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Effects of Irrigation and Rainfall on the Population Dynamics of Rift Valley Fever and Other Arbovirus Mosquito Vectors in the Epidemic-Prone Tana River County, Kenya

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Abstract

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis that is found in most regions of sub-Saharan Africa, and it affects humans, livestock, and some wild ungulates. Outbreaks are precipitated by an abundance of mosquito vectors associated with heavy persistent rainfall with flooding. We determined the impact of flood-irrigation farming and the effect of environmental parameters on the ecology and densities of primary and secondary vectors of the RVF virus (RVFV) in an RVF-epidemic hotspot in the Tana River Basin, Kenya. Mosquito sampling was conducted in farms and villages (settlements) in an irrigated and a neighboring nonirrigated site (Murukani). Overall, a significantly higher number of mosquitoes were collected in farms in the irrigation scheme compared with villages in the same area ($P < 0.001$), or farms ($P < 0.001$), and villages ($P = 0.03$) in Murukani. In particular, key primary vectors of RVFV, *Aedes mcintoshi* Marks and *Aedes ochraceous* Theobald, were more prevalent in the farms compared with villages in the irrigation scheme ($P = 0.001$) both during the dry and the wet seasons. Similarly, there was a greater abundance of secondary vectors, particularly *Culex univittatus* Theobald and *Culex pipiens* (L.) in the irrigation scheme than in the Murukani area. Rainfall and humidity were positively correlated with mosquito densities, particularly the primary vectors. Adult floodwater mosquitoes and *Mansonia* spp. were collected indoors; immatures of *Ae. mcintoshi* and secondary vectors were collected in the irrigation drainage canals, whereas those of *Ae. ochraceous* and *Aedes sudanensis* Theobald were missing from these water bodies. In conclusion, irrigation in RVF endemic areas provides conducive resting and breeding conditions for vectors of RVFV and other endemic arboviruses.

Key words: Rift Valley fever, *Ae. mcintoshi*, irrigation scheme, ecology, rainfall

Rift Valley fever (RVF) is a severe acute mosquito-borne viral zoonosis that affects ruminants and humans (Linthicum et al. 1985). Its epizootics occur in eastern, western, and southern Africa every 5–15 yr, following heavy, persistent rainfall that results in the hatching of large numbers of floodwater *Aedes* mosquitoes, which are known to maintain and transmit the virus (Davies 1975). Animals are infected through bites of infected mosquitoes, whereas humans are typically affected when they come in close contact with body fluids and tissues of infected animals and via mosquito bites, leading mainly to mild or asymptomatic cases (Pepin et al. 2010). Since being first identified in 1930 in the Rift Valley of Kenya (Daubney et al. 1931), recurring outbreaks have led to high morbidity and mortality in human populations. This is in addition to significant economic losses associated with livestock morbidity and mortality and abortions together with losses incurred from trade bans and restrictions (Pepin et al. 2010). In the past couple of decades, two major outbreaks of RVF (1997–1997 and 2006–2007) have occurred in the eastern Africa, resulting in massive public health and economic impacts on

the affected countries (Woods et al. 2002, Nguku et al. 2010). Recent studies conducted during interepidemic periods have also indicated active transmission of the virus between epidemics in Kenya (Lichoti et al. 2014, Owange et al. 2014).

Kenya's economy is agriculture based, despite 80% of the country being composed of arid to semiarid lands (ASAL). These lands are characterized by low and erratic rainfall averaging 100–1,200 mm per annum and high evapotranspiration rates. Because of frequent droughts coupled with the growing human population requiring expansion of food production, communities can no longer rely on rain-fed agriculture to meet their subsistence needs (Blank et al. 2002). The government drafted sessional paper No. 8 of 2012 with plans to reverse the effects of long-term underdevelopment by putting in place policy frameworks that would fast-track sustainable development in ASALs through increased access to skills and technologies for irrigated agriculture (GOK 2012) in the effort to introduce irrigation-fed agriculture. However, public health impact assessment focused only on diseases such as malaria and schistosomiasis to a small extent (Blank et al. 2002). As such, there is limited knowledge on how irrigation would influence the risk of other diseases such as RVF.

The population dynamics of mosquitoes is mainly driven by rainfall, humidity and temperature. Heavy rainfall results in flooding and availability of breeding habitats that trigger mass hatching of floodwater mosquitoes' eggs (Davies et al. 1985, Linthicum et al. 1987). Although humidity favors the survival of mosquito populations, the high temperatures strongly influence development of aquatic stages. Some of the surviving mosquitoes, already infected with RVFV, transmit the virus to nearby domestic animals (Davies and Highton 1980), initiating virus amplification and transmission. The primary vectors of RVF in East Africa include *Aedes mcintoshi* Marks and *Aedes ochraceous* Theobald, and the secondary vectors include *Culex pipiens* (L.), *Culex univittatus* Theobald, and *Mansonia uniformis* Theobald (Linthicum et al. 1985, Turell et al. 2008, Sang et al. 2010).

The RVFV is considered to be transovarially carried in eggs of infected *Aedes* mosquitoes, which can survive for several years in dry soil (O'Malley 1990). The floodwater *Aedes* have been observed to lay their eggs preferably in temporary ground pools on low-lying depressions known as dambos that are commonly found in RVF-endemic/-epidemic areas (Linthicum et al. 1985; Davies et al. 1985). Flooding of the dambos induces synchronized hatching of a large number of eggs. Past isolations of RVFV from *Ae. mcintoshi* males and females reared from larvae provided evidence for vertical transmission (Logan et al. 1991). Prolonged flooding results in subsequent inhabitation by opportunistic mosquito vector species of the *Culex* and *Anopheles* genera (secondary vectors). The humid and cloudy conditions that persist during prolonged rains enhance survival of the adult *Aedes* over more feeding and oviposition cycles (Davies et al. 1985). This allows greater amplification of the virus in the vertebrate population. The transmission of other arboviruses such as West Nile (WNV), Semliki Forest (SFV), Sindbis virus (SINV), Bunyamwera virus (BUNV), Pongola virus (PGAV), Babanki virus (BBKV), and Ndumu virus (NDUV) also occurs, aiding the co-circulation with RVFV among mosquitoes during the outbreak (Crabtree et al. 2009). Subsequent surveillance studies targeting mosquito vectors have indicated continued transmission of these viruses between RVF-epidemic periods (Ochieng et al. 2013).

