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## An update on the mosquito species composition and diversity in western and North Western Uganda

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### Abstract

Although the west and north western parts of Uganda are historically known homes to a number of mosquito species and arboviruses associated with morbidity and mortality, early studies were highly focal and limited to specific collection methods. We aimed to update mosquito species composition in areas where a febrile illness study had shown evidence of arboviruses circulating.

Adult mosquito sampling was done outside and inside houses using light traps baited with solid carbon dioxide and pyrethrum spray respectively. All collected mosquitoes were identified using appropriate morphological identification keys.

A total of 22,455 mosquitoes from 89 species, 22 sub species and 11 genera were collected from Arua and Kasese districts. Overall abundance was found to be higher in Kasese (n=13446, 59.9%) than Arua district (n = 9009, 40.1%), though no significant differences were observed across villages in Arua and Kasese districts (Kruskal Wallis,  $X^2 = 2$ ,  $df = 3$ ,  $p > 0.05$ ). Collection numbers were highest for genus *Coquillettidia* (n = 7942, 35.4%), followed by *Culex* (n = 7642, 34.03%), *Mansonia* (n = 3414, 15.2%), *Anopheles* (n = 1970, 8.8%) and *Aedes* (n = 1349, 6.01%). Other species were across 6 genera *Eretmopodites* (n = 59, 0.26%), *Uranoteania* (n = 36, 0.16%), *Lutzia* (n = 26, 0.12%), *Mimomyia* (n = 13, 0.06%), *Aediomyia* (n = 3, 0.01%) and *Toxorhynchites* (n = 1, 0.004%) appeared low in both districts. Species richness was comparatively higher in Kasese than Arua district, however across villages, it was evenly distributed with no significant differences observed, and species diversity was significantly higher in Arua than Kasese (Mann Whitney U test,  $p < 0.05$ ). A number of species identified here have been implied in arbovirus transmission. Moreover, we show the first description of *Culex (Culex) litwakae* Harbach mosquito in Uganda, a species previously described in the coastal regions of Kenya. The existence of a mosquito species previously not documented in Uganda suggests a likelihood of many invasive species whose potential to transmit viruses to humans and animals remains largely unknown.

**Keywords:** Mosquitoes, arbovirus, species composition, *Culex litwakae*, Uganda

### Introduction

Mosquitoes of family *Culicidae* breed and occupy a wide range of ecological habitats, have different host blood feeding preferences and dispersal abilities<sup>[1]</sup>. This is likely to influence species composition and diversity which in turn will affect the risk of vector borne disease transmission. Monitoring species composition and diversity indices will not only provide baseline data for determining human and animal populations at risk of virus infection but also provide vital information for detecting a possible mosquito borne disease outbreak<sup>[2]</sup>. The mosquito species composition of Western and North western Uganda needed to be updated, indeed these regions are home to several arboviruses and mosquito species of public health importance<sup>[3, 6]</sup>. A number of mosquito species whose distribution was only known in the neighbouring Democratic Republic of Congo (DRC) are now commonly found in Uganda<sup>[7]</sup>. Others whose local endemicity 50 years ago were confined in the high land areas of western Uganda at  $\geq 6000$ ft have of recent been described in other regions of Uganda<sup>[4, 7]</sup>.

Some of the mosquito borne viruses first described in these regions have over the years emerged into new areas of the world including Europe, Asia and America where they are associated with significant morbidity<sup>[8-11]</sup>. Thus, important gaps in knowledge exist and these need to be addressed. Related studies in both regions showed potential for additional novel pathogens described from diverse animal hosts some of which are linked to mosquitoes as potential vectors<sup>[12, 17]</sup>.

Previous studies were highly focal and limited to areas of yellow fever seropositivity [6, 18, 23]. Moreover many of these studies were conducted more than 40 years ago and limited to Bwamba county with only two documented studies in the West Nile region [5, 24] (Table 1, Figure 1).

Recent entomological studies in Arua district were limited to the Rhino camp adjacent the Albert Nile while those in part of Kasese district were conducted in the middle of Queen Elizabeth National Park far away from human settlement and communities [4, 5]. Findings from these studies cannot easily be expanded to the vast West Nile region that stretches from the Albert Nile to the borders of Democratic Republic of Congo (DRC) as well as South Sudan while those in Kasese district cannot give a true picture of the risk of arbovirus spill over from animal to human populations. Collection methods used in the previous surveys were limited, they targeted mainly outdoor light seeking mosquito species [25]. A central aim of the ArboViral Infection Study (AVI) study by Makerere University/Uganda Virus Research Institute Centre of Excellence in Infection and Immunity Research and Training (MUII-Plus) and MRC University of Glasgow Centre for Virus Research (CVR) was to update mosquito species composition and diversity in areas where a previous Acute Febrile illness study (AFI) (UVRI unpublished) had shown evidence of emerging and novel viruses.

## Materials and Methods

Mosquito collections were done in Arua and Kasese districts in the North Western and Western Uganda respectively. In Arua district, sample collection was done in the four villages of Yedu, Oniba, Ambala and Barize while in Kasese district, it was done in Kidodo, Kirembe, Kyondo and Road Barrier villages. To our knowledge there has not been any documentation about the mosquitoes in Adumi sub county and areas adjacent QENP in Kasese district. The study sites are shown in Figure 1.

## Mosquito sampling

Different methods including indoor spraying, light traps, larvae and ovitrap collections were carried out inside, outside houses and in people's gardens. Mosquito collections were carried out on variable days, generally, all collections in both districts were done when it was dry. In Arua district, a total of two collections were done, the first collection was from 21<sup>st</sup> November 2017 to 30<sup>th</sup> November 2017, the second was from 1<sup>st</sup> February 2019 to 10<sup>th</sup> February 2019. In Kasese district, the first round of mosquito sampling was from 19<sup>th</sup> March 2018 to 28<sup>th</sup> March 2018, the second round was from 1<sup>st</sup> May 2019 to 10<sup>th</sup> May 2019. Outdoor collections included ovitrap setting, larvae search and Centers for Disease Control (CDC) light trap setting for trapping of adult mosquitoes. In each of the randomly selected villages, CDC light traps baited with dry ice (solid carbon dioxide) in an insulated modified Igloo drink cooler (John. W. Hock Company, Gainesville, FL) were hung in bushes, peridomestic gardens and near people's houses. Each of the light traps were set in the evening at about 5:00 pm, left overnight and removed the following morning at about 8:00 am. Larvae searches were done both inside and outside houses. Both artificial containers and existing water pools were searched for immature stages of mosquitoes. Larvae and pupae were scooped using a dipper and raised to adults for morphological identification.

For collection of indoor biting and resting species, household heads of houses that had been randomly selected were

approached the previous evening and permission sought for access into their houses. The following day all cooking utensils, food stuffs and people were evacuated before spraying was done. White sheets were laid inside houses and spraying done both inside and outside using Mortein insecticide (Reckitt Benckiser South Africa). After 10 minutes, the houses were opened, knocked down mosquitoes were picked using forceps, packed in 2.0 ml cryo vials and kept in dry ice for later transportation to the laboratory for morphological identification.

For ovitrap collections, black ovitraps lined with brown paper and half filled with water were laid outside people's houses and removed on the last day of mosquito sampling in the field. They were set to dry and later immersed in water to allow mosquito eggs hatch. These were reared to adults, collected and stored at -8 °C for later identification. All collected mosquito larvae were raised to the adult stage and identified. Identification was done using appropriate morphological keys using a Stereo microscope (Discovery V12) mounted on a chill table [7, 26, 27]. Mosquitoes were sorted, pooled according to place of collection, species, sex, feeding status and stored at -8 °C.

