

## Rift Valley Fever Virus Epidemic in Kenya, 2006/2007: The Entomologic Investigations

Rosemary Sang,\* Elizabeth Kioko, Joel Lutomiah, Marion Warigia, Caroline Ochieng, Monica O'Guinn, John S. Lee, Hellen Koka, Marvin Godsey, David Hoel, Hanafi Hanafi, Barry Miller, David Schnabel, Robert F. Breiman, and Jason Richardson

Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya; United States Army Medical Research Unit (USAMRU), Village Market, Nairobi, Kenya; USAMRIID, Frederick, Maryland; Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, Foothills Campus, Fort Collins, Colorado; Vector Biology Research Program, United States Naval Medical Research Unit No. 3, Cairo, Egypt; International Emerging Infections Program, CDC-Kenya

**Abstract.** In December 2006, Rift Valley fever (RVF) was diagnosed in humans in Garissa Hospital, Kenya and an outbreak reported affecting 11 districts. Entomologic surveillance was performed in four districts to determine the epidemic/epizootic vectors of RVF virus (RVFV). Approximately 297,000 mosquitoes were collected, 164,626 identified to species, 72,058 sorted into 3,003 pools and tested for RVFV by reverse transcription-polymerase chain reaction. Seventy-seven pools representing 10 species tested positive for RVFV, including *Aedes mcintoshi/circumluteolus* (26 pools), *Aedes ochraceus* (23 pools), *Mansonia uniformis* (15 pools); *Culex poecilipes*, *Culex bitaeniorhynchus* (3 pools each); *Anopheles squamosus*, *Mansonia africana* (2 pools each); *Culex quinquefasciatus*, *Culex univittatus*, *Aedes pempaensis* (1 pool each). Positive *Ae. pempaensis*, *Cx. univittatus*, and *Cx. bitaeniorhynchus* was a first time observation. Species composition, densities, and infection varied among districts supporting hypothesis that different mosquito species serve as epizootic/epidemic vectors of RVFV in diverse ecologies, creating a complex epidemiologic pattern in East Africa.

### INTRODUCTION

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis, which periodically causes disease outbreaks in humans and livestock and is known to have been endemic in sub-Saharan Africa since 1912.<sup>1</sup> The zoonosis is caused by RVF virus (RVFV), a member of the *Phlebovirus* genus of the *Bunyaviridae* family. The virus was first isolated in 1930 from a sheep during an epizootic at a farm by Lake Naivasha in the Rift Valley Province of Kenya.<sup>2</sup> Transmission to humans is thought to occur through direct contact with tissues and blood of infected animals, and by the bite from an infected mosquito.

Before the 1977 outbreak in Egypt, RVF was considered a disease of livestock with little impact on humans.<sup>3</sup> Since then, periodic outbreaks associated with widespread involvement of livestock and acute febrile illness with hemorrhagic syndrome in humans have been reported in many African countries, as well as Saudi Arabia and Yemen.<sup>4–11</sup> The last major RVF outbreak occurred in eastern Africa in 1997–1998 and affected Tanzania, Somalia, and Kenya, where an estimated 27,500 human cases, and  $\geq 170$  deaths, were reported to have occurred.<sup>11</sup> Although some of these cases have since been attributed to Ngari virus.<sup>12</sup> Entomologic investigations during that outbreak were minimal and many questions remained unanswered regarding the epidemic/epizootic vectors of the virus in Kenya and elsewhere.

Outbreaks of RVF have previously been associated with unusually heavy rainfall, the extensive flooding of low lying grassland depressions called *dambos*, and the mass emergence of flood water *Aedes* mosquitoes.<sup>13</sup> Rift Valley fever virus has been isolated from more than 40 species of mosquitoes from eight genera,<sup>6,14</sup> and laboratory studies indicate that numerous species of mosquitoes and sand flies are susceptible to oral infection, some of which are able to transmit RVFV by bite.<sup>6,15–19</sup>