A number of studies conducted in East and Central Africa suggest that irrigation can negatively affect the health of local populations (Oomen et al. 1990). Introduction of massive water reservoirs, water distribution canals, and drainage systems can increase the number of breeding sites for mosquitoes and the duration of their breeding season, whereas elevated relative humidity can extend the average longevity of mosquitoes, hence affecting the transmission intensity and incidence rates of diseases. Similarly, the induced hydrological changes could cause outbreaks of other water-related, vector-borne diseases such as schistosomiasis, liver flukes, filariasis, and onchocerciasis (Oomen et al. 1990).

In this study, we assessed the impact of introduced irrigation farming in Bura, Tana River County, on the ecology and population dynamics of RVFV vectors and other arboviruses. Although this county is administratively located in Coast Province, it neighbors Garissa County, a major RVF outbreak hotspot in Kenya. Both Tana River and Garissa counties were heavily affected by the 1997–1998 and 2006–2007 outbreaks. Bura and Hola, the main irrigation schemes located in former pastoral regions, are major government projects being implemented in Kenya with the goal of improving food security.

Materials and Methods

Study Site Description

The Bura irrigation scheme comprises 10,000 acres divided into several blocks, each measuring between 216 and 720 acres, and contains 2,200 homesteads in 11 villages. One of the villages within the irrigation scheme was not sampled because it was not under either irrigation or farming during our visits. Each block is further subdivided into 3–10 farming units, each measuring 72 acres. The scheme is supplied with irrigation water from river Tana through the main canal that branches into several branch canals that supply several manmade water reservoirs. From these, the water is supplied to the farms through additional manmade canals including the block feeder (BF) canals, which carry water from the reservoirs to the blocks and unit feeder (UF) canals that branch from the BF canals and supply water into the farming units through the unit furrows, which are the smallest canals that run through the fields to irrigate the farms. Any excess water in the farms is drained into the river through the unit drains (UDs) at the opposite end of the UF canals, block drains, and finally village drains (VDs). Murukani village is situated ~15 km from the Bura irrigation scheme, and is a settlement area that is nonirrigated and relies on rainfall for minimal farming. The area is inhabited by a predominantly pastoralist community that keeps some livestock, including sheep, goat, and cattle, which usually move far from the village in search of pasture during the dry season. Sampling points in farms and villages are presented (Fig. 1).

Sampling Design

Adult mosquitoes were collected four times from 26 September to 2 October 2013, from 14 to 21 November 2013, from 24 April to 1 May 2014, and from 24 November to 1 December 2014 for a total of 31 trap nights. The first sampling period was conducted during the dry season (7.2 mm of rainfall in September 2013) with minimal irrigation, whereas the second and third periods (14 to 21 November 2013 and 24 April to 1 May 2014) were sampled during the dry season (38.8 and 35.2 mm of rainfall, respectively) with enhanced irrigation. The fourth sampling conducted between 24 November and 1 December 2014 was during heavy rains (183.6 mm in November) that were accompanied by flooding (Fig. 2).

Adult Mosquito Collection

Adult mosquitoes were collected using CO_2-baited CDC light traps (John W. Hock, Gainesville, FL), which were set from 4 p.m. to

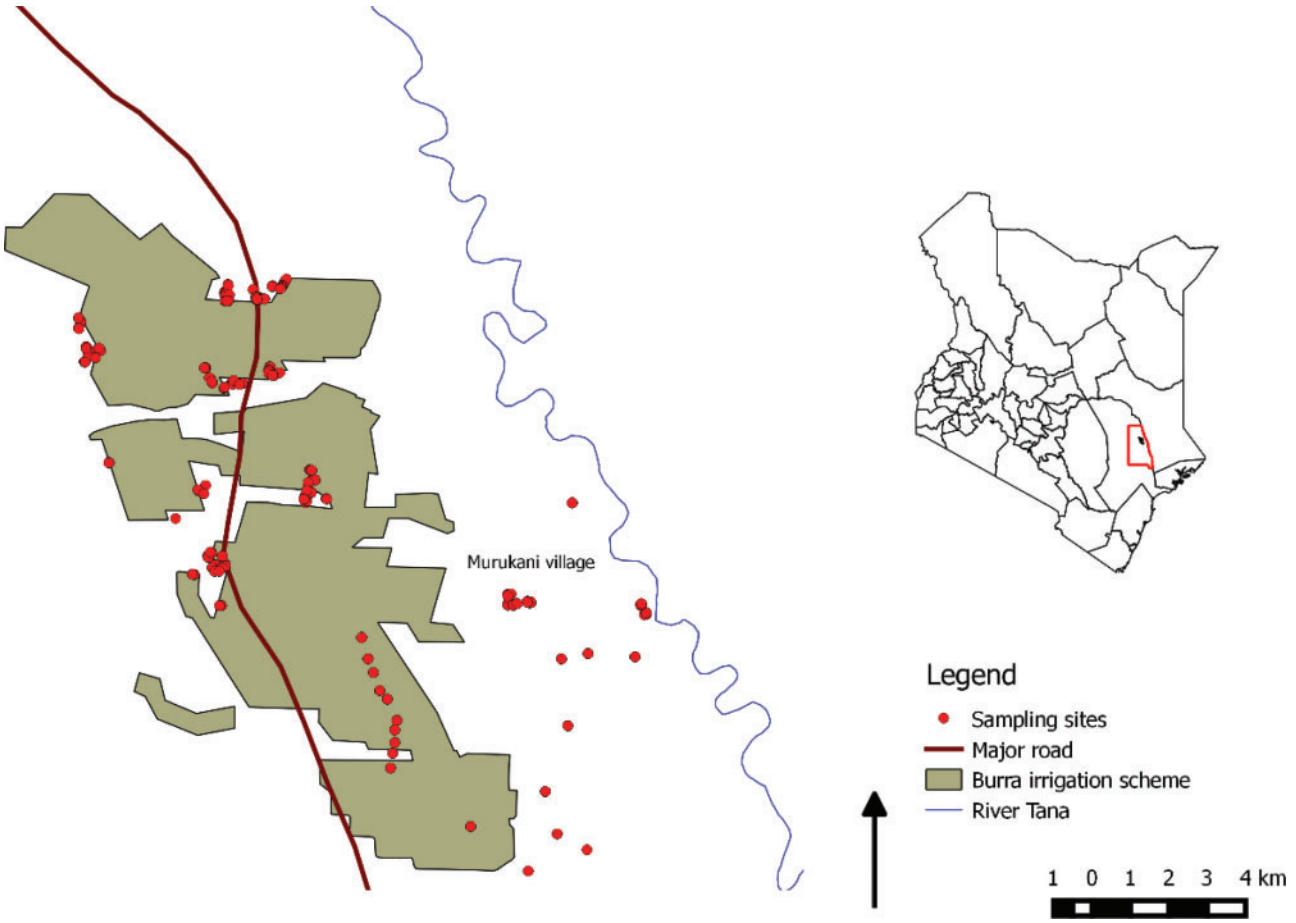

Fig. 1. A map showing the locations of the sampling points in the Bura irrigation scheme and Murukani village. The inset map shows the location of the study area (with boundaries in red line) in Kenya.