## Ethical clearance

This study was part of the ArboViral Infection Study (AVI) that was cleared by the Uganda National Council for Science and Technology (UNCST) under study No: HS 2485.

## Data analysis

Data was recorded by Excel and used to calculate species richness, composition, diversity and abundance. Graphs were drawn using GraphPad Prism version 7.0a (GraphPad Software, Inc., San Diego, CA, USA). Species richness was calculated as number of species per genus, species composition was expressed as a proportion of the number of a particular mosquito species in relation to the total number of mosquitoes in that village expressed as a percentage, while mosquito abundance referred to the total number of mosquitoes in that village. Diversity measures computed included Simpson's diversity and Shannon's diversity indices. Simpson's diversity index (D) was calculated using the formula below:

Simpson Diversity Index (D) =  $1 - \sum(n(n-1)/N(N-1))$ , where D is the Simpson's or Species diversity, n is the number of individuals of a particular mosquito species and N is the total number of adult mosquitoes all combined. Species richness, diversity and abundance were compared between districts and across villages using Mann Whitney U and Kruskal Wallis tests. A Mann Whitney U test was used to compare diversity between the two districts while Kruskal Wallis test was used to determine how species richness, diversity and abundance varied across villages in each of the respective districts.

## Results and Discussion

### Mosquito collections and genera across districts and villages

We investigated species richness, composition, diversity and relative abundance of mosquitoes across eight villages in Arua and Kasese districts. We also compared species between the two districts in areas thought to be at risk of virus spillover to the human population.

A total of 22,455 mosquitoes from 89 species, 22 sub species and 11 genera were collected from Arua and Kasese districts. The 11 genera included *Aedes*, *Aediomyia*, *Anopheles*,

*Coquillettidia*, *Culex*, *Eretmopodites*, *Lutzia*, *Mansonia*, *Mimomyia*, *Toxorhynchites* and *Uranoteania*. Out of the 89 species, 33 were collected from both districts, the rest were unique to each district (Supplementary Table S1 and Table S2). Overall mosquito abundance was higher in Kasese (n=13446, 59.9%) than Arua district (n=9009, 40.1%). Both districts combined, mosquito collections were highest in genus *Coquillettidia* (n=7942, 35.4%), followed by *Culex* (n=7642, 34.03%), third was *Mansonia* (n=3414, 15.2%), *Anopheles* (n=1970, 8.8%) and *Aedes* (n=1349, 6.01%). Other species in the remaining 6 genera of *Eretmopodites* (n=59, 0.26%), *Uranoteania* (n=36, 0.16%), *Lutzia* (n=26, 0.12%), *Mimomyia* (n=13, 0.06%), *Aediomyia* (n=3, 0.01%) and *Toxorhynchites* (n=1, 0.004%) appeared in low numbers in both districts (Figure 2).

In Arua district, mosquito abundance was highly uneven with no significant differences (Kruskal Wallis,  $X^2=2$ , df =3,  $p>0.05$ ) across the four sampled villages. Yedu village (n=3732, 41.4%) showed the highest number of mosquitoes, followed by Barize (n=2462, 27.3%), Oniba (n=1709, 19%) while the lowest was Ambala village (n=1106, 12.3%).

A total of 62 species from genera *Aedes*, *Aediomyia*, *Coquillettidia*, *Culex*, *Eretmopodites*, *Lutzia*, *Mansonia* and *Uranoteania* were collected from Arua district. Although species richness varied from village to village, it did not differ significantly across the four study sites (Kruskal Wallis test,  $X^2=0.57$ , df =3,  $p>0.05$ ). Highest number of species in Arua district came from Yedu village (46 species), second highest from Barize (43 species), followed by Ambala (40 species) and least number of species collection were from Oniba village (31 species) (Figure 3).

Number of species in Arua district were highest in the genus *Culex* (24 species, n=4056, 38.7%), followed by *Anopheles* (10 species, n=1,222, 16.1%) and *Aedes* (10 species, n=866, 16.1%) which had the same number of species, fourth highest was genus *Coquillettidia* (7 species, n=2040, 11.3%). The rest of the species were few in number and belonged to genera *Uranoteania* (4 species, n=17, 6.45%), *Mansonia* (3 species, n=720, 4.84%), *Eretmopodites* (2 species, n=59, 3.23%), *Lutzia* (1 species, n=26, 1.61%) and *Aediomyia* (1 species, n=1, 1.61%).

In Kasese district, mosquito abundance did not differ significantly across the four villages (Kruskal Wallis,  $X^2=2.08$ , df =3,  $p>0.05$ ). A total of 13446 mosquitoes from genera *Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Mansonia*, *Mimomyia*, *Toxorhynchites* and *Uranoteania*, 16 sub species and 74 species were collected from Kidodo, Kirembe, Kyondo and road barrier villages in Kasese district. Highest number of mosquito collection came from Kyondo (n=6414, 47.7%), followed by Kidodo (n=3017, 22.4%), Kirembe (n=2755, 20.5%) and the least number of collections was in road barrier village (n=1260, 9.4%).

Species richness was higher in Kasese (74 species, n=13,446) than Arua (62 species, n=9009) district. It also did not differ significantly across the sampling units (Kruskal Wallis test,  $X^2=0.97$ , df =3,  $p>0.05$ ). Highest numbers of species came from Kirembe and Kyondo (47 species), followed by Kidodo (35 species) and least collection was from road barrier (23 species) (Figure 3). Genus *Culex* was the most diverse genus with 26 species (n=3586, 35.1%) followed by *Aedes* (22 species, n = 483, 29.7%), *Anopheles* (10 species, n=748,13.5%), *Coquillettidia* (8 species, n=5902,10.8%), *Mansonia* (3 species, n=2694, 4.1%), *Mimomyia* (2 species, n=13, 2.7%), *Uranoteania* (2 species, n=19, 2.7%) to

*Toxorhynchites* (1 species, n=1, 1.4%). Although the genus *Coquillettidia* ranked third in terms of number of species it had the highest number of mosquitoes from 8 species and 1 sub species.

### Species diversity

Generally, species diversity was significantly higher in Arua than Kasese district (Mann Whitney U test,  $p<0.05$ ) (Figure 4). In Arua district, species diversity was highest in Oniba (Simpson Diversity Index 1- D = 0.93, 31 species), followed by Ambala (1- D = 0.92, 40 species), Yedu (1-D = 0.91, 46 species) and least in Barize (1- D = 0.9, 43 species) village. In Kasese district, species diversity was highest in Road barrier (Simpson Diversity Index 1- D = 0.85, 23 species), followed by Kirembe (1- D = 0.81, 47 species) and least in the two villages of Kidodo (1- D = 0.8, 35 species) and Kyondo (1- D = 0.8, 47 species). Although Kirembe (47 species) and Kyondo (47 species) had the highest number of species, they were not the most biologically diverse villages. Their diversity indices were affected by the large number of *Coquillettidia* (*Coquillettidia*) *fuscopenata* Theobald.

Out of the 11 genera documented, genus *Culex* was the most diverse with five subspecies of *Culicomyia* (4 species), *Culex* (11 species), *Eumelanomyia* (2 species), *Kitzmelleria* (1 species) and *Oculeomyia* (3 species) and 29 species. The second most diverse was genus *Aedes* with four subspecies of *Aedimorphus* (15 species), *Stegomyia* (6 species), *Neomelaniconion* (3 species) and *Dunnius* (1 species) and 21 species.

