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Surveillance of Culicine Mosquitoes in Six Villages of Taita-Taveta County, Kenya, With Host Determinations From Blood-Fed Females

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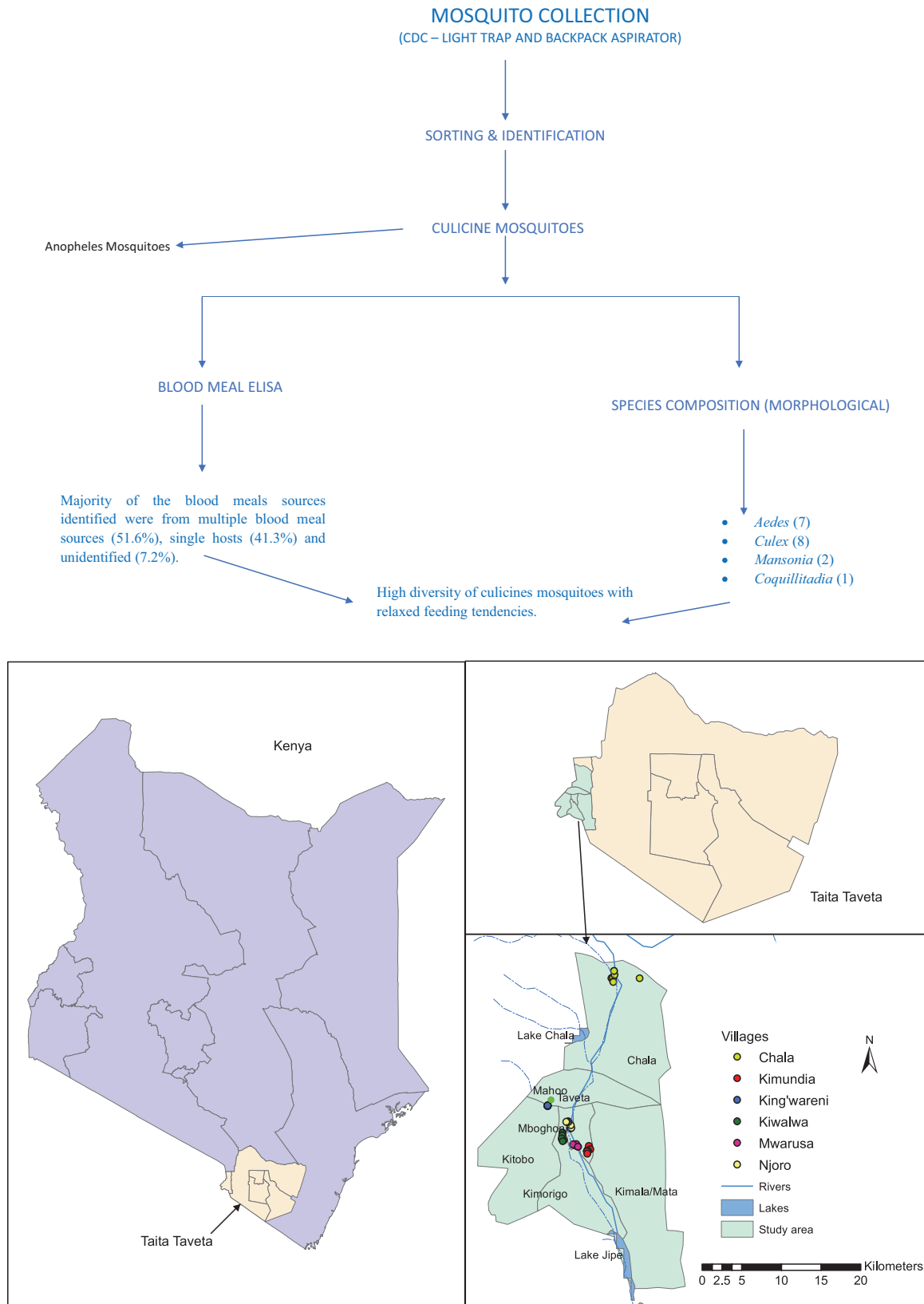
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Abstract

Culicine mosquitoes are vectors of human disease-causing pathogens like filarial worms and several arthropod-borne viruses (arboviruses). Currently, there has been an increase in emerging and re-emerging vector-borne diseases along coastal Kenya, which has been of major concern in public health. This study aimed at determining culicine mosquito species abundance, diversity and their host feeding preferences in Taita-Taveta County, Coastal Kenya. Entomological sampling was done during the long-wet season (March and May) and long dry season (June to October) 2016–2018. Mosquito sampling was done using CDC light traps and Backpack aspiration for indoor and outdoor environments. All culicine mosquitoes collected were identified morphologically and categorized according to their physiological status. Blood fed culicine mosquitoes were tested for bloodmeal sources using ELISA. In total, 3,278 culicine mosquitoes were collected, of which 738 (22.5 %) were found indoors and 2,540, (77.5 %) outdoors. The mosquitoes consisted of 18 species belonging to four genera: *Aedes* (7), *Culex* (8), *Mansonia* (2), and *Coquillettidia* (1). Overall, there was high mosquito species diversity (H) in outdoors ($H = 2.4339$) than in indoors ($H = 2.2523$), whereas even distribution (EH) was higher in indoors ($EH = 0.9064$) than outdoors ($EH = 0.8266$). Majorly the bloodmeals identified were from multiple host sources with (51.6%), single hosts (41.3%), and unidentified (7.2%). This study has demonstrated a high diversity of culicine mosquitoes with relaxed feeding tendencies. These mosquitoes are contributing to mosquito biting nuisance and the likelihood of exposure of populations to diseases of public health.