6 a.m. In total, ~10 traps were set per night in farms and villages in the Bura irrigation scheme and Murukani area. A total of 441 traps (307 outdoors and 134 indoors) were set during the study period. Of the 307 traps set outdoors, 271 were spread across the 10 villages that were sampled in the irrigation scheme (127 in the farms and 144 within the villages), whereas 36 were set in Murukani (18 in the farms and 18 in the villages). For the indoor sampling, 115 traps were used in the irrigated area, whereas 19 were used in Murukani. The mosquitoes were collected every morning and transferred to a site laboratory for sorting. Mosquitoes collected as adults and those emerging from larvae (described below) were immobilized using 99.5% triethylamine (Sigma-Aldrich, St. Louis, MO) and preserved in liquid nitrogen (LN_2) awaiting transportation to Kenya Medical Research Institute (KEMRI) laboratory for identification and processing.

Larval Mosquito Collection

Mosquito larvae were collected using standard larval dippers measuring 350 ml (BioQuip, Inc., Compton, CA) during both the rain and dry seasons. The larvae were collected from all open-water bodies within the irrigation scheme system including UFs except from the main reservoir and branch canals that were examined and found to harbor no mosquito larvae. Similarly, rain-fed water collections were sought for larval sampling. The collected larvae were transferred in well-labeled (date of collection and type of water body/canal) Whirl-Pak bags (Universal Medical, Norwood, MA) to the site laboratory where they were reared to adults in 4-liter-capacity plastic cages. Rearing was done as per the site and date of collection. Emerging adults were knocked down and cryopreserved in liquid nitrogen for subsequent identification and analysis for viruses. All adult mosquito trapping and larval collection sites were georeferenced for mapping. All the field data for adult mosquito and larvae sampling were captured using a designed data capture tool.

Mosquito Identification

All mosquitoes collected as adults and those emerging from larvae were identified to species using the keys of Edwards (1941), Gillies and de Meillon (1968), Jupp (1986), and Harbach (1988), and pooled up to 25 mosquitoes per pool. All identification was performed on chilled ice packs to preserve the virus for isolation work in cell culture. Identified mosquitoes were preserved at −70°C until analysis.

Mosquito Processing and Virus Isolation in Cell Culture

Laboratory studies were conducted to determine the parasites types carried by the sampled mosquitoes. Mosquito pools were homogenized in a biosafety level 2 laboratory at KEMRI's Center for Virus Research using 4.5-mm-diameter copper beads (BB-caliber air gun shot) in 1 ml of Eagle's Minimum Essential Medium (MEM), with Earle's salts and reduced NaHCO3 (Sigma-Aldrich, St. Louis, MO) supplemented with 15% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich), 2% L-glutamine (Sigma-Aldrich), and 2%