### Comparison of mosquito collection methods

The choice of mosquito collection method is likely to influence mosquito virus surveillance outcomes. This is because different species have different behaviour and are attracted by different cues. A study to compare methods of mosquito collection in South Africa, showed 96% of the collections were by light traps baited with carbon dioxide, however in terms of pathogen transmission gravid traps were more informative [28]. Four methods including light traps baited with carbon dioxide, indoor collection using insecticide, egg and larvae sampling were used in this study. Use of light traps and indoor collection method targeted adult species which are key to informing horizontal virus transmission. Out of the 22455 mosquitoes collected in both districts, 20602 (91.75%) were collected using light traps baited with solid carbon dioxide, while (n=1044, 4.65%) were by indoor collection and (n=809, 3.6%) by larvae and ovitrap method (Table 2). 502 (71.5%) mosquitoes from indoor collections were *Anopheles* (*Anopheles*) *gambiae* Giles and *Anopheles* (*Anopheles*) *funestus*, which are potential vectors for o'nyong nyong virus (ONNV) [29], 30 (4.3%) were *Culex* species *Culex* (*Culex*) *pipiens* Linnaeus, *Culex* (*Culex*) *quinquefasciatus* Say, *Culex* (*Culex*) *decens* Theobald and *Culex* (*Culex*) *univitattus* which are potential vectors for West Nile virus (WNV). In Kasese district, out of the 342 mosquitoes collected from inside houses, (n=118, 34.5%) were *An. gambiae* and *Anopheles funestus*, (n=191, 55.8%) were *Culex pipiens*, *Culex quinquefasciatus* and *Culex decens* all of which are vectors for WNV. Species collected inside houses are either anthropophilic or known bridge vectors that have over the years played significant role in virus transmission.

### Identification of *Culex (Culex) litwakae* mosquito species

In the course of identification of mosquitoes from Barize village of Arua district, we came across two *Culex* species which could not be speciated using the available identification keys [7]. This species was also identified in parallel in a collection from the Ogwapoke village of Kitgum district (Mutebi and Mossel, personal communication [Figure 1]). The head had erect dark scales, antennae was normal, the proboscis was lighter in the middle (not pale) and dark towards the distal ends. The scutum was brown with a few golden-brown scales, pleura was lighter brown than the scutum, one lower mesepimeral bristle, no post spiracular and pre-alar scales and the middle of the sternopleura had broad cream-colored scales. The abdomen was blunt, typical of the *Culex* sub genus. The tergites were un banded with basal lateral spots. Harbach describes tergites with variable banding or basal medial pale spots of variable size [30]. We observed one of the specimens with a complete absence of basal medial pale scales. The venter was pale with a dark line of black medial scales, sometimes reduced to significantly fewer, but clearly identifiable, black scales, as opposed to the uniformly pale venter of *Culex (Culex) antennatus* (Becker) (Table 3). Tarsi were all dark with simple claws, hind tibia had a pale apical spot. The basal portions of the halteres were pale yellow and the knob of the halteres was dark (Figure 5).

These characteristics neither place the species as *Cx. antennatus* nor *Cx. pipiens* but suggest an intermediate phenotype with features of both *Cx. antennatus* and *Cx. pipiens* as previously described by the existing identification keys [7]. Although the species has been described in the coastal regions of Kenya [30], this is the first time it has been documented in Uganda. The presence of *Cx. litwakae* in Uganda as an emerging species should be further investigated by larger studies. Although not a known vector of pathogens, it closely resembles *Cx. pipiens* and *Cx. antennatus*, species which are previously described in the transmission of several arboviruses [5]. More detailed studies are needed to understand its biology and potential and potential for transmitting pathogens.

### Public health importance of mosquito species identified

Out of the 74 species identified from Kasese, 40 (54.1%) have been incriminated in transmission of a wide range of viruses affecting either animals or humans (supplementary Table S3). In Arua district, 33 (53.2%) out of 62 species are potential vectors of a wide range of viruses (supplementary Table S4). Although Uganda is a hotspot for many arboviruses, for several years the country has experienced several outbreaks of yellow fever virus (YFV), ONNV and Rift Valley fever virus (RVFV), while WNV and chikungunya virus (CHIKV) continue to be reported during sentinel surveillance activities. A number of species that have been previously involved in such outbreaks were identified in both districts. In Uganda, yellow fever transmission is associated with *Aedes (Stegomyia) simpsoni* and *Aedes (Stegomyia) africanus*, ONNV outbreaks associated with *Anopheles (Anopheles) gambiae* and *Anopheles (Anopheles) funestus*, RVFV transmission associated with a range of *Aedes* species. Fewer *Aedes* collection was made due to the diurnal behaviour of

*Aedes* mosquitoes as opposed to our traps which are often set in the evening. The vector status and transmission potential of a number of mosquito species remain largely obscure as there is limited transmission studies. There is a likelihood that the number of vector species is even higher than reported.

To our knowledge, this is the first mosquito survey in Adumi (Arua district) and settled communities close to Queen Elizabeth National Park in Uganda. Out of the 89 species from both districts, 33 to 40 species are potential vectors for human animal viruses. Several mosquito species that have been implicated in the transmission of YFV, RVFV, CHIKV and ONNV which have caused outbreaks in Uganda, were among those identified in our collections. Species diversity in both areas remain high suggesting the presence of suitable habitats for diverse mosquito proliferation. The presence of diverse mosquito vector species in the area surveyed show that the risk of virus spill over into the human population exists however a multiplicity of factors must be in place for an outbreak to occur. Larger mosquito studies with a wide range of collection methods are needed for a more comprehensive study that would form the basis of virus surveillance in both districts. The light trap collection method remains an important method of mosquito collection, however this should be supplemented with other collection methods like indoor collection and gravid trap collection for generating more informative results in terms of species composition and virus circulation. Although majority of the species were found in both study areas, there were those unique to each study site suggesting these communities are quite different. The identification of *Culex litwakae* a mosquito species previously not known in Uganda suggest a likelihood that there could be species not yet documented or new introductions into the country. The presence of such likely emerging species should be further investigated by larger studies.