Graphical abstract



Key words: abundance, bloodmeal, Culicine, diversity

Over 80% of the population in the world is at the risk of vector-borne diseases including arboviruses, malaria and lymphatic filariasis (WHO 2017). Mosquitoes have played a significant role in the transmission of these pathogens. Notably, the subfamily *Culicinae* has been incriminated as a major vector of lymphatic filariasis (Derua et al. 2017) and arboviruses such as Rift Valley Fever virus, dengue fever virus, yellow fever virus, and West Nile Virus, (Sang et al. 2010, LaBeaud et al. 2011, Mwaengo et al. 2012, Lutomiah et al. 2016). Culicine mosquitoes of the genus *Aedes* and *Culex* encompasses the key species responsible for pathogen transmission. They are responsible in global transmission of diseases of public health interest resulting to lack of health security to both animals and human populations. Bloodmeals are crucial for mosquito oogenesis and reproduction but also an avenue for pathogens uptake from an infected host and eventual transmission to a susceptible one. The preference of the bloodmeal is influenced by several factors including host availability, nutritional requirements, host preferences of the species, and vector density (Zimmerman et al. 2006). For instance, *Culex quinquefasciatus* (Say) exhibits relaxed feeding tendencies, relying mostly on avian hosts for bloodmeals though in some instances feeding equally on both birds and mammalian hosts hence associated with multiple bloodmeal sources. This feeding tendencies and adaptation greatly increase the chances of lymphatic filariasis and arboviruses transmission (Kilpatrick et al. 2006, Marm Kilpatrick et al. 2006, Savage et al. 2007, Garcia-Rejon et al. 2010, Sawabe et al. 2010). Mosquito species abundance and diversity are known to be influenced by factors, such as climate, seasonality, availability of microhabitats for breeding, and physicochemical parameters of breeding sites (Jones et al. 2008, Muturi et al. 2008, Akram et al. 2009, Kim and Tsuda 2010, Midega et al. 2010). Tropical countries have environmental factors that favor an abundance of breeding sites and fast biological development of mosquitoes. Some of these factors contribute to the extensive proliferation of mosquitoes ranging from sporadic floods, irrigation canals, presence of several lakes/rivers, and low altitudes especially around coastal regions (Karungu et al. 2019). Culicine mosquitoes have a wide range of breeding areas; they breed mostly inside or near houses (peri-domestic habitats) in waters with high organic materials, such as canals, ditches, rivers, lakes, swamps, tree holes, and runoff from agricultural plants (Philbert and Ijumba 2013). *Culex quinquefasciatus*, for instance, prefers to breed in highly organic surface water like domestic collections of water, flooded open drains, flooded latrines, overflow water from houses, kitchens, as well as in-ground – pools, ditches, and shallow wells (Weinstein et al. 1997). *Aedes aegypti* (Linnaeus) on the other hand breeds in stagnant water in old tires, poorly discarded plastic bottles/cans, storage water tanks, and coconut husks (Philbert and Ijumba 2013).

Recently, coastal region, Kenya, has been a hot spot for emerging and re-emerging arboviral diseases including dengue fever and chikungunya virus. Culicine mosquitoes are believed to be the major drivers of these arboviral disease transmission dynamics (Petersen and Powers 2016; Agha et al. 2017a, b). Emerging and re-emerging of these infectious diseases are often due to arboviruses that are maintained in a zoonotic cycle between mosquito vectors and wildlife species, with spill over to humans in areas where human and wildlife population borders each other (Lindahl et al. 2017). Taita-Taveta County being within the Kenyan coastal region where the emergence and re-emergence of infectious diseases has been reported, it has forest ecology, urban ecology and agro-ecosystem ecology, which contributes to proliferation of mosquitoes. It also borders Tsavo National park, which poses high-risk of spill over because there is human and wildlife population interface. Taita-Taveta County also uses agrochemicals such as herbicides and pesticides that can increase the risk of insecticide resistance to insecticides

used in public health for control of mosquitoes such as pyrethroids. Major studies done in Taita-Taveta County since the WHO Global Malaria Eradication Campaign (between 1955 and 1960) (Wilson 1960) were majorly on Anopheles species and no effort has been made to study Culicine mosquitoes which are also important in disease transmission and biting- nuisance. There is no published data on Culicine mosquito species composition, abundance, diversity, and host feeding preferences in Taita-Taveta County. Concerns about the role of local mosquito vectors in the introduction of vector-borne disease to anthropologic environments have created the need for conducting studies on mosquito vector diversity and host preferences for bloodmeal source to improve our understanding of the disease transmission cycles and design effective vector control tools.

Materials and Methods

Study Area

The study was conducted in Taita-Taveta County in the Coastal region of Kenya. The County lies between latitude 3° 24'00"S and longitude 37°41'00"E. Taita-Taveta borders Tsavo national park which is rich in a variety of wild animals and birds. The main economic occupation of the inhabitants in the County are mainly mixed farming, livestock, trade/business, and waged labor. Most households in the study areas prefer keeping goats, chickens, and cattle. The houses are mainly made of concrete or mud walls and iron sheets or palm leaf (Makuti) roofing. The area experiences bimodal rainfall pattern with the mean annual rainfall ranges between 200 and 1,200 mm. Temperature ranges from 21°C to 31°C. Agricultural activities in the county rely on water from four rivers; Tsavo, Lumi, Njoro, Kitobo, and spring water from the foot of Mt. Kilimanjaro.