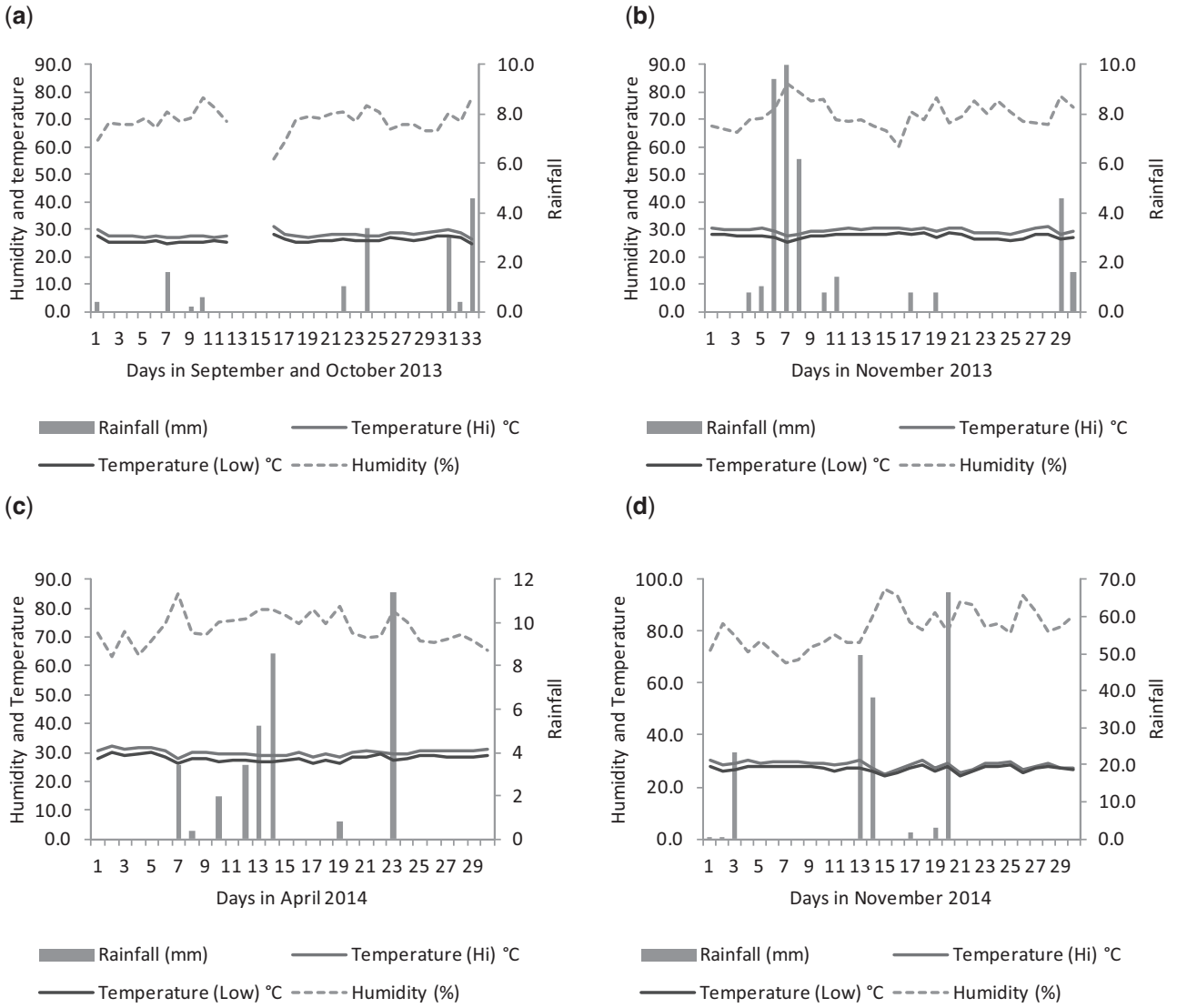

Fig. 2. Environmental conditions prevailing in Bura during the four sampling occasions – (**a**) during the dry season in September–October 2013, (**b**) dry season in November 2013, (**c**) dry season in April 2014, and (**d**) wet season in November 2014.

antibiotic/antimycotic solution (Sigma-Aldrich) with 10,000 U penicillin, 10 mg streptomycin, and 25 g amphotericin B per milliliter. The homogenates were clarified by centrifugation at 12,000 rpm (Eppendorf centrifuge 5417R) for 10 min at 4°C and the supernatants transferred into 1.5-ml cryogenic vials. Each mosquito pool supernatant (50 µl) was inoculated in a single well of a 24-well culture plate containing a confluent monolayers of Vero cells (CCL81) grown in MEM, which was supplemented with 10% FBS and 2% L-glutamine and 2% antibiotic/antimycotic solution. The inoculated cultures were incubated for 45 min to allow for virus adsorption, and each sample maintained in MEM was supplemented with 2% FBS and 2% antibiotic/antimycotic solution. The cultures were incubated at 37°C in 5% CO_2 and monitored daily, through Day 14, for cytopathic effects as an indication of virus infection.

Virus Detection and Analysis by RT-PCR

RNA was extracted from 250-µl of each cell culture supernatant using the Trizol-LS-chloroform extraction method (Chomczynski and Sacchi 1987). The final RNA pellet was dissolved in 11 µl of nuclease-free water at room temperature and stored on ice or frozen at −80°C, ready for RT-PCR. To convert extracted RNA into cDNA, 10 µl of RNA and 2 µl of random hexamer (100 nmol) were combined in a dome-topped PCR tube and placed in a thermocycler programmed at 70°C for 10 min to denature the sample and then cooled to 4°C for 5 min. The following components were added to the tubes: 4 µl of 5X first strand buffer (Invitrogen), 0.01 µmole of deoxynucleotide (dNTPs; Invitrogen), 0.02 µmole of dithiothreitol (DTT; Invitrogen), 10 U of RNase Out inhibitor (Invitrogen), and 100 U of SuperScript III reverse transcriptase (Invitrogen). These components were then incubated in the thermocycler under the following conditions: 25°C for 15 min, 42°C for 50 min, 70°C for 15 min, and 4°C hold temperature. The final volume for this reaction was 20 µl, which was used for various PCR amplifications using primers targeting virus genera or specific arboviruses.

The PCR amplification of targeted viral sequences in the cDNA was performed in a 25-µL reaction containing 12.5 µl of Amplitaq Gold PCR Master Mix (Applied Biosystems), 25 picomoles each of forward and reverse primers, 2 µl of the cDNA, and 9.5 µl of water to top up to 25 µl. The cDNA was tested using primers targeting

flavivirus, alphavirus, and orthobunyavirus arbovirus genera, and when a sample tested positive for a genus, it was tested with primers targeting conserved genes in the specific viruses belonging to the genus, as previously described (Kuno et al. 1996, Ibrahim et al. 1997, Bryant et al. 2005, Eshoo et al. 2007). Where a sample gave a positive result with virus-specific or orthobunyavirus, flavivirus, or alphavirus primers, the amplicon was purified directly from the PCR reaction or from the gel using Wizard SV Gel and PCR Clean-Up System kit (Promega). Sequencing was outsourced and performed using ABI-PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA), using both the forward and reverse genus primers.

Data Analysis

Meteorological Data

Meteorological data were obtained from the Bura irrigation scheme's meteorological station, which measures a wide range of weather variables at 2-h intervals. Variables processed for this analysis included rainfall, temperature (mean, high, and low), humidity, wind speed, solar radiation, and Temperature Humidity Wind (THW) index (an apparent temperature estimate based on humidity and wind speed). Daily summaries (sum for rainfall and averages for the other variables) for these data were obtained by collapsing the data by date.

In addition to the meteorological variables identified above, other variables included the sampling site—farms and villages (settlements) in the irrigated (Bura) and nonirrigated (Murukani) sites—and season and phase of irrigation described as active, none, and low. The active phase referred to the sampling period when there was intensive irrigation, often during the dry season, whereas the no-irrigation phase (none) represented the dormant stage, particularly during the wet season when rain-fed agriculture could be done. The low phase of irrigation represented periods when minimal irrigation was being done, particularly for subsistence farming.

Vector Data

Data on adult mosquitoes were cleaned and the species grouped into three clusters depending on the species. The clusters included all mosquitoes, primary vectors of RVFV (*Ae. mcintoshi* and *Ae. ochraceus*), and vectors for WNV that included *Cx. univittatus* and *Cx. pipiens*. Dates, species names, and geo-coordinates were also verified, with the geo-coordinates being formatted to decimal degrees. The number of mosquito catches per trap per day was computed by aggregating the number of mosquitoes caught by trap ID, mosquito cluster, and geo-coordinates. The numbers obtained (mosquito catches/trap/day) were log-transformed using the function $log_{10}(n+1)$, with n representing the aggregated trap catches. This variable was used as an outcome in univariable and multivariable analyses.

Descriptive Analysis

Tables were used to summarize species and numbers of adult mosquitoes caught in different sites by the irrigation phase and those trapped indoors using CDC light traps. Univariable analyses were also used to test crude correlations between each predictor variable and the outcomes for the respective mosquito cluster.