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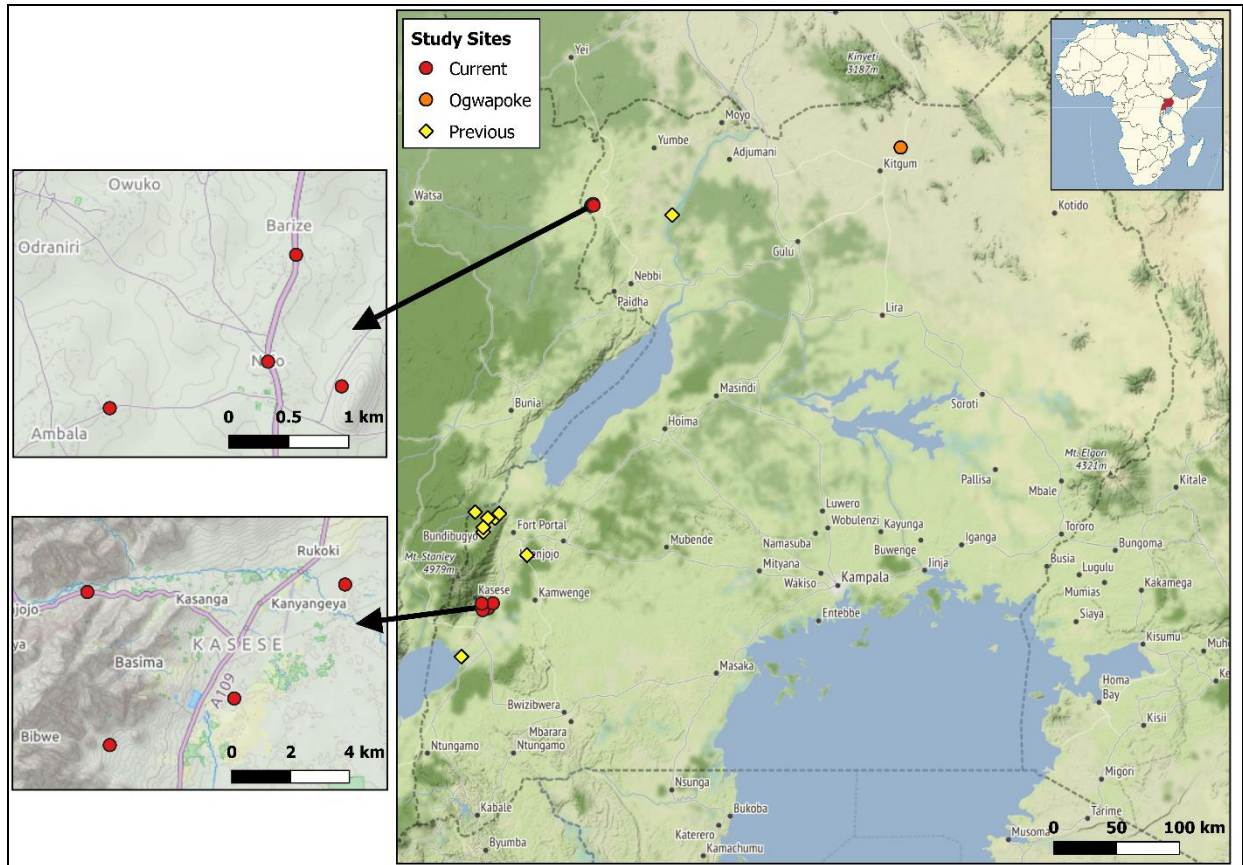


Fig 1: Map of Uganda showing sample collection sites

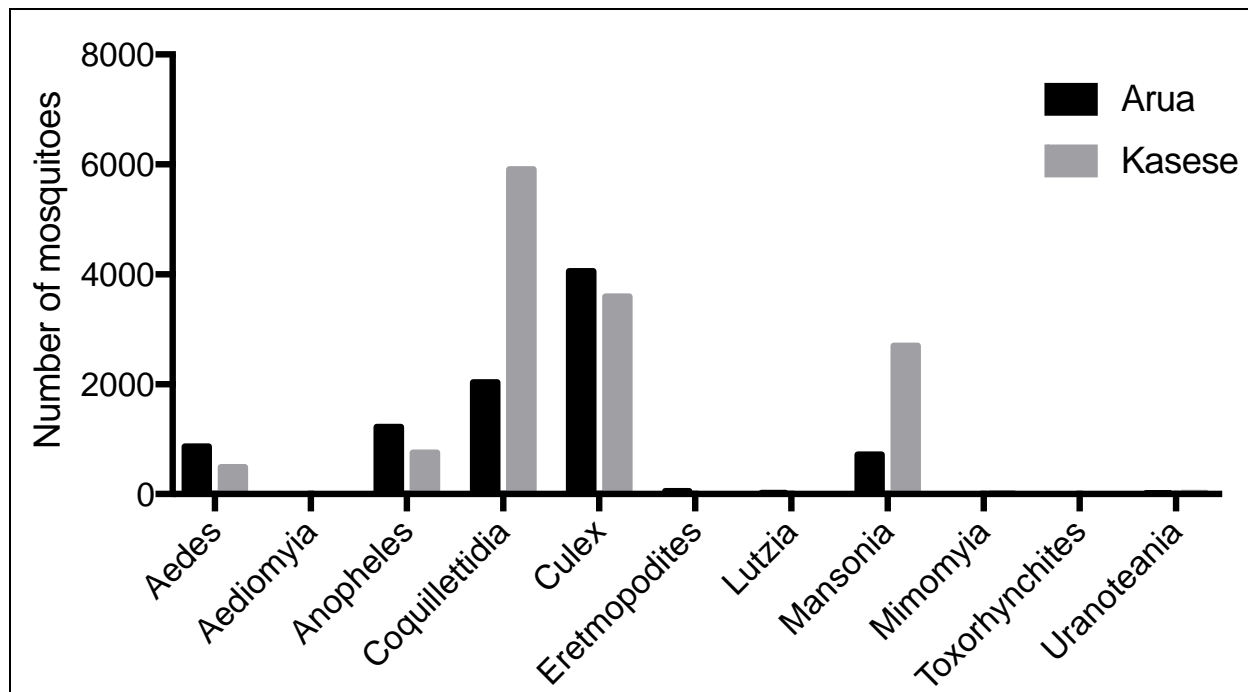


Fig 2: Number of mosquitoes per genus in Arua and Kasese districts

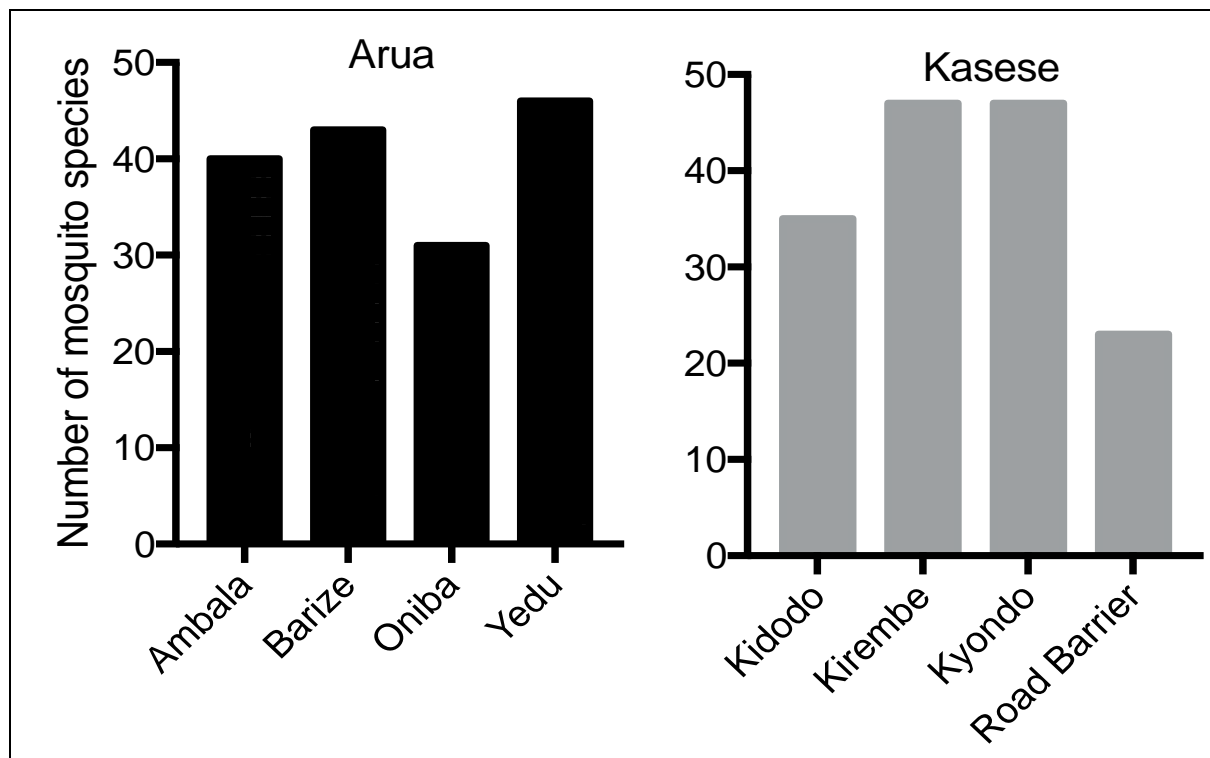


Fig 3: Number of mosquito species collected per village.