Evidence suggests that in certain *Aedes* species of the subgenera *Neomelanicion* and *Aedimorphus*, the female mosquitoes may transmit RVFV vertically to their eggs.<sup>20</sup> When these mosquitoes lay their eggs in flooded areas (including *dambos*), transovarially infected adults may emerge and transmit RVFV to nearby domestic animals, including sheep, goats, cattle, and camels. High viremias in these animals may then lead to the infection of secondary arthropod vector species including various *Culex* species, followed by the spread of this virus to additional animals and humans.<sup>13,21,22</sup>

Given the recent global spread of emerging pathogens such as severe acute respiratory syndrome (SARS), West Nile, and Chikungunya viruses, it is critical to understand the mechanisms underlying their maintenance and transmission in nature. Before this outbreak, climatic modeling studies predicted heavy rains in Kenya in October 2006 and that the heavy rains would potentially trigger an RVF outbreak.<sup>23</sup> The advanced warning of increased RVF risk in East Africa enabled rapid emergency response planning that resulted in a comprehensive field investigation during the peak of virus transmission and spread of the disease.

**The outbreak.** In mid-November of 2006, heavy and persistent rainfall led to flooding throughout much of northeastern Kenya and Somalia. Satellite-based monitoring indicated significant Normalized Difference Vegetation Index (NDVI) anomalies, and increased likelihood of RVF transmission.<sup>24</sup> In mid-December, mosquito surveillance was initiated in Garissa District of the North Eastern Province of Kenya the focus of the 1997–1998 outbreak. Concurrently, the Kenyan Ministry of Health received reports of unexplained fatalities associated with fever and generalized bleeding in Garissa.<sup>25</sup> Rift Valley fever virus RNA or immunoglobulin M (IgM) antibodies against RVF virus were detected in blood specimens from 10 of the initial 19 patients tested (Nguku P, unpublished data). Reports of livestock deaths and unexplained animal abortions in the area provided further evidence of an RVF outbreak. The entomologic investigation team from the Kenya Medical Research Institute (KEMRI) and the U.S. Army Medical Research Unit-Kenya was expanded to include members from the Centers for Disease Control and Prevention Division of

\*Address correspondence to Rosemary Sang, Arbovirology/Hemorrhagic Fevers Laboratory, Centre for Virus Research, Kenya Medical Research Institute (KEMRI), P.O. Box 54628, Nairobi, Kenya. E-mail: rsang@kemri.org

Vector-Borne Infectious Diseases (CDC-DVBID), Fort Collins, CO and the Navy Medical Research Unit No. 3 Cairo, Egypt. The team focused on evaluating the entomologic parameters that contributed to the epidemic; specifically, to determine the mosquito species composition, the abundance of known and potential mosquito vector species, and to conduct virus testing to identify the species most likely involved in virus transmission in the affected areas. Our goal was to learn more about the vectors involved in virus maintenance and transmission during outbreaks, information that could be used to forecast risk and facilitate improvement of prevention and response tools for use in preventing or controlling future outbreaks.

## MATERIALS AND METHODS

**Study sites.** Studies were undertaken at four ecologically distinct communities in eastern, central, and western Kenya where RVFV activity was detected in humans or livestock (Figure 1A). The first cases of RVF were reported from the Garissa district, which is located in the North Eastern Province of Kenya bordered by the Tana River to the west and Somalia to the east (Figure 1B). The district is characterized as an arid area with Somali *Acacia-Commiphora* bushlands and thickets. Rainfall is sporadic, averaging approximately 200–500 mm per year, with occasional torrential storms causing extensive flooding. The average temperatures range from 20 to 38°C and the altitude varies from 70 to 400 m above sea level. The soil is generally sandy with scattered areas of dark clay that tend to retain water after the rains and serve as watering holes and grazing land for livestock and wild animals. The sparse population (~7 people/km<sup>2</sup>)<sup>26</sup> of the district is found concentrated around the water sources and also around small market centers. The people are largely nomadic, moving between districts with their large herds in search of water and pasture land. The collection of arthropods was conducted between December 15, 2006 and March 3, 2007 at 20 sites within an estimated 100 km radius of the provincial capital of Garissa (Figure 1B).