Multivariable models for each mosquito cluster were fitted using the generalized linear model with an identity link. Lagged values of meteorological variables including cumulative rainfall estimates for up to 4 wk before the date of sampling were tested. A 3-wk cumulative rainfall was found to be suitable for this analysis, as it returned the highest log-likelihood estimate.

Linearity assumption for all continuous predictors was tested by generating and fitting quadratic forms of these variables in the models. All the continuous variables except rainfall met this assumption (i.e., their quadratic terms were not significant in the models). The 3-wk cumulative rainfall was therefore categorized into the following three levels: $\leq$8.8, >8.8–31.4, and >31.4, with 8.8 and 31.4 representing second and third quartiles of the cumulative rainfall values, respectively.

Parsimonious models were constructed through the backward elimination technique where a variable that was found as being nonsignificant in the full model was removed, starting with those that were perceived as being highly insignificant. This was repeated at each stage of model building until an optimal model was realized. Wald test was used to determine the significance of a variable, and an alpha value of 0.05 was used as the cutoff value. First-order interaction terms were also created and their significance tested using the same method. Robust standard errors were generated to help fix heteroskedasticity that could have arisen from repeated sampling of sites within the study area.

Residual analyses were conducted to evaluate the models generated. Deviance residuals and fitted values were generated and scatter plots between these two outputs generated to gauge: 1) cases that were poorly predicted by the model and hence appeared as outliers in the residual analysis, and 2) whether the assumption of homoskedasticity was met—indicated by a pattern of residuals against predicted values.

Results

Outdoor Sampling

A total of 39,224 mosquitoes were sampled (35,068 in the irrigation scheme and 4,156 in Murukani (Table 1). Within the irrigation scheme, more mosquitoes (n = 27,975) were collected in the farms than in the villages (n =7,093). In contrast, in Murukani, fewer mosquitoes (n = 1,281) were collected from the farms than from the villages (n = 2,875). The key primary vectors comprising *Ae. mcintoshi* and *Ae. ochraceous*, as well as the secondary vectors, particularly *Cx. univittatus* and *Cx. pipiens* (which are also vectors of WNV), were most abundant in the irrigated farms compared with the three other sites (Table 1).

Univariable analyses suggested that mosquito densities in the farms were significantly higher than those in the villages in the irrigation scheme. These analyses further show a positive correlation between rainfall and mosquito densities. High solar radiation intensity and high wind speed have consistent effects of reducing mosquito densities for all mosquito species. In contrast, humidity was positively correlated with mosquito densities (Table 2).

Multivariable Analysis

Multivariable analysis suggest that rain, area, and humidity were significantly associated with mosquito apparent density. Irrigation intensity and rainfall are significantly and positively associated and therefore caused multicollinearity in the model (indicated by high standard errors). Rainfall was therefore kept and irrigation removed from the model. Rainfall had a similar effect across the three groups of mosquitoes identified. The effect of area varied by the mosquito group; when all mosquitoes were aggregated, the densities observed in the irrigated farms were significantly higher than those observed in the villages within the scheme or Murukani. The densities of the primary vectors of RVF and those of WNV observed in the irrigated

Table 1. Mosquitoes sampled as adults from the Bura irrigation scheme and Murukani study sites, Bura, Tana River County

Species	Sept./Oct. 2013				Nov. 2013				April 2014				Nov./Dec. 2014			
	Irrigation scheme		Murukani		Irrigation scheme		Murukani		Irrigation scheme		Murukani		Irrigation scheme		Murukani	
	Farm	Village	Farm	Village	Farm	Village	Farm	Village	Farm	Village	Farm	Village	Farm	Village	Farm	Village
Ae. aegypti	6	19	0	0	1	1	0	0	5	9	0	0	5	11	0	0
Ae. mcintoshi	1792	300	0	0	1342	195	26	50	249	143	3	1	3897	471	172	501
Ae. neomelanocon spp.	21	0	0	0	21	15	0	0	0	0	0	0	0	0	0	0
Ae. ochraceus	0	0	0	0	35	24	0	12	37	18	6	8	636	431	105	430
Ae. sudanensis	170	236	0	3	100	39	2	1	51	28	2	2	375	120	32	99
Ae. stegomyia spp.	3	17	0	0	0	6	0	0	0	0	0	0	23	1	0	1
Ae. tricholabis	258	39	0	3	235	59	0	0	31	9	3	4	495	166	127	286
Aedes spp.	62	1	0	0	22	52	17	1	5	4	0	0	6	5	0	3
Aedomyia furfurea	234	37	0	0	10	0	0	0	225	22	0	4	2250	96	49	22
Aedomyia spp.	11	5	0	0	11	4	0	0	0	0	0	0	0	0	5	0
An. funestus	272	139	6	1	11	0	0	0	347	186	6	66	615	415	42	41
An. gambiae	6	195	0	0	1	0	0	0	6	112	0	21	131	59	2	47
An. squamosus	0	0	0	0	1	0	0	0	2	0	0	0	2	21	4	10
Anopheles spp.	21	1	1	0	16	32	1	2	3	23	0	3	50	11	6	14
Cx. annulioris	0	220	0	0	4	0	0	0	495	345	0	5	341	214	33	11
Cx. bitaeniorhynchus	81	49	0	0	1	0	0	0	80	2	0	0	2	0	0	0
Cx. pipiens	107	217	7	3	34	4	0	0	76	54	7	4	7006	756	163	737
Cx. poicilipes	58	40	8	4	22	2	0	0	37	25	8	4	121	31	82	32
Cx. univittatus	401	108	6	1	343	17	0	0	41	60	6	1	409	118	10	55
Cx. vansomereni	253	38	0	0	227	220	0	0	33	2	0	0	261	23	106	13
Culex spp.	0	269	1	5	206	27	1	3	457	0	1	5	1292	271	215	353
Mn. africana	489	0	0	0	484	39	0	0	16	52	0	0	131	11	4	2
Mn. uniformis	54	16	1	0	37	0	0	0	26	13	1	0	63	15	1	0
Mansonia spp.	84	18	0	3	82	38	3	0	0	2	0	0	13	0	0	1
Total	4383	1964	30	20	3246	774	50	69	2222	1109	43	128	18124	3246	1158	2658

September–October 2013 – dry season with very minimal irrigation; November 2013 – dry season with enhanced irrigation; April 2014 – minimal rain with enhanced irrigation; November–December 2014 – heavy rainfall with no irrigation.