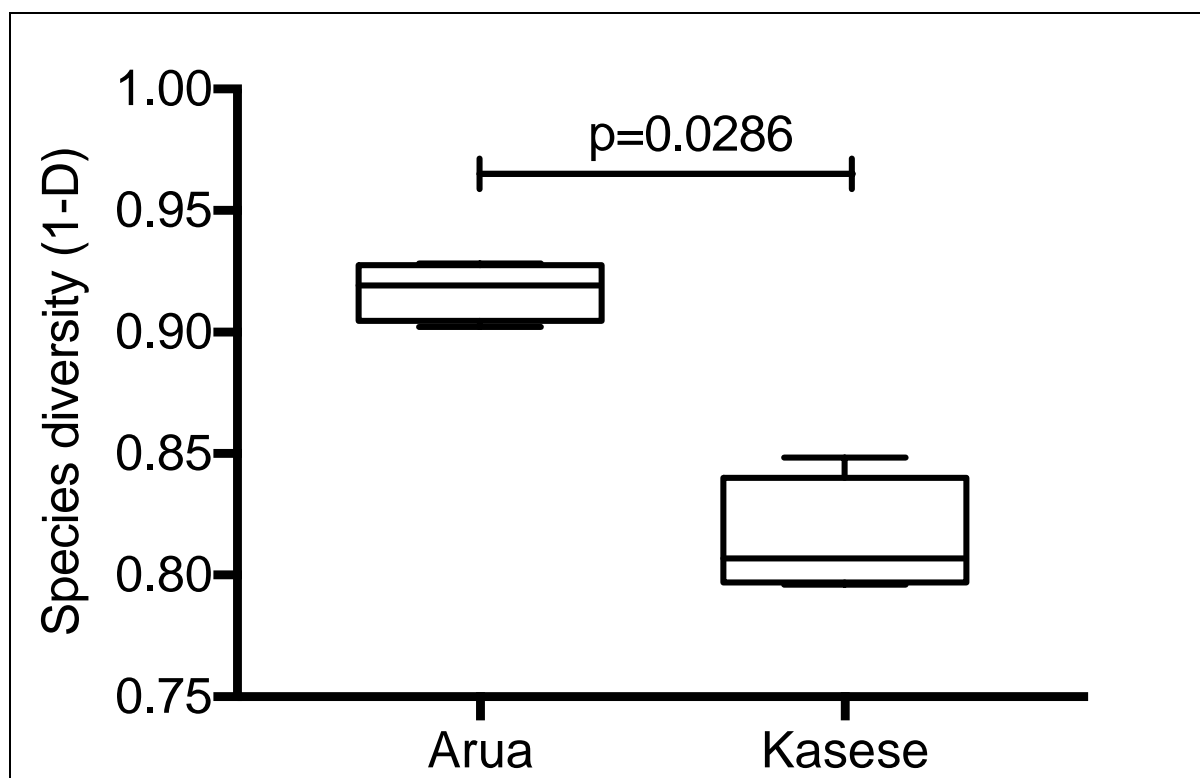
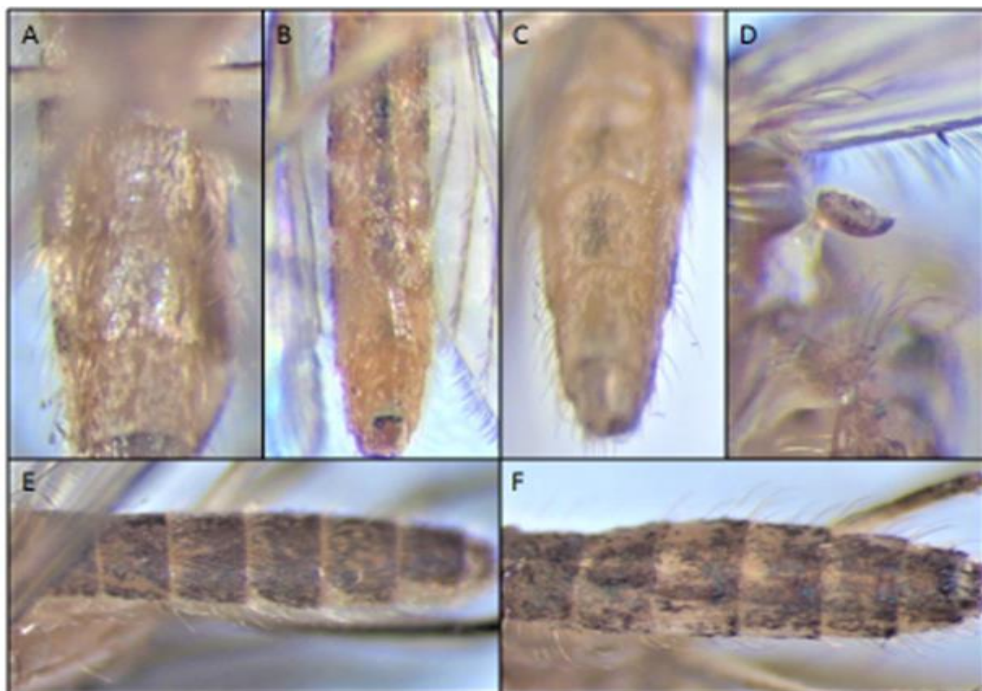


Fig 4: Mosquito species diversity in villages of Arua and Kasese districts (Mann Whitney U test  $p < 0.05$ )



**Fig 5:** Morphological characteristics of *Cx. (Cx.) litwaka*. (A) The uniformly pale venter of *Cx. (Cx.) antennatus*. (B, C) The venter of *Cx. (Cx.) litwaka* is similar to *Cx. (Cx.) pipiens*. (D) *Cx. (Cx.) litwaka* haltere with a light stem and dark knob. (E) *Cx. (Cx.) litwaka* with dark tergum like *Cx. (Cx.) antennatus* and sometimes *Cx. (Cx.) pipiens*. (F) *Cx. (Cx.) litwaka* tergum with basal medial white spots (also sometimes like *Cx. (Cx.) pipiens*).

**Table 1:** Areas in Western and North Western Uganda of previous mosquito studies.

North western Uganda (West Nile region)	Latitudes	Longitudes	References
Rhino camp	2° 58'N	31° 24' E	[5]
Sunguru	3° 01'N	30° 48' E	[14]
Omugo	3° 17'N	31° 07' E	[8]
Western Uganda			
<i>Aedes simpsoni</i>	0° 48'N	30° 08' E	[32]
Bwamba virus	0° 48'N	30° 05' E	[33]
Ntaya virus	0° 42'N	30° 3' E	[34]
Bunyamwera virus	0° 51'N	29° 59' E	[35]
Uganda S	0° 44'N	30° 3' E	[35]
Semliki Forest Virus	0° 44'N	30° 3' E	[36]
Mweya QENP	0° 11'S	29° 54' E	[4]
Sempaya, SNP	0° 49'N	30° 10' E	[4]

**Table 2:** Number of mosquitoes per collection method.

Collection method	Name of district		Total	%
	Arua	Kasese		
Light trap baited with CO <sub>2</sub>	7584	13018	20602	91.75
Indoor collection	702	342	1044	4.65
Larvae collection	86	3	89	0.4
Ovitrap	637	83	720	3.21
Total	9009	13446	22,455	100

**Table 3:** Differential\_morphological characteristics shared between the three *Culex* species

	<i>Cx. (Cx.) antennatus</i>	<i>Cx. (Cx.) litwaka</i>	<i>Cx. (Cx.) pipiens</i>	References
Venter	Pale	Pale with black scales tending to form a medial line, sometimes reduced	Pale with black scales tending to form a medial line, sometimes reduced	[7, 30]
Haltere Knob	Dark	Dark	Yellow	[7, 30]
Tergites	Dark medially	Tergites unbanded, often basal medial pale spots, sometimes reduced	Banded sometimes unbanded	[7, 30]

## Supplementary Tables

Table S1: Mosquito species composition in the four villages of Kasese district.