Six villages were sampled for adult mosquitoes; Chala, Kimundia, King'wareni, Kiwalwa, Mwarusa, and Njoro. Kiwalwa is a highly populated riverine ecosystem. Mwarusa is a flat, swampy area with river Lumi flowing along the edge of the village. Kimundia is swampy, households are sparsely distributed, and some sections of this village are flooded during the rainy seasons. Some parts of Njoro are wet, particularly where river Njoro flows through, while the rest of the village is dry and dusty. Chala is a dry area though, with modern houses, agriculture is done through drip and localized irrigation. The criteria for the selection of these villages were based on riverine, nonriverine ecosystem, and drainage system. The entomological sampling design was based on rainfall pattern, the main wet season (March and May), and dry season (June to October) 2016–2018. During each season, sampling was done once, totaling to six visits per household over the entire period.

Mosquito Collection

Entomological sampling was done using CDC backpack aspirators model 1412 (John W. Hock Company, Gainesville, FL) and Centers for Disease Control (CDC) light traps (John W. Hock Company), which were set indoor and outdoor. CDC light trap, mosquito collection was conducted in 10 randomly selected houses between 1800 and 0600 h. One light trap was hanged at the foot-end of the bed and a second light trap was placed outside the house. Three hundred and twenty light traps were used during the entire sampling frame. Sampling using Backpack aspiration technique was done in the same 10 randomly selected houses in each village. The indoor collection was done in the bedroom and living room. Mosquitoes were aspirated from the walls and all hanging clothing was shaken to expose any uncollected mosquitoes. In each house,

Table 1. Culicine mosquito species composition and abundance in the six villages of Taita-Taveta County, Coastal Kenya

Mosquito species	Chala		Kimundia		Kivalwa		Mwarusa		Njoro		King'wareni		χ^2		P-value	
	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor
<i>Aedes hirsutus</i>	0	0	293 (21)	40 (2.5)	12 (6.4)	5 (3.5)	21 (1.7)	7 (1.1)	37 (9.7)	1 (1.4)	0	0	105.4	-	<0.001	<0.001
<i>Aedes aegypti</i>	0	0	3 (0.2)	0	0	0	0	0	0	0	0	0	-	-	0.95	0.95
<i>Aedes mcintoshi</i>	0	0	139 (9.7)	1 (0.6)	39 (21)	7 (4.9)	4 (3.2)	1 (1.6)	32 (8.4)	3 (4.1)	0	0	70.3	-	<0.001	0.002
<i>Aedes pemaensis</i>	0	0	2 (0.1)	0	0	0	0	0	0	0	0	0	-	-	0.98	0.98
<i>Aedes simpsoni complex</i>	0	0	1 (0.07)	0	0	2 (1.4)	0	0	0	1 (1.4)	0	0	-	-	0.96	0.17
<i>Aedes straitipes</i>	0	0	0	0	7 (3.7)	1 (0.7)	0	0	0	0	0	0	-	-	0.35	0.42
<i>Aedes tricholabis</i>	0	0	8 (0.6)	1 (0.6)	0	0	0	0	0	0	0	0	-	-	0.56	0.64
<i>Culex annulirostris</i>	0	0	5 (0.4)	0	0	0	0	0	0	1 (1.4)	0	0	-	-	0.79	0.23
<i>Culex quinquefasciatus</i>	90 (98)	31 (100)	781 (55)	74 (4.5)	124 (66)	117 (82)	93 (74)	52 (83)	264 (69)	49 (67)	326 (100)	265 (100)	54.3	25.1	<0.001	<0.001
<i>Culex culicicoma</i>	0	0	0	0	0	0	1 (0.8)	0	0	0	0	0	-	-	0.09	0.09
<i>Culex poicilipes</i>	0	0	20 (1.4)	0	0	0	1 (0.8)	0	0	0	0	0	-	-	0.02	0.02
<i>Culex fuscoccephala</i>	0	0	3 (0.2)	0	0	0	0	0	0	0	0	0	-	-	0.99	0.99
<i>Culex straitipes</i>	0	0	1 (0.07)	0	0	0	0	0	0	0	0	0	-	-	0.97	0.97
<i>Culex vansomereni</i>	0	0	8 (0.6)	0	0	0	0	0	0	1 (1.4)	0	0	-	-	0.59	0.23
<i>Culex univittatus</i>	0	0	105 (7.4)	55 (21)	0	9 (6.3)	0	1 (1.6)	20 (5.2)	12 (16)	0	0	52.1	-	<0.001	<0.001
<i>Coquillettidia aurites</i>	1 (1)	0	8 (0.6)	0	1 (0.5)	0	1 (0.8)	1 (1.6)	18 (4.5)	1 (1.4)	0	0	-	-	<0.001	0.07
<i>Ma. africana</i>	0	0	0	0	2 (1)	0	0	0	1 (0.3)	1 (1.4)	0	0	-	-	0.03	0.23
<i>Ma. uniformis</i>	1 (1)	0	49 (3.4)	12 (7.4)	2 (1)	2 (1.4)	5 (4.0)	1 (1.6)	11 (2.9)	3 (4.1)	0	0	-	-	0.001	<0.001
Total	92	31	1,426	163	187	143	126	63	383	73	326	265	-	-	-	-

mosquito aspiration was done for between 5 and 10 min depending on the size of the house. The collection was done between 0600 and 1000 h. The traps were removed in the morning and all cups containing mosquitoes kept in the cool box for transportation to the KEMRI field laboratory in Taveta.