Table 2. Outputs of univariable models used for an initial analysis of three mosquito data sets

Variable	Levels	All mosquito species		Primary RVF vectors[a]		WNV vectors[b]	
		Frequency	β (95% CI[c])	Frequency	β (95% CI[c])	Frequency	β (95% CI[c])
Rainfall (mm)	≤8.8	62	−3.06 (−3.55 to −2.57)[e]	16	−1.44 (−2.16to −0.72)[e]	14	−2.09 (−2.91 to −1.28)[e]
	>8.8–31.4	56	−0.82 (−1.33 to −0.31)[e]	45	−0.99 (−1.47 to −0.52)[e]	25	−1.44 (−2.08 to −0.81)[e]
	>31.4	153	0.00	114	0.00	92	0.00
Irrigation intensity	High	129	0.00	96	0.00	58	0.00
	Low	62	−2.33 (−2.81 to −1.84)[e]	16	−0.53 (−1.18 to −0.12)	14	−0.82 (−1.56 to −0.09)[e]
	None	80	1.34 (0.89 to 1.78)[e]	63	1.58 (1.19 to 1.97)[e]	59	1.90 (1.45 to 2.36)[e]
Site	Irrigation farm	106	0.00	78	0.00	62	0.00
	Murukani farm	17	−1.27 (−2.30 to −0.23)[e]	9	−0.20 (−1.17 to –0.76)	5	0.02 (−1.43 to –1.47)
	Murukani village	22	−0.89 (−1.81 to –0.04)	15	−0.12 (−0.89 to –0.65)	10	0.36 (−0.70 to –1.43)
	Irrigation village	122	−1.14 (−1.67 to −0.62)[e]	70	−0.99 (−1.45 to −0.54)[e]	52	−0.86 (−1.45 to −0.27)[e]
Solar radiation			−0.01 (−0.02 to −0.01)[e]		−0.01 (−0.012 to −0.006)[e]		−0.01 (−0.014 to −0.006)[e]
THW index[d]			0.49 (0.37 to 0.61)[e]		0.04 (−0.09 to –0.17)		0.03 (−0.14 to –0.20)
Wind speed			−0.31 (−0.38 to −0.24)[e]		−0.26 (−0.33 to −0.19)[e]		−0.30 (−0.37 to −0.23)[e]
Humidity			0.09 (0.06 to 0.12)[e]		0.08 (0.06 to 0.11)[e]		0.11 (0.08 to 0.14)[e]

[a] *Aedes mcintoshi* and *Ae. ochraceus*

[b] *Culex univittatus* and *Cx. pipiens*

[c] Confidence interval

[d] THW index – apparent temperature estimated by adjusting temperature based on humidity and wind speed

[e] Significant at alpha = 0.05.

Table 3. Outputs of multivariable models fitted to three types of mosquito data sets—all mosquitoes, primary vectors of RVF, and vectors of WNV

Variable	Levels	All mosquito species				Primary RVF vectors[a]				WNV vectors[b]			
		β	95% CI[c]		$P > \|Z\|$	β	95% CI[c]		$P > \|Z\|$	β	95% CI[c]		$P > \|Z\|$
			Lower	Upper			Lower	Upper			Lower	Upper	
Rain[d]	≤8.8	−2.85	−3.34	−2.37	0.00	−1.41	−2.05	−0.78	0.00	−1.52	−2.01	−1.03	0.00
	>8.8–31.4	−0.41	−1.08	0.26	0.23	−0.43	−0.99	0.12	0.13	−0.56	−1.17	−0.04	0.07
	>31.4	0.00				0.00				0.00			
Area	Murukani farm	−1.26	−2.02	−0.50	0.00	−0.43	−1.02	0.16	0.15	−0.48	−1.56	0.60	0.39
	Murukani village	−0.75	−1.43	−0.08	0.03	−0.34	−0.93	0.23	0.24	−0.21	−1.25	0.83	0.69
	Irrigation village	−1.17	−1.59	−0.76	0.00	−1.14	−1.58	−0.72	0.00	−0.78	−1.22	−0.36	0.00
	Irrigation farm	0.00				0.00				0.00			
Humidity		0.03	−0.01	0.07	0.17	0.05	0.02	0.09	0.00	0.08	0.04	0.11	0.00
Constant		2.40	−0.76	5.55	0.14	−0.31	−3.02	2.40	0.82	−2.24	−5.13	0.65	0.13
Log pseudolikelihood		−496.08				−278.97				−222.317			

[a]*Aedes mcintoshi* and *Ae. ochraceus*.
[b]*Culex univittatus* and *Cx. pipiens*.
[c]Confidence interval.
[d]Cumulative rainfall categorized into three classes.

Table 4. Mosquito species collected indoors in the irrigation scheme and Murukani

Species	Oct./Nov. 2013		April 2014		Nov./Dec. 2014	
	Irrigation scheme Indoor	Murukani Indoor	Irrigation scheme Indoor	Murukani Indoor	Irrigation scheme Indoor	Murukani Indoor
Ae. mcintoshi	0	0	2	0	12	9
Ae. ochraceus	0	0	0	0	7	15
Ae. sudanensis	1	0	6	0	3	3
Ae. tricholabis	0	0	1	0	1	13
Culex spp.	12	0	46	3	18	32
Cx. annulioris	0	0	125	0	12	9
Cx. ethiopicus	0	0	1	0	0	0
Cx. poicilipes	0	0	0	0	0	23
Cx. univittatus	0	0	20	0	12	5
Cx. vansomereni	1	0	3	2	14	2
Mn. africana	0	0	13	0	3	1
Mn. uniformis	0	0	4	0	0	0
Total	14	0	221	5	82	112

farms were however not significantly different from those observed in Murukani.

However, solar radiation and wind speed are not significant, whereas THW was not included because it was closely correlated with humidity, which was kept in the models (Table 3).

Indoor Sampling

Although *Culex* spp. are routinely sampled indoors, of interest was the capture of floodwater mosquitoes and *Mansonia africana* and *Mn. uniformis* indoors both in the irrigation scheme and Murukani (Table 4).

Larval Sampling

Larval sampling data show that most of the larvae were collected in November–December 2014 during the rainy season. BFs, UDs, and rain pools (RPs) supported the breeding of RVFV primary vectors, particularly *Ae. mcintoshi*. Only one *Ae. ochraceus* larva was sampled in a RP during November–December 2014 collection. In general, the secondary vectors of RVFV, including *Cx. univittatus*, *Cx. pipiens*, and *Culex poicilipes* Theobald, used all the irrigation canals as breeding habitats (Table 5).