Genus	Sub genus	Species	Kidodo	Kirembe	Kyondo	Road Barrier
<i>Aedes</i>	<i>Aedimorphus</i>	<i>abnormalis</i>		25		
		<i>apicoargenteus</i>		1		
		<i>argenteopunctatus</i>		2		4
		<i>cumminsi</i>	2	65	7	
		<i>dentatus</i>		8		
		<i>domesticus</i>		10		
		<i>gibbinsi</i>		42	7	
		<i>hirsutus</i>			76	
		<i>leptolabis</i>		7	1	
		<i>natronius</i>			1	
		<i>phylolabis</i>		5		
		<i>quasiunivittatus</i>			5	
		<i>tarsalis</i>		24		
		<i>tricholabis</i>		5		
	<i>Aedimorphus</i>				1	
	<i>Dunnius</i>	<i>kummi</i>			1	
	<i>Neomelaniconion</i>	<i>albocephalus</i>			2	
		<i>circumluteolus</i>	9	10	25	1
		<i>mcintoshii</i>			3	
	<i>Stegomyia</i>	<i>aegypti</i>	51		25	50
		<i>africanus</i>			4	
		<i>simpsoni</i>		1		1
		<i>vitattus</i>			2	
<i>Anopheles</i>	<i>Anopheles</i>	<i>coustani</i>	37	37	84	
		<i>implexus</i>		1		
		<i>maculpalpis</i>		4		21
		<i>moucheti</i>	3	2		105
		<i>paludis</i>	1			2
		<i>tenebrosus</i>			15	
		<i>zymesi</i>			49	
		<i>ziemanni</i>			150	
	<i>Cellia</i>	<i>funestus</i>	1	25	4	11
		<i>gambiae</i>	3	16	162	15
<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>annetti</i>	1			
		<i>aurites</i>	3	51	1	86
		<i>cristata</i>				75
		<i>fraseri</i>	262	89	28	12
		<i>fuscopennata</i>	1157	1042	2458	425
		<i>maculipennis</i>	1			
		<i>metallica</i>		104	10	94
		<i>microannulata</i>	1	2		
<i>Culex</i>	<i>Culex</i>	<i>antennatus</i>	209	53	412	
		<i>argenteopunctatus</i>				2
		<i>bitaeniorhynchus</i>	1	2	1	
		<i>decens group</i>	139	336	221	73
		<i>duttoni</i>	12	13	41	2
		<i>ingrami</i>			5	
		<i>macfei</i>	4			
		<i>mirificus</i>		4		
		<i>neavei</i>	52	399	279	110
		<i>perfuscus</i>	9	39	26	
		<i>pipiens</i>	230	30	150	1
		<i>poicilipes</i>	2	1	36	
		<i>pruina</i>			48	
		<i>quinquefasciatus</i>	7	16	4	
		<i>trifilatus</i>		15		
		<i>univittatus</i>	7	53	11	
		<i>vansomeroni</i>		2		
		<i>ventrillon</i>	1			
		<i>watti</i>		7		
	<i>Culex spp</i>			85		
	<i>Culicomyia</i>	<i>cinerellus</i>	10			
		<i>cinereus</i>	50	17	80	55
		<i>nebulosus</i>	10		35	



	<i>Eumelanomyia</i>	<i>insignis</i>	4	8	6	
		<i>rubinotus</i>		10	1	
	<i>Kitzmelleria</i>	<i>moucheti</i>	4			
		<i>annulioris consmilis</i>	28	35	58	25
<i>Mimomyia</i>	<i>Etorleptomyia</i>	<i>mediolineata</i>			10	
<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i>		1	1	
		<i>nigerrima</i>	157	5	719	31
		<i>uniformis</i>	548	44	1138	50
<i>Mimomyia</i>	<i>Mimomyia</i>	<i>mimoyiaformis</i>			3	
<i>Toxorhynchites</i>	<i>Afrorhynchus</i>	<i>brevipalpis</i>			1	
<i>Uranoteania</i>	<i>Uranoteania</i>	<i>mashoneansis</i>		1		9
<i>Uranoteania</i>	<i>Uranoteania</i>	<i>palidocephala</i>	1	1	7	

**Table S2:** Mosquito species composition in the four villages of Arua district.

Genus	Sub genus	Species	Ambala	Barize	Oniba	Yedu
<i>Aedes</i>	<i>Aedimorphus</i>	<i>Apicoargenteus</i>				1
		<i>argenteopunctatus</i>				7
		<i>durbanensis</i>		3		2
		<i>leptolabis</i>		1		
		<i>Aedimorphus</i>	1			
<i>Aedes</i>	<i>Neomelaniconion</i>	<i>circumluteolus</i>		57	2	53
	<i>Stegomyia</i>	<i>aegypti</i>	22	249		209
		<i>africanus</i>		1		2
		<i>simpsoni</i>	5	143		100
		<i>vitattus</i>	1			2
<i>Aediomyia</i>	<i>Lepiothauma</i>	<i>furfurea</i>	1			2
<i>Anopheles</i>	<i>Anopheles</i>	<i>coustani</i>	15	65	33	34
		<i>implexus</i>			5	
		<i>maculpalpis</i>	6	14	11	26
		<i>moucheti</i>	3	17	18	2
		<i>pharoensis</i>			3	2
		<i>theileri</i>	4	9	26	52
		<i>ziemanni</i>	30	41	8	49
	<i>Cellia</i>	<i>funestus</i>	58	33	178	108
		<i>gambiae</i>	47	26	194	82
	<i>Zavortinkus</i>	<i>longipalpis</i>				3
<i>Anopheles</i>	<i>Anopheles</i>	<i>spp</i>			20	
<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>aurites</i>		17	11	9
		<i>cristata</i>	115		23	1
		<i>fraseri</i>	64	41	119	45
		<i>fuscopennata</i>	26	591	56	726
		<i>maculipennis</i>	1	22	20	84
		<i>metallica</i>	1			57
		<i>pseudoconopas</i>	5			6
<i>Culex</i>	<i>Culex</i>	<i>antennatus</i>	11	3	66	39
		<i>argenteopunctatus</i>				3
		<i>aurantapex</i>	2			
		<i>bitaeniorhynchus</i>	1			
		<i>decens group</i>	194	145	176	549
		<i>duttoni</i>	9	48	46	30
		<i>litwakae</i>		2		
		<i>mirificus</i>	1			
		<i>neavei</i>	24	106	56	191
		<i>perfuscus</i>	61	6	10	5
		<i>pipiens</i>		3	1	
		<i>poecilipes</i>	3			
		<i>quinquefasciatus</i>		1		
		<i>triflatus</i>	3	29		7
		<i>univittatus</i>	36	156	115	254
		<i>watti</i>		3		
		<i>Culex spp</i>	26			38
	<i>Culicomyia</i>	<i>cinerellus</i>				
		<i>cinereus</i>	82	73	84	177
		<i>nebulosus</i>	2	2	1	
	<i>Eumelanomyia</i>	<i>insignis</i>	3	24	30	5
		<i>rubinotus</i>		22		11
	<i>Kitzmelleria</i>	<i>moucheti</i>	4	8	4	8
	<i>Oculeomyia</i>	<i>annulioris</i>	43	115	30	323