Mosquito Identification

All mosquitoes collected were morphologically identified in the field as culicine and anopheles and preserved using silica gel and later transported to KEMRI, Center for Geographic Medicine Research Coast for laboratory analysis. Only Culicine mosquitoes

Table 2. Culicine mosquito species diversity and evenness for the six villages in Taita-Taveta County, Kenya

Distribution	Village	Indoor	Outdoor
Shannon's diversity index (<i>H</i>)	Chala	0.1312 (1)	0.1245 (3)
	Kimundia	0.6913 (7)	1.1207 (16)
	Kin'wareni	0.3675 (1)	0.2634 (1)
	Kiwalwa	0.4589 (7)	0.2729 (8)
	Mwarusa	0.2630 (6)	0.1923 (7)
	Njoro	0.3402 (10)	0.4602 (8)
Shannon's equitability (<i>EH</i>)	All villages	2.2523 (12)	2.4339 (19)
	Chala	0.000 (1)	0.1133 (3)
	Kimundia	0.3552 (7)	0.4042 (16)
	Kin'wareni	0.000 (1)	0.000 (1)
	Kiwalwa	0.2358 (7)	0.1312 (8)
	Mwarusa	0.1468 (6)	0.0988 (7)
	Njoro	0.1477 (10)	0.2213 (8)
	All villages	0.9064 (12)	0.8266 (19)

were used for this study since the Anopheles mosquitoes were analyzed separately for other study. The collected Culicine were sorted according to sex and species using morphological characteristics as described earlier (Edwards 1941). The females were further grouped into their physiological status (unfed and blood-fed) by examining their abdomen under a stereomicroscope. All blood-fed mosquitoes were kept in labeled vials and preserved awaiting further analysis.

Bloodmeal Analysis

The blood-fed culicine mosquitoes were screened for bloodmeal using direct enzyme-linked immune sorbent assay (ELISA) method as earlier described by (Beier et al. 1988, Mwangangi et al. 2003, Karisa et al. 2019). A panel of antibodies against human, bovine, chicken, and goat were used for bloodmeal source. Positive controls included serum for each host tested, and different combinations of human, bovine, goat, and chicken serum mixtures in PBS. Results were read visually through color change (homogenous greenish-blue color for positive and clear for negative samples).

Data Analysis

Stata statistical package (StataCorp. 2011, *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP) (StataCorp 2011) was used to clean, manage, and analyze the data sets. Species composition and spatial heterogeneity were analyzed using χ^2 . Statistical differences among different species was considered significant when the *P*-value was below 0.05 ($P < 0.05$). The Shannon diversity index (*H*) was used to describe mosquito species diversity in the study sites, Taita-Taveta County, as described earlier (Muturi et al. 2006b). Shannon index accounts for both diversity and evenness of the species present.

Table 3. Bloodmeal sources for blood fed Culicine mosquitoes collected in six sites of Taita-Taveta County, Coastal Kenya

Village	Mosquito species	Number of tested	Human (%)	Goat (%)	Bovine (%)	Chicken (%)	Mixed (%)	Unknown (%)
Chala	<i>Culex quinquefasciatus</i>	9	0	0	3 (33.3)	0	4 (44.4)	2 (22.2)
Kimundia	<i>Aedes hirsutus</i>	210	3 (1.4)	5 (2.4)	68 (32.4)	1 (0.5)	117 (55.7)	16 (7.6)
	<i>Aedes mcintoshi</i>	43	3 (7.0)	5 (11.6)	10 (23.3)	1 (2.3)	23 (53.5)	1 (2.3)
	<i>Aedes taylori</i>	222	12 (5.4)	2 (0.9)	44 (19.8)	0	157 (70.7)	7 (3.2)
	<i>Aedes tricholabis</i>	1	0	0	1 (100.0)	0	0	0
	<i>Coquillettidia aurites</i>	5	1 (20.0)	1 (20.0)	0	0	3 (60.0)	0
	<i>Culex fuscocephala</i>	2	0	0	0	0	2 (100.0)	0
	<i>Mansonia</i>	10	1 (10.0)	0	2 (20.0)	0	7 (70.0)	0
	<i>Culex poicilipes</i>	1	0	0	0	0	1 (100.0)	0
	<i>Culex quinquefasciatus</i>	330	15 (4.5)	14 (4.2)	133 (40.3)	5 (1.5)	150 (45.5)	13 (3.9)
	<i>Culex univittatus</i>	6	0	1 (16.7)	2 (33.3)	0	2 (33.3)	1 (16.7)
King'wareni	<i>Aedes mcintoshi</i>	2	0	0	0	0	2 (100.0)	0
	<i>Culex quinquefasciatus</i>	33	4 (12.1)	2 (6.1)	14 (42.4)	2 (6.1)	9 (27.3)	2 (6.1)
Kiwalwa	<i>Aedes hirsutus</i>	11	1 (9.1)	1 (9.1)	6 (54.5)	0	2 (18.2)	1 (9.1)
	<i>Aedes mcintoshi</i>	3	0	0	0	0	2 (66.7)	1 (33.3)
	<i>Mansonia</i>	1	0	1 (100.0)	0	0	0	0
Mwarusa	<i>Culex quinquefasciatus</i>	142	43 (30.3)	5 (3.5)	10 (7.0)	7 (4.9)	55 (38.7)	22 (15.5)
	<i>Aedes hirsutus</i>	5	0	0	1 (20.0)	0	3 (60.0)	1 (20.0)
	<i>Aedes mcintoshi</i>	2	0	0	1 (50.0)	0	1 (50.0)	0
Njoro	<i>Culex quinquefasciatus</i>	136	9 (6.6)	22 (16.2)	23 (16.9)	0	68 (50.0)	14 (10.3)
	<i>Aedes hirsutus</i>	2	0	1 (50.0)	0	0	0	1 (50.0)
	<i>Coquillettidia aurites</i>	1	0	1 (100.0)	0	0	0	0
	<i>Mansonia</i>	1	0	0	0	0	1 (100.0)	0
	<i>Culex quinquefasciatus</i>	62	10 (16.1)	4 (6.5)	10 (16.1)	0	31 (50.0)	7 (11.3)
	<i>Culex univittatus</i>	1	0	0	0	1 (100.0)	0	0
	Total		1,241	102 (8.2)	65 (5.2)	328 (26.4)	17 (1.4)	640 (51.6)