Virus Isolation and Identification

Of the 2,156 mosquito pools that were inoculated in cell culture, eight NDUV isolates were obtained from mosquito species that were collected in November 2014 in the farms in the irrigation scheme. One isolate each was obtained from pools of male *Aedes sudanensis* Theobald, *Culex bitaeniorhynchus* Giles, and *Culex vansomereni* Edwards; two isolates were obtained from two pools of *Cx. pipiens*, whereas three were obtained from two pools of male and one pool of female *Aedomyia furfurea* (Table 6).

The identity of these isolates was confirmed by sequencing the diagnostic fragment using the primer pair, VIR2052F and VIR2052R, which targets a 150 base pair region of the nsP4 gene (Eshoo et al. 2007), a region that is highly conserved across all alphaviruses. As expected, the sequences of the study isolates showed a very close relatedness to each other forming a distinct clade and were also most closeley related to the members of the

Table 5. Mosquitoes sampled as larvae from diverse irrigation system water bodies during irrigation period and in the short rains of November–December 2014

Species	Sept./Oct. 2013						April 2014				Nov./Dec. 2014						
	RP	BF	UF	UD	RG	Total	VD	UD	Ufr	Total	BF	FC	UD	RP	UF	Total	MRP
Ae. aegypti	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Ae. mcintoshi	2	105	0	55	0	162	0	46	0	46	0	0	0	16	0	16	0
Ae. ochraceus	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Aedes spp.	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0
An. funestus	0	0	0	0	0	0	0	0	36	36	3	50	0	9	9	71	0
An. gambiae	6	0	4	0	0	10	22	4	24	50	0	6	0	253	5	264	2
An. squamosus	0	0	0	0	0	0	5	1	1	7	0	0	0	0	0	0	0
Coquillettidia spp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Culex spp.	3	0	0	3	0	6	0	2	0	2	0	0	1	50	1	52	0
Cx. annulioris	0	0	0	0	0	0	23	18	1	42	0	0	0	0	0	0	0
Cx. bitaeniorhynchus	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Cx. pipiens	0	2	8	0	5	15	122	4	15	141	0	129	31	95	6	261	0
Cx. poicilipes	0	0	0	0	1	1	0	0	0	0	0	2	7	1	1	11	0
Cx. tigripes	0	0	0	0	0	0	0	0	7	7	0	0	5	0	0	0	0
Cx. univittatus	15	116	5	99	1	236	0	23	18	41	33	53	32	0	150	268	0
Cx. vansomereni	0	0	8	0	0	8	0	0	0	0	9	0	0	35	25	69	0
Uranotaenia spp.	0	0	0	9	0	9	0	0	0	0	0	0	0	0	0	0	0
Hodgesia spp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Total	26	225	25	166	7	449	172	102	102	376	46	240	77	460	197	1,014	2

BF, block feeder; FC, Feeder canal; MRP, Murukani rain pool; RG, Road gulley; RP, rain pool; UD, unit drain; UF, unit feeder; Ufr, unit furrow; and VD, village drain.

Table 6. Mosquito species from Bura irrigation scheme from which NDUV was isolated

Species	Sex	Positive pool	Total pools assayed
Ao. furfurea	M	2	33
Ao. furfurea	F	1	206
Ae. sudanensis	M	1	16
Cx. vansomereni	F	1	95
Cx. pipiens	F	2	460
Cx. bitaeniorhynchus	F	1	12
Total		8	822

clade containing sequences obtained from GenBank, including isolates from Uganda (Masembe et al. 2012) and Kenya (Fig. 3).

Discussion

The government of Kenya (GOK) is expanding agricultural production by using the vast ASALs in the northern half of the country to attain food security and improved economic growth for local communities, through irrigation farming (GOK 2012). However, no initial impact assessment is being conducted to evaluate potential impacts of irrigation on the environment, human and animal health. It is anticipated that introduction of massive irrigation in ASALs, which are characterized by high temperatures, would significantly change the environmental conditions and vector-pathogen-host interactions.

This study has showed an overall increase in the abundance of *Ae. mcintoshi*, *Ae. ochraceus*, *Aedes tricholabis* Edwards and *Cx. pipiens* in the irrigation scheme compared with that in Murukani, which acted as the control site. These mosquito species are important vectors of arboviral diseases such as RVF and WNV. At both sites, high numbers of mosquitoes were collected during the November–December 2014 wet season. This is because of the availability of many natural breeding habitats and the artificial ones being flooded for longer periods, allowing for proliferation of large mosquito populations that also survive for longer because of high levels of humidity. In Murukani, an unirrigated area, which is situated only 15 km from the periphery of the irrigation scheme, there was a higher proportion of primary vectors sampled during active irrigation compared with when there was no active irrigation in the irrigation scheme.

Aedes mcintoshi was found to breed better during the dry season with minimal irrigation compared with when irrigation was enhanced, suggesting that the water level in the irrigation canals could probably be an important factor influencing the breeding of *Ae. mcintoshi*. A single *Ae. ochraceus* larva was sampled during heavy rainfall with no irrigation. *Aedes mcintoshi*, whose immature stages are normally associated with natural low-lying dambos, was the most predominant adult floodwater *Aedes* species sampled during times when the farms were under irrigation. This species was found breeding in manmade irrigation canals, suggesting that it has adapted better to breeding in altered habitats, following cultivation of many dambo habitats. Dambos are characteristically found in semiarid and arid RVF hotspot areas and in endemic zones; floodwater *Aedes* mosquitoes in these areas lay drought-resistant eggs in the dambo habitats, and the adults swarm in nearby vegetation following emergence (Linthicum et al. 1985, Logan et al. 1991).

The consequences of adaptation of floodwater *Aedes* mosquitoes to use irrigation canals for breeding for disease transmission needs to be studied further by evaluating the vectorial capacity of the population in the irrigation scheme and other possible inherent genetic fitness changes that may occur. However, it was interesting that the other floodwater mosquitoes including *Ae. ochraceous* and *Ae. sudanensis* that are known to play a role in RVF transmission, although sampled as adults in CDC light traps, were missing in the drainage system of the irrigation scheme. Future studies should also seek to determine where they breed in the irrigation scheme.