		<i>annulioris consmilis</i>	63	140	183	158
<i>Eretmopodites</i>	<i>Eretmopodites</i>	<i>chrysogaster</i>		1		14
		<i>quinquivitattus</i>	43			1
<i>Lutzia</i>	<i>Metalutzia</i>	<i>tigripes</i>	13		3	10
<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i>				5
		<i>nigerrima</i>	3	11		68
		<i>uniformis</i>	69	221	176	167
<i>Uranoteania</i>	<i>Uranoteania</i>	<i>alboabdominalis</i>				2
		<i>mashoneansis</i>	3	1		2
		<i>palidocephala</i>	2			
		<i>nivipous</i>		6		

**Table S3:** Viruses of medical importance that have been isolated from mosquito species (collection from Kasese district)

Genus	Sub-genus	Species	Viruses
<i>Aedes</i>	<i>Aedimorphus</i>	<i>abnormalis</i> Edwards	WSLV [37], NDUV [37], MOSV [37], CHIKV [37], SPOV [37], MIDV [37], PGAV [37]
		<i>apicoargenteus</i>	ZIKAV [37]
		<i>argenteopunctatus</i> (Theobald)	SHOV [31, 37], CHIKV [37], NRIV [37], MIDV [37], WSLV [37], PGAV [37], SFV [37]
		<i>cumminsii</i> (Theobald)	SPOV [4, 37], PGAV [4, 37], LEBV [4, 37], RVFV [4, 37], CHIKV [4, 37], WSLV [37], PGAV [4], DENV-2 [38]
		<i>domesticus</i> (Theobald)	BUNV [37], WSLV [38]
		<i>natronius</i> Edwards	UGAS [38]
	<i>Neomelanicion</i>	<i>tarsalis</i> (Newstead)	WSLV [38], SHOV [38], PGAV [38], PATAV [38], NGOV [38], KEDV [38], MIDV [38], ZIKAV [38]
		<i>albothorax</i> (Theobald)	WNV [38]
		<i>circumluteolus</i>	RVFV [4], WSLV [4], SPOV [4], SHOV [4], PGAV [4], NDUV [38], MIDV [38], LEBV [38], INGV [31], BUNV [34], BWAV [34], WNV [31], GERV [38]
	<i>Stegomyia</i>	<i>mcintoshii</i>	WSLV [38], RVFV [38], NRIV [31]
		<i>aegypti formosus</i> (Walker)	ZIKAV [39], YFV [39], WNV [39], CHIKV [39], ORUV [38]
		<i>africanus</i>	ZIKAV [38], YFV [38], WNV [38], CHIKV [38], ORUV [31], BBKV [31], BOZOV [31, 38]
		<i>simpsoni</i> group	YFV [38], BBKV [38], NRIV [38]
	<i>Anopheles</i>	<i>Anopheles</i>	<i>vitattus</i>
<i>coustani</i> Laveran			BWAV [4], WNV [4,5], PGAV [4, 27], NRIV [4], CHIKV [4]
<i>paludis</i> Theobald			GOMV [31], BOUV [31]
<i>zymesi</i> Edwards			CHIKV [31], WNV [31], PGAV, NRIV
<i>Cellia</i>		<i>ziemanni</i> Greunberg	CHIKV [31], WNV [4,5], PGAV [4,5], NRIV [4,5]
		<i>funestus</i> complex	TANV [4,5], TATV [34], PGAV [4,5], ORUV [4,5], ONNV [33], NDOV, BWAV [34], BUNV [34], BOZV [31]
		<i>gambiae</i> s.l	TATV [34], ORUV [4,5], BWAV [4,5], BUNV [34], ONNV [33], NRIV [4,5], ILEV [34], CHIKV [4, 37], MIDV [4,5], NDOV [4,5], BGIV [31], ZIKAV [4,5]
<i>Coquillettidia</i>		<i>aurites</i> (Theobald)	TATV [34], USUV [4, 37]
		<i>fuscopenata</i> (Theobald)	RVFV [5], YFV [4,5], CHIKV [4, 37], SINV [4,5]
		<i>maculipennis</i> (Theobald)	CHIKV [4, 37]
<i>Culex</i>	<i>metallica</i> (Theobald)	WNV [4,5], MIDV [4,5], BBKV [4,5]	
	<i>Culex</i>	<i>antennatus</i> (Becker)	WNV [4,5], RVFV [4,5], WSLV [4,5], SINV [4,5], PGAV [4,5], NRIV [4,5], BARV [31]
		<i>decens</i> group	MOSV [4,5], MPOV [4,5], CHIKV [4,5], WNV [4,5], BBKV [4,5]
		<i>neavei</i> Theobald	SPOV [4,5], WNV [4,5], INGV [4,5], MOSV [4,5], USUV [4,5], KOUV [4,5]
		<i>perfuscus</i> Edwards	GOMV [31], USUV [5], ORUV [4,5], NOLAV [4,5], MOSV [4,5], USUV [4,5], WNV [4,5], BBKV [4,5], SINV [4,5], WSLV [4,5], BAGV [4,5], BGNV [4,5]
		<i>pipiens</i> Linnaeus	TVTIV [4,5], SFV [5], LMV [4,5], JBEV [4,5], HPV [5], TAHV [5], WNV [5], BUNV [4, 37], BANV [5], LACV [5]
		<i>poicilipes</i>	WNV [4,5]
		<i>pruina</i> Theobald	YAOV [31], MOSV [5], KAMV [5], WNV [5], BOZV [5]
		<i>quinquefasciatus</i> Say	WNV [5], CHIKV [4, 37], WANV [5], SLEV, OROV [5], EEEV [5], KUNV [5], RRV [5], VEEV [5], SINV [5], AMTV [5]
	<i>univittatus</i>	WNV [4, 37], SINV [4, 37], INGV [31], WSLV [4, 37], SPOV, USUV [5], ACDV [31]	
<i>Culicomyia</i>	<i>cinereus</i> Theobald	CHIKV [4, 37], BBKV [4,5], MIDV [4,5]	
	<i>nebulosus</i> Theobald	MIDV [5], BGIV [5], BBKV [5]	
<i>Eumelanomyia</i>	<i>rubinotus</i> Theobald	AMTV [4, 37], BANV [5], GERV [40], RVFV [5]	
<i>Oculeomyia</i>	<i>annulioris consmilis</i> Newstead	MIDV [4,5], WSLV [5]	
<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i> (Theobald)	SPOV [5], PGAV [5], LEBV [5], BWAV [40], BBKV [4,5], WSLV [4,5], PGAV [4,5], MIDV [4,5], USUV [4,5], BUNV [40]

		<i>uniformis</i> (Theobald)	YATAV [31], SPOV [5], SANV [31], RRV [5], PGAV [5], PUCV [5], NDUV [4, 37], MALV [31], WSLV [5], BBKV [4, 37], BUNV [40], AMTV [4, 37]
<i>Uranoteania</i>	<i>Pseudofilcabia</i>	<i>mashoneansis</i> Theobald	WSLV [5]