The city of Kilifi, located in Kilifi district, Coast Province, is 318 km south of Garissa (Figure 1C). The district contains a moderately dense population (~114 people/km<sup>2</sup>)<sup>26</sup> and the vegetation is characterized by a mix of East African mangroves and northern Zanzibar-Inhambane coastal forests that are comprised of dense woods, swamps, dry scrub, and commercial plantations.<sup>27,28</sup> Annual rainfall for the district ranges from 750 to 1,200 mm, while the average temperature maximum is 30°C.<sup>29</sup> The soils are fertile and subsistence farming of corn, coconut, goats, chicken, and cattle is widespread. The collection of vectors was conducted between 12 January and 1 February 2007 at 10 homesteads that were associated with confirmed or suspected RVF cases or at sites nearby (Figure 1C). Nine of the homesteads were within a 120 km transect of each other and no further than 6 km from the coast. The remaining homestead was inland, about 30 km west of the other homesteads.

Kirinyaga is located in the highland region of the Central Province of Kenya, approximately 100 km northeast of Nairobi, on the southern slope of Mount Kenya (Figure 1A). At 1,113 to 1,623 m above sea level, the collection sites were typified by East African montane forests and northern *Acacia-Commiphora* bushlands and thickets. The mean daily temperatures range from 16 to 26°C, with an annual rainfall

of approximately 950 mm.<sup>30</sup> This densely populated district is home to more than 500,000 people (> 300 people/km<sup>2</sup>).<sup>26</sup> The primary occupation is agriculture, including subsistence farming of corn, beans, and potatoes; cash crop farming of tea and rice, and the raising of both exotic and indigenous livestock. Mosquitoes were collected from four locations around the district from 6 to 8 February 2007 (Figure 1A).

Baringo District is located in the Rift Valley Province of Kenya, 250 km northwest of Nairobi. The low lying arid part of Baringo consists of northern *Acacia-Commiphora* bushlands and thickets and has experienced severe land degradation caused by uncontrolled grazing. Harsh physical and climatic conditions have led to a sparsely populated district (average of 31 people/km<sup>2</sup>)<sup>26</sup> where the local inhabitants, classified as agro pastoralists, subsist mainly on limited crop production and livestock rearing. The collection of arthropods was conducted at three sites near Lake Baringo (elevation ~980 m) where the annual rainfall ranges from 300 to 700 mm, and the daily temperature varies between 16 and 42°C.<sup>31</sup> Trapping was conducted from 13 to 15 February 2007 around flooded marshland ~2.2 km west of Lake Baringo and along the Molo river, south of the lake (Figure 1D).

**Vector collection and identification.** Arthropods were collected from areas where confirmed or suspected RVF cases were previously reported. Mosquitoes and sand flies were sampled using CO<sub>2</sub>-baited CDC light traps placed outdoors approximately 1 hour before sunset and collected 1–3 hours after sunrise the next day. Mosquitoes were typically anesthetized using triethylamine,<sup>32</sup> identified to species, and pooled (≤ 25 mosquitoes per pool) by species, sex, collection date, and site and frozen at –80°C for later testing. When large numbers of mosquitoes were trapped, they were killed by freezing, immediately stored in 15 mL centrifuge tubes, and transported in a liquid nitrogen shipper to the laboratory where they were identified on ice and pooled as indicated for testing. During mosquito identification, all specimens with blood in their abdomens (blood fed) were sorted out and preserved singly in vials for subsequent blood meal analysis in a separate study.

In selected locations, ticks were collected from infested animals, placed in 15 mL centrifuge tubes, stored in a liquid nitrogen shipper, and transported to the laboratory for identification and testing. Mosquitoes and ticks were identified to species using various taxonomic keys.<sup>33–38</sup>

Representative pinned specimens of the important species were sent to Walter Reed Biosystematics unit in Silver Spring, MD and to the taxonomy unit of the Arthropod Borne and Infectious Diseases laboratory, CDC, Fort Collins, CO, for verification of the identification.

**Parity determination.** Parity was determined for a limited number of mosquitoes because of concerns that some mosquito collections might be composed largely of newly emerged, unfed females that would likely be uninfected. A sub-sample of pools of probable vector species, and of species with a high relative abundance was evaluated to estimate parous rates. Females from selected pools were placed on a microscope slide and their ovaries dissected into a drop of distilled water.<sup>39</sup> After drying, the ovaries were graded as parous (evidence of previous blood feeding and egg production) or nulliparous (no evidence of egg