Equally interesting was the sampling of floodwater mosquitoes indoors. Although it is reasonably arguable that these mosquitoes

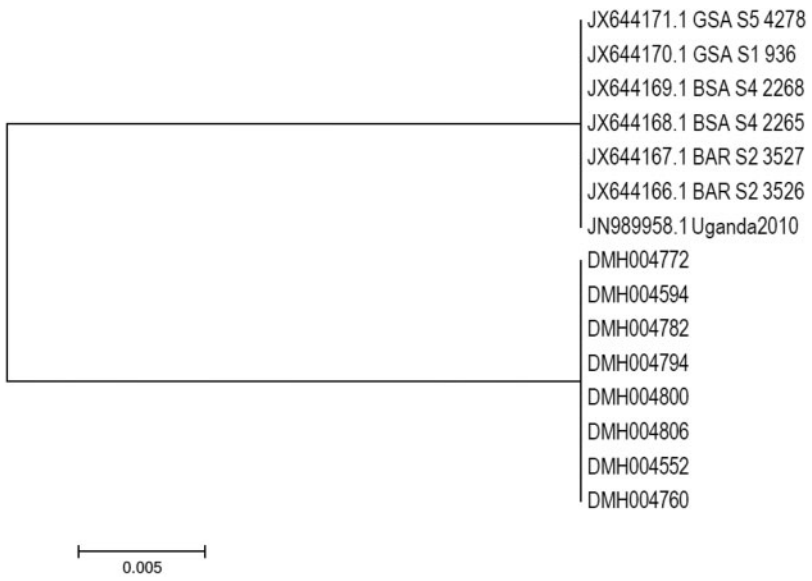

Fig. 3. Evolutionary relationships of taxa. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei 1987). The evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences. Eight sequences belong to the study. The seven sequences are from GenBank. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

were attracted indoors by the CO_2 from the CDC light traps, this is the first observation of house entering by these species, calling for further studies to assess their resting behavior, as this has implications on disease epidemiology. However, although a rare occurrence, the *Mansonia* species has been sampled indoors by CDC light traps in The Gambia (Kirby et al. 2008). Although this study has shown the potential for RVF transmission to occur when the farms are undergoing irrigation, it is clear that vector densities are much higher during times of elevated rainfall because of an increase in natural breeding habitats, as well as the observed high humidity that favors mosquito survival. It is, therefore, during this period when the risk of disease transmission is elevated, thus calling for close monitoring through enhanced surveillance. This is confirmed by the isolation of the NDUV from multiple pools of mosquitoes collected during the November 2014 period during enhanced rainfall and mosquito densities. NDUV has previously been isolated from several mosquito species including *Ae. mcintoshi*, *Ae. ochraceus*, *Ae. tricholabis*, *Ae. sudanensis*, *Cx. pipiens*, and *Mn. uniformis* (Crabtree et al. 2009, Ochieng et al. 2013, Lutomiah et al. 2014a, Lutomiah et al. 2014b). Therefore, isolation of this virus from *Ao. furfurea*, *Cx. vansomereni*, and *Cx. bitaeniorhynchus* widens the range of potential vectors. Its isolation from male *Ae. sudanensis* and *Ao. furfurea* also supports existing evidence that it is maintained in nature by transovarial transmission (Lutomiah et al. 2014b). NDUV was first isolated in South Africa in 1959, and antibodies to the virus have been detected in humans (Kokernot et al. 1961). Therefore, the circulation of this virus at the irrigation scheme calls for determination of its impact on human health.

Based on the observed abundance of vectors, WNV, SINV, Usutu virus (USUV), BUNV, and Ngari (NRI) are also likely to be transmitted in this area. WNV and SINV are transmitted principally by *Cx. pipiens* and *Cx. univittatus* (Taylor et al. 1955, Jupp 2001, Lutomiah et al. 2011), which were collected in abundance in farms. WNV is the most important causative agent of viral encephalitis worldwide (Chancey et al. 2015) and is associated with fever in 20% of the victims and severe symptoms in <1% of the cases (Hayes et al. 2005). SINV, which causes febrile illnesses characterized by arthralgia in humans, was first isolated in Egypt (Taylor et al. 1955) and has long been associated with epidemics (McIntosh et al. 1976, Skogh and Espmark 1982, Storm et al. 2014, Bergqvist et al. 2015). In Kenya, SINV has also been isolated from mosquitoes collected in the neighboring Garissa County (Ochieng et al. 2013). *Culex pipiens* and *Cx. univittatus* are ornithophillic, a situation that portends risk of introduction of exotic arboviruses through migratory birds. In addition to *Cx. pipiens* (Busquets et al. 2008, Calzolari et al. 2010, Tamba et al. 2011, Jöst et al. 2011), *Mn. africana* has also been identified as a vector of USUV (Cornet et al. 1979, Hubálek, 1994). Like WNV and SINV, USUV is associated with fever and rash (Adam and Digoutte 2014). BUNV has been isolated from *Ae. mcintoshi* and *Ae. ochraceus* (Crabtree et al. 2009, Ochieng et al. 2013) and is associated with febrile illness and infrequent central nervous system involvement (Gonzalez and Georges 1988), whereas NGV has been associated with hemorrhagic disease (Bowen et al. 2001). Only few *Ae. aegypti*, a principal vector of dengue, chikungunya, and yellow fever, were collected, probably because of the methods used that were inappropriate for this species.

It is also possible for irrigation to affect nearby environs, although the extent of the impact may not be easily appreciated. Therefore, future studies should not only focus on the effects of irrigation on the immediate vicinity but also the extent beyond the schemes and what this portends for disease epidemiology.

In conclusion, irrigation particularly in RVF endemic areas has a favorable influence on primary and secondary vectors of RVF. The mosquito adaptation to changing environmental conditions and habitats created through land use change must be monitored to forestall emergence of increased vectors populations and potential for emergence of new/elevated disease burden. In addition, vectors of other arboviruses like WNV, SINV, and orthobunyaviruses, among others, seem to favor irrigated farms, creating an elevated risk for these viruses in the modified ecosystem and putting the farming populations at risk of febrile illnesses. It is possible to monitor preferred breeding sites in the irrigation scheme so that they can be targeted for control.

As implementation of irrigation projects is developed, continued surveillance is recommended to monitor the changing trends in mosquito vector adaptation and increased densities and, therefore, detect disease transmission at an early stage in order to prevent major outbreaks. UFs and UDs were the most productive for the larvae of diverse mosquito species, including those known to transmit arboviral disease. This suggests that focus should be on monitoring these water bodies to control the vectors.

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