The host preference was calculated by expressing the number of mosquitoes positive for each specific host as a proportion of the total mosquitoes tested. This was categorized based on the focal point of collection (indoor and outdoor).

Results

Indoor and Outdoor Culicine Mosquito Species Composition/Abundance

In total, 3,278 adult mosquitoes were collected in both indoor (22.5%, $n = 738$) and outdoor (77.5%, $n = 2,540$) environments during the study period. Eighteen species of culicine mosquitoes belonging to genera (*Aedes*, *Culex*, *Coquillettidia*, and *Mansonia*) were collected in the six sites. The genus *Aedes* comprised of seven species, whereas *Culex* comprised of eight species, *Coquillettidia* comprised of one species, and finally, *Mansonia* comprised two species. *Culex quinquefasciatus* was significantly reported in high numbers both indoor ($\chi^2 = 25.1$, $df = 6$, $P < 0.001$) and outdoor ($\chi^2 = 54.3$, $df = 6$, $P < 0.001$) across the six sites. From the six sites, Kimundia and King'wareni had the highest number of mosquitoes collected outdoor (43.5%) and indoor (8.1%), respectively (Table 1). Fewer mosquito samples were collected during the dry season compared with wet season, thus we could not report the results per season.

Culicine Mosquito Species Diversity and Evenness

Overall, there was high mosquito species diversity (H) in outdoors ($H = 2.4339$) than in indoors ($H = 2.2523$), whereas evenness (EH) was higher in indoors ($EH = 0.9064$) than outdoors ($EH = 0.8266$) (Table 2).

Indoor Species Diversity and Evenness

Kimundia had a higher diversity of mosquito species was observed, with ($H = 0.6913$) compared with the other sites. Similarly, high species equitability was observed in the same site with EH being

(0.3552). Lower species diversity and equitability were reported in Chala ($H = 0.1312$) and ($EH = 0.0000$), respectively. King'wareni also had low mosquito's species equitability with EH of 0.0000 (Table 2).

Outdoor Species Diversity and Evenness

High species diversity and equitability were observed in Kimundia ($H = 1.1207$) and ($EH = 0.4042$), respectively, compared with the other sites. Lower species diversity was observed in Chala ($H = 0.1245$) and evenness was observed in King'wareni ($EH = 0.0000$) (Table 2).

Bloodmeal Sources of Culicine Mosquitoes

Overall, 1,241 mosquitoes were tested for bloodmeal sources using direct ELISA method. These included; *Cx. quinquefasciatus* ($n = 712$), *Aedes hirsutus* ($n = 228$), *Aedes taylori* ($n = 222$), *Aedes mcintoshi* ($n = 50$), *Mansonia* ($n = 12$), *Culex univittatus* ($n = 7$), *Coquillettidia aurites* ($n = 6$), *Culex fuscocephala* ($n = 2$), *Culex poicilipes* ($n = 1$), and *Aedes tricholabis* ($n = 1$). Out of the 1,241 mosquitoes tested for bloodmeal sources, 512 (41.3%) had fed on a single host including human (8.2%, $n = 102$), goat (5.2%, $n = 65$), bovine (26.4%, $n = 328$), and chicken (1.4%, $n = 17$), whereas (51.6%, $n = 640$) identified were from mixed/multiple bloodmeal sources. The rest 7.2% were from unknown sources (Table 3). The mosquito species displayed feeding behavior involving bloodmeals from a single host including; *Ae. hirsutus*, *Ae. mcintoshi*, *Aedes taylori*, *Ae. tricholabis*, *Cx. quinquefasciatus*, and others (Table 4).

Most of the multiple feeders preferred bovine-goat combination with a proportion of (45.8%) followed by a combination of bovine-human-goat (24.1%). The least preferred multiple hosts were bovine-chicken-human, chicken-goat and bovine-chicken with 0.6, 1.1, and 1.6%, respectively (Table 5). *Culex quinquefasciatus* showed different diverse trophic preferences but predominantly fed on bovine-goat and bovine-human-goat combinations (Tables 5 and 6).