**Table S4:** Viruses of medical importance that have been isolated from mosquito species (collection from Arua district)

Genus	Sub-genus	Species	Viruses
<i>Aedes</i>	<i>Aedimorphus</i>	<i>apicoargenteus</i>	ZIKAV [4,5]
		<i>argenteopunctatus</i> (Theobald)	SHOV [31], CHIKV [4,5], NRIV [4,5], MIDV [4,5], WSLV [4,5], PGAV [4,5], SFV [4,5]
	<i>Neomelaniconion</i>	<i>circumluteolus</i> (Theobald)	RVFV [5], WSLV [5], SPOV [5], SHOV [5], PGAV [4,5], NDUV [4,5], MIDV [4,5], LEBV [31], INGV [31], BUNV [34], BWAV [34], WNV [4,5], GERV [4,5]
		<i>aegypti formosus</i> (Walker)	ZIKAV [4,5], YFV [4,5], WNV [4,5], CHIKV [4,5], ORUV [4,5]
	<i>Stegomyia</i>	<i>africanus</i>	ZIKAV, YFV, WNV, CHIKV, ORUV, BBKV, BOZOV [31]
		<i>simpsoni</i>	YFV [4,5], BBKV [4,5], NRIV [4,5]
		<i>vitattus</i>	BBKV, YFV
<i>Anopheles</i>	<i>Anopheles</i>	<i>coustani</i>	CHIKV [4,5], WNV [4,5], PGAV [4,5], NRIV [4,5]
	<i>Cellia</i>	<i>funestus</i>	TANV [31], TATV [34, 40], PGAV [31], ORUV [4,5], ONNV [33], NDOV, BWAV [34, 40], BUNV [34], BOZV [31]
		<i>gambiae</i>	TATV [34], ORUV [4, 37], BWAV [40], BUNV [40, 34], ONNV [33], NRIV [4,5], ILEV [34], CHIKV [31], MIDV [4,5], NDOV [4,5], BGIV [4,5], ZIKAV [31]
<i>Coquillettidia</i>	<i>Coquillettidia</i>	<i>aurites</i>	BWAV, TATV [34], USUV [5]
		<i>fuscopenata</i> (Theobald)	RVFV [5], YFV [5], CHIKV [5], SINV [5]
		<i>maculipennis</i> (Theobald)	CHIKV [5]
		<i>metallica</i> (Theobald)	MIDV [31], WNV [31], BBKV [31]
<i>Culex</i>	<i>Culex</i>	<i>antennatus</i> (Becker)	AMTV [5], WNV [4, 37], RVFV [31], WSLV [31], SINV [31], PGAV [5], NRIV [5], BARV [31]
		<i>decens</i> group	MOSV [31], MPOV [5], CHIKV [5], WNV [4,5], BBKV [4,5]
		<i>neavei</i> Theobald	SPOV [4,5], WNV [4,5], INGV [31], MOSV [31], USUV [4,5], KOUV [31]
		<i>perfuscus</i> Edwards	GOMV [31], USUV [4,5], ORUV [4,5], NOLAV [31], MOSV [4,5], USUV [34], WNV [5], BBKV [4,5], SINV [4,5], WSLV [4,5], BAGV [31], BGNV [31]
		<i>pipiens pipiens</i> Linnaeus	TVTIV [31], SFV [4,5], LMV [31], JBEV [4,5], HPV [4,5], TAHV [4,5], WNV [4,5], BUNV [4,5], BANV [4,5], LACV [31]
		<i>poicilipes</i>	WNV [31]
		<i>quinquefasciatus</i> Say	WNV [5], CHIKV [31], WANV [31], SLEV [31], ORUV [5], EEEV [31], KUNV [31], RRV [5], VEEV [5], SINV [5]
		<i>univitattus</i>	WNV [4,5], SINV [4,5], INGV [4,5], WSLV [4,5], SPOV [4,5], USUV [4,5], ACDV [4,5]
<i>Culicomyia</i>	<i>Culicomyia</i>	<i>cinereus</i> Theobald	CHIKV [4,5], BBKV [34], MIDV [4,5]
		<i>nebulosus</i> Theobald	MIDV [4,5], BGIV [4,5], BBKV [4,5]
	<i>Eumelanomyia</i>	<i>rubinotus</i> Theobald	AMTV [4,5], BANV [4,5], GERV [4,5], RVFV [4,5]
	<i>Oculeomyia</i>	<i>annulioris</i> Theobald	MIDV [4,5], WSLV [34]
		<i>annulioris consmilis</i> Newstead	MIDV [4,5], WSLV [34]
<i>Eretmopodites</i>	<i>Eretmopodites</i>	<i>chrysoaster</i> Graham	MIDV [4,5], SFV [4,5], RVFV [4,5], YFV [4,5]
<i>Lutzia</i>	<i>Metalutzia</i>	<i>tigripes</i> De Grandpre & De Charmony	SINV [4,5], BBKV [4,5], BIAV [4,5]
<i>Mansonia</i>	<i>Mansonioides</i>	<i>africana</i> (Theobald)	SPOV [4,5], PGAV [4,5], LEBV [4,5], BWAV [4,5], BBKV [4,5], BUNV [40], YATAV [4,5], SPOV [4,5], SANV [4,5], RRV [4,5], PGAV [4,5], PUCV [4,5], NDUV [4,5], MALV [4,5], BWAV [40], AMTV [4,5]
		<i>uniformis</i> (Theobald)	
<i>Uranoteania</i>	<i>Uranoteania</i>	<i>palidocephala</i> Theobald	WSLV [4,5]

ACDCV-Acado virus, AMTV-Arumowot virus, BAGV-Bagaza virus, BANV-Banzi virus, BARV-Barur virus, BARV-Barur virus, BBKV-Babanki virus

BGIV-Bangui virus, BGNV- Bangoran virus, BIAV-Bobia virus, BOUV-Boubou virus, BOZOV-Bozo virus, BUNV- Bunyamwera virus, BWAV-Bwamba virus, CHIKV-chikungunya virus, DENV-2-dengue virus serotype-2, EEEV- Eastern equine encephalomyelitis virus, GERV-Germiston virus, GOMV- Gomoka virus, HPV- Hart Park virus, ILEV-ileshe virus, INGV- Ingwavuma virus, JBEV- Japanese Encephalitis virus, KEDV- Kedougou virus, KOUV-Koutango virus, KUNV- Kunjin virus, LACV-La Crosse virus, LEBV- Lebombo virus, LMV- Las Maloyas virus, MALV-Malakal virus, MIDV-Middleburg virus, MOSV-Mossuril virus, MPOV-M'poko virus, NDUV-Ndumu virus, NGOV-Ngoupe virus, NOLAV- Nola virus, NRIV-Ngari virus, ONNV-o'nyong- nyong virus, ORUV-Orungo virus, PATAV-Pata virus, PGAV-Pongola virus, PUCV-Puchong virus, RRV-Ross River virus, RVFV-Rift Valley fever virus, SANV-Sango virus, SFV-Semliki Forest virus, SHOV- Shokwe virus, SINV-Sindbis virus, SLEV- St. Louis encephalitis virus, SPOV-Spondweni virus, TAHV-Tahyna virus, TANV- Tanga virus, TATV-Tataguine virus, TVTV- Trivittatus virus, UGAS-Uganda S virus, USUV-USutu virus, VEEV- Venezuelan equine encephalitis virus, WANV- Wanowrie virus, WNV-West Nile virus, WSLV- Wesselsbron virus, YAOV-Yaounde virus, YATAV-Yata virus, YFV-yellow fever virus, ZIKAV-Zika virus

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