1.22)
Jun	12	16	0.75 (1.12)	1	4	0.25 (0.87)	1	1	1.00 (1.22)
Jul	7	10	0.70 (1.09)	1	1	1.00 (1.22)	2	2	1.00 (1.22)
Aug	9	9	1.00 (1.22)	0	0	0.00 (0.71)	0	0	0.00 (0.71)
Sep	24	24	1.00 (1.22)	0	0	0.00 (0.71)	0	0	0.00 (0.71)
Total (Mean ± S.D of transformed values)	220	301	(0.73 ± 0.16 [a])	94	312	(0.31 ± 0.26 [a])	12	16	(0.33 ± 0.43[a])

HBI: Human blood index; HBI: No of *Anopheles* found with human blood divided by total no of *Anopheles* with blood; Data transformation formula X1= √X+0.5, where X1 is the transformed value and X is the actual value. Data were transformed to accommodate zero values and attain normal distribution. Mean HBI with the same letter superscripts are not significantly ($p > 0.05$) different.

ephemeral sunlit pools preferred by this species [35]. The number of *An. coluzzii* mosquitoes (17) found throughout the year were very low leading to reduced chances and actual observation of zero sporozoite rate among the *An. coluzzii* mosquitoes collected. The occurrence of low *An. coluzzii* (2.5%) incidence in the present study conformed to earlier reports of 6-9% *An. coluzzii* in Gaa-Bolorunduro [23] and other communities in Kwara state [39]. Lower incidences of *An. coluzzii* in these communities contrasts high *An. coluzzii* occurrence (40-100%) in other places like Lagos [40,41] which has extensive flooded areas suitable for *An. coluzzii* breeding. *Anopheles coluzzii* mosquitoes prefer to breed in flooded relatively permanent breeding sites [42,43].

In addition to year-long species composition, our study, for the first time, determined anthropophilic indices and sporozoite infection rates of *Anopheles* mosquitoes in Gaa-Bolorunduro community. Sporozoite infection rates identified in *An. gambiae* (1.60%) and *An. arabiensis* (0.9%) mosquitoes suggest the implication of these predominant species (*An. arabiensis* 50.8%, *An. gambiae* 46.7%) as the main vectors responsible for malaria transmission in Gaa-Bolorunduro. The lower human blood index of *An. arabiensis* compared to the *An. gambiae* species suggests lesser *An. arabiensis* human blood feeding that probably led to the lower *An. arabiensis* sporozoite rates (0.9%) compared to *An. gambiae* (1.60%). However, the annual mean human blood index of *An. arabiensis* was not significantly lower than those of the more anthropophagic *An. gambiae* and *An. coluzzii* species. This corroborates the assertion that *An. arabiensis* feeds often enough on humans to mediate intense transmission but also extensively enough on outdoor alternative hosts to be resilient against the use of indoor personal protection measures [13]. Lower human blood indices of *An. arabiensis* compared to *An. gambiae* have also been reported in other communities in Africa [7-8,44]. Overall, the results of this study suggest that the presence of cattle population influenced *Anopheles* species composition towards higher *An. arabiensis* mosquito occurrence in Gaa-Bolorunduro as compared to *An. gambiae* predominance in non-cattle rearing communities in Kwara State. The high numbers of *An. arabiensis* and *An. gambiae* mosquitoes found resting indoors in Gaa-Bolorunduro could be targeted using indoor control measures such as LLIN. However, the exophilic tendencies of *An. arabiensis* mosquitoes could also be triggered by full implementation of the universal LLIN coverage in this community. In this study, we did not conduct outdoor mosquito surveillance and bovine blood meal assessments. Non-detection of human blood in some of these blood-fed mosquitoes suggests that the vectors probably fed on the available alternative cattle host in the study community. Nevertheless, confirmation of the presence of cattle blood meal in these engorged mosquitoes is still required for suggesting appropriate complementary outdoor mosquito control measures such as the application of effective insecticide veterinary formulation to kill the mosquito when they land on the cattle.

Conclusions

This study identified higher indoor occurrence but lower human blood indices in *An. arabiensis* compared to *An. gambiae* and *An. coluzzii* mosquitoes in cattle rearing Gaa-Bolorunduro community in Kwara State, Nigeria. The prevalent indoor resting sporozoite infected *An. arabiensis* and *An. gambiae* mosquito species identified in this study can be targeted with appropriate and consistent use of long-lasting insecticide-treated bed nets on a community scale. Further studies on bovine blood meal assessments and outdoor mosquito collections are required for recommending complementary outdoor malaria vector control measures in this cattle rearing community.

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Authors' Contributions

STA and AO designed the study. AO and AOO coordinated the field collections. TAO supervised the laboratory analyses. AKO collated and analyzed the data. AO drafted the manuscript. All authors read and approved the final version of the manuscript.

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Annex – Supplementary Items

Supplementary Table 1. Derivations of the seasonal mean numbers of each *Anopheles* species collected.

Dry season	**Actual (Transformed) No. of *An. gambiae***	**Actual (Transformed) No. of *An. arabiensis***	**Actual (Transformed) No. of *An. coluzzii***
Oct	24 (4.95)	30 (5.52)	0 (0.71)
Nov	28 (5.34)	38 (6.20)	5 (2.35)
Dec	81 (9.03)	104 (10.22)	0 (0.71)
Jan	24 (4.95)	62 (7.91)	6 (2.55)
Feb	20 (4.53)	48 (6.96)	0 (0.71)
Mar	20 (4.53)	31 (5.61)	0 (0.71)
(Mean ± S.D of transformed values)	(5.56 ± 1.73)	(7.07 ± 1.79)	(1.29 ± 0.90)
Wet season			
Apr	40 (6.36)	16 (4.06)	0 (0.71)
May	10 (3.24)	5 (2.35)	3 (1.87)
Jun	16 (4.06)	4 (2.12)	1 (1.22)
Jul	10 (3.24)	1 (1.22)	2 (1.58)
Aug	10 (3.24)	0 (0.71)	0 (0.71)
Sep	29 (5.43)	0 (0.71)	0 (0.71)
(Mean ± SD of transformed values)	(4.26 ± 1.34)	(1.86 ± 1.28)	(1.13 ± 0.51)

Data were transformed to accommodate zero values and attain normal distribution. Data transformation formula $X1 = \sqrt{X+0.5}$, where X1 is the transformed value and X is the actual value.