Table 4. Single host bloodmeal among *Culicine* mosquitoes in Taita-Taveta County, Coastal Kenya

Species	Location	Number of Tested	Human (%)	Goat (%)	Bovine (%)	Chicken (%)
<i>Culex quinquefasciatus</i>	Indoor	110	62 (56.4)	6 (5.5)	30 (27.3)	12 (10.9)
	Outdoor	225	19 (8.4)	41 (18.2)	163 (72.4)	2 (0.9)
	Overall	335	81 (24.2)	47 (14.0)	193 (57.6)	14 (4.2)
<i>Aedes hirsutus</i>	Indoor	9	0 (0.0)	1 (11.1)	8 (88.9)	0 (0.0)
	Outdoor	78	4 (5.1)	6 (7.7)	67 (85.9)	1 (1.3)
	Overall	87	4 (4.6)	7 (8.0)	75 (86.2)	1 (1.1)
<i>Aedes mcintoshi</i>	Indoor	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
	Outdoor	19	3 (15.8)	4 (21.1)	11 (57.9)	1 (5.3)
	Overall	20	3 (15.0)	5 (25.0)	11 (55.0)	1 (5.0)
<i>Aedes taylori</i>	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	58	12 (20.6)	2 (3.4)	44 (75.9)	0 (0.0)
	Overall	58	12 (20.6)	2 (3.4)	44 (75.9)	0 (0.0)
<i>Aedes tricholabis</i>	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	1	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)
	Overall	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
<i>Mansonia</i>	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	4	1 (25.0)	1 (25.0)	2 (50.0)	0 (0.0)
	Overall	4	1 (25.0)	1 (25.0)	2 (50.0)	0 (0.0)
<i>Culex univittatus</i>	Indoor	3	0 (0.0)	1 (33.3)	1 (33.3)	1 (33.3)
	Outdoor	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
	Overall	4	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)
All species combined	Indoor	123	62 (50.4)	9 (7.3)	39 (31.7)	13 (10.6)
	Outdoor	389	40 (10.3)	56 (14.4)	289 (74.3)	4 (1.0)
	Overall	512	102 (19.9)	65 (12.7)	328 (64.1)	17 (3.3)

Table 5. Multiple host bloodmeal among Culicine mosquitoes in Taita-Taveta County, Coastal Kenya

Species	Location	Number of tested	BC (%)	BCG (%)	BCH (%)	BCHG (%)	BG (%)	BH (%)	BHG (%)	CG (%)	CH (%)	CHG (%)	HG (%)
<i>Culex quinquefasciatus</i>	Indoor	72	0 (0.0)	1 (1.4)	0 (0.0)	1 (1.4)	26 (36.1)	3 (4.2)	17 (23.6)	1 (1.4)	8 (11.1)	4 (5.6)	11 (15.3)
	Outdoor	245	3 (1.2)	6 (2.4)	1 (0.4)	7 (2.9)	124 (50.6)	22 (9.0)	53 (21.6)	3 (1.2)	0 (0.0)	4 (1.6)	22 (9.0)
<i>Aedes hirsutus</i>	Overall	317	3 (0.9)	7 (2.9)	1 (0.3)	8 (2.5)	150 (47.3)	25 (7.9)	70 (22.1)	4 (1.3)	8 (2.5)	8 (2.5)	33 (10.4)
	Indoor	13	1 (7.7)	0 (0.0)	0 (0.0)	3 (23.1)	7 (53.8)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Aedes mcrittoshi</i>	Outdoor	109	2 (1.8)	1 (0.9)	2 (1.8)	10 (9.2)	48 (44.0)	10 (9.2)	33 (30.3)	1 (0.9)	2 (1.8)	0 (0.0)	0 (0.0)
	Overall	122	3 (2.5)	1 (0.8)	2 (1.6)	13 (10.7)	55 (45.1)	12 (9.8)	33 (27.0)	1 (0.8)	2 (1.6)	0 (0.0)	0 (0.0)
<i>Aedes taylori</i>	Indoor	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	26	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)	13 (50.0)	4 (15.4)	6 (23.1)	0 (0.0)	1 (3.8)	0 (0.0)	1 (3.8)
<i>Culex fusco</i>	Overall	28	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	14 (50.0)	4 (14.3)	7 (25.0)	0 (0.0)	1 (3.6)	0 (0.0)	1 (3.6)
	Indoor	2	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Mansonia</i>	Outdoor	155	4 (2.6)	8 (5.2)	1 (0.6)	2 (1.3)	68 (43.9)	22 (14.2)	38 (24.5)	2 (1.3)	1 (0.6)	2 (1.3)	7 (4.5)
	Overall	157	4 (2.5)	8 (5.1)	1 (0.6)	3 (1.9)	68 (43.3)	22 (14.0)	38 (24.2)	2 (1.3)	1 (0.6)	3 (1.9)	7 (4.5)
<i>Culex poicilipes</i>	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Culex univittatus</i>	Overall	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
All species combined	Outdoor	8	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	5 (62.5)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
	Overall	8	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	5 (62.5)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)
All species combined	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Outdoor	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
All species combined	Overall	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)
	Indoor	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
All species combined	Outdoor	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Overall	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
All species combined	Indoor	89	1 (1.1)	1 (1.1)	0 (0.0)	5 (5.6)	34 (38.2)	5 (5.6)	18 (20.2)	1 (1.1)	8 (9.0)	5 (5.6)	11 (12.4)
	Outdoor	551	9 (1.6)	15 (2.7)	4 (0.7)	21 (3.8)	259 (47.0)	59 (10.7)	136 (24.7)	6 (1.1)	5 (0.9)	6 (1.1)	31 (5.6)
Overall	640	10 (1.6)	16 (2.5)	4 (0.6)	26 (4.1)	293 (45.8)	64 (10.0)	154 (24.1)	7 (1.1)	13 (2.0)	11 (1.7)	42 (6.6)	

Table 6. Mixed bloodmeal sources of the blood-fed Culicine mosquitoes collected in Taita-Taveta, Coastal Kenya

Site	Species	BC (%)	BCG (%)	BCH (%)	BCHG (%)	BG (%)	BH (%)	BHG (%)	CG (%)	CH (%)	CHG (%)	HG (%)	Total	
Chala Kimundia	<i>Culex quinq</i>	0	0	0	1 (25.0)	0	1 (25.0)	1 (25.0)	0	0	0	1 (25.0)	4	
	<i>Aedes hirsutus</i>	2 (1.7)	1 (0.9)	2 (1.7)	11 (9.4)	53 (45.2)	12 (10.3)	33 (28.2)	1 (0.9)	2 (1.7)	0	0	117	
	<i>Aedes mcintoshi</i>	0	0	0	1 (4.3)	13 (56.5)	3 (13.0)	6 (26.1)	0	0	0	0	23	
	<i>Aedes taylori</i>	4 (2.5)	8 (5.1)	1 (0.6)	3 (1.9)	68 (43.3)	22 (14.0)	38 (24.2)	2 (1.3)	1 (0.6)	3 (1.9)	7 (4.5)	157	
	<i>Coq.aurites</i>	0	0	0	0	0	1 (33.3)	2 (66.7)	0	0	0	0	3	
	<i>Culex fusco</i>	0	0	0	0	0	0	2 (100.0)	0	0	0	0	2	
	<i>Mansonia</i>	0	0	0	1 (14.3)	5 (71.4)	0	1 (14.3)	0	0	0	0	0	7
	<i>Culex poicilipes</i>	0	0	0	0	0	0	0	0	1 (100.0)	0	0	0	1
	<i>Culex quinq</i>	0	2 (1.3)	0	0	85 (56.7)	14 (9.3)	28 (18.7)	2 (1.3)	1 (0.7)	3 (2.0)	15 (10.0)	150	
	<i>Culex univittatus</i>	0	0	0	0	1 (50.0)	0	1 (50.0)	0	0	0	0	0	2
King'wareni	<i>Aedes mcintoshi</i>	0	0	0	0	0	0	0	0	1 (50.0)	0	1 (50.0)	2	
	<i>Culex quinq</i>	0	0	0	0	6 (66.7)	0	3 (33.3)	0	0	0	0	9	
Kiwalwa	<i>Aedes hirsutus</i>	0	0	0	2 (100.0)	0	0	0	0	0	0	0	2	
	<i>Aedes mcintoshi</i>	0	0	0	0	1 (50.0)	1 (50.0)	0	0	0	0	0	2	
	<i>Culex quinq</i>	0	0	0	0	26 (47.3)	3 (5.5)	8 (14.5)	1 (1.8)	6 (10.9)	1 (1.8)	7 (12.7)	55	
Mwarusa	<i>Aedes hirsutus</i>	1 (33.3)	0	0	0	2 (66.7)	0	0	0	0	0	0	3	
	<i>Aedes mcintoshi</i>	0	0	0	0	0	0	1 (100.0)	0	0	0	0	1	
	<i>Culex quinq</i>	3 (4.4)	5 (7.4)	0	2 (2.9)	19 (27.9)	4 (5.9)	22 (32.3)	1 (1.5)	1 (1.5)	3 (4.4)	8 (11.8)	68	
Njoro	<i>Culex quinq</i>	0	0	1 (3.2)	2 (6.5)	14 (45.2)	0	8 (25.8)	0	0	1 (3.2)	2 (6.5)	31	
	<i>Mansonia</i>	0	0	0	0	0	0	0	0	0	0	1 (100.0)	1	
	Total	10 (1.6)	16 (2.5)	4 (0.6)	26 (4.1)	293 (45.8)	64 (10.0)	154 (24.1)	7 (1.1)	13 (2.0)	11 (1.7)	42 (6.6)	640	

B, bovine; C, chicken; G, goat; H-human.

Discussion

In this study, we sought out to understand culicine mosquito species composition, abundance, diversity, and distribution within Taita-Taveta County. Our data present the first comprehensive analysis of culicine mosquito vector species density and composition in the region. The six villages involved in the study had significant spatial heterogeneity in terms of mosquito densities and species. The highest abundance was reported in areas where agricultural activities were carried out and in swampy areas, such as Kimundia, King'areni, and Kiwalwa. Kimundia recorded the highest mosquito density, which could be attributed to ideal breeding habitats of culicine mosquito species. The mosquito larval ecology including nature of breeding habitats and the human-related activities such as irrigated agriculture with poor water channels and nonengineered irrigation canals contributed significantly to mosquito abundance and diversity. Previous studies done in Kenya have reported a positive relationship between habitat type, diversity, and mosquito species richness (Muturi et al. 2007). Similar results have been reported in Mwea irrigation scheme, Kenya, where there was high mosquito density which was due to agriculture activities (Mutero et al. 2004). This present study also supports an observation by (Afrane et al. 2012) that larval abundance, survival, and production of adult mosquitoes in irrigated vegetable farms are quite high and, therefore, contributing to high adult mosquito density. This demonstrates that mosquito productivity is a function of the availability of mosquitoes breeding habitats with more mosquitoes being found in areas with available stagnant water (Muturi et al. 2006a, Mwangangi et al. 2010).

Culicine mosquitoes are the most common with diverse flexible breeding habitats. *Culex quinquefasciatus*, *Ae. hirsutus*, *Cx. univittatus*, and *Ae. mcintoshi* were highly sampled mosquito species from all the six sites of Taita-Taveta County. *Cx. quinquefasciatus* mosquitoes for instance are known to breed in habitats with high organic content mostly in irrigation farms, swamps, rivers, latrines, uncovered septic tanks, drainage, rain pools, ditches, tree holes, and banana axles (Subra 1981, Irving-Bell et al. 1987, Aigbodion et al. 2011), which were present in the study area. The presence of these mosquito species in the study area poses a potential risk of mosquito-borne diseases such as lymphatic filariasis and RVFV to the human population (Mwandawiro et al. 1997, Woods et al. 2002, LaBeaud et al. 2011, Sang et al. 2017). Besides the potential for pathogen transmission, culicine mosquitoes are mostly involved in biting nuisance especially outdoors where most adult vector control tools are not in place. With the presence of vectors for bancroftian filariasis and arboviruses, outdoor mosquito control initiatives should be put in place to target mosquitoes of diverse feeding and resting behaviors. This could be done by exploring larval source management strategies integrated with scale-up of long-lasting insecticide-impregnated nets (LLINs) at the universal level through integrated vector management (IVM) package (WHO 2012).

Mosquito feeding preferences is a key determinant of disease transmission (Garcia-Rejon et al. 2010, Sawabe et al. 2010, Janssen et al. 2015). Host preference for culicine mosquitoes in Taita-Taveta County showed that these mosquitoes were relaxed feeders taking bloodmeals from several and available vertebrate hosts. When the human host was not readily available, culicine mosquitoes sought for alternative hosts available which are often in close proximity to human dwellings (Ijumba and Lindsay 2001). Culicine mosquitoes in Taita-Taveta County showed a high host preference on bovine (single host) and bovine/goat (multiple hosts) (zoophilic nature) over human hosts (anthropophilic nature). These results were in agreement with the previous study that reported high zoophilic nature of

culicine mosquito in Mwea irrigation scheme, Kenya (Muturi et al. 2008). The low anthropophilic and high zoophilic nature of culicine mosquitoes observed in our study could be both advantageous and disadvantageous to bancroftian filariasis and RVF transmission, respectively. Low anthropophilic nature of *Cx. quinquefasciatus* may disfavor transmission of Bancroftian filariasis. This could be possible due to the loss of a significant number of worms to the wrong host, or by failing to pick up enough microfilaria that can sustain transmission. Bancroftian filariasis transmission is very inefficient because there is no parasite multiplication in the mosquito and continuous exposure to many infective bites is necessary for transmission to occur (Hairston and de Meillon 1968, Bockarie et al. 2002). On the negative, high zoophilic nature of *Aedes* and *Culex* mosquito species poses a high risk of Rift Valley Fever (RVF) virus transmission to domestic animals including cattle, goats, and sheep (Tchouassi et al. 2016). The feeding pattern for these culicine mosquito species in Taita-Taveta County, therefore, present an ideal condition for RVF, and efforts should be made to establish the risk factors of the disease in similar areas and to develop sustainable mosquito surveillance and control systems.

Our results demonstrated that nearly all the species examined fed on multiple hosts within a single gonotrophic cycle. High mosquito density in these areas where agricultural activities are highly practised enforces the use of bed nets and other protective measures against mosquito bite consequently reverting mosquitoes to feeding on domestic animals because humans are not easily accessible (Muriu et al. 2008). Interestingly, 7.2% of the bloodmeal samples were not from any of the four hosts tested an indication that culicine mosquitoes in this area have a wide host range. Other livestock and animals present in the study area were dogs, cats, wild birds, house rats, and rodents, but due to logistical limitations and unavailability of anti-seras against these mentioned animals, we were restricted to conducting ELISA tests against them. These findings highlight the need to include a variety of possible hosts when conducting mosquito host choice studies.

This study demonstrated a marked difference in species composition, abundance, and distribution of culicine mosquitoes in the six villages of Taita-Taveta County, Kenya. The study demonstrated that culicine mosquitoes in Taita-Taveta County were highly zoophilic and that multiple feeding within the same gonotrophic cycle was common among these species. The results of this study show that there is a need to scale up vector control interventions to target outdoor mosquitoes which will ensure a significant reduction in mosquito populations.

Ethical Considerations

The study was approved by the KEMRI Scientific and Ethics Review Unit (SERU) Protocol (KEMRI/SERU/CGMRC/035/3219). Oral consent was also obtained from the household members before mosquito collection commenced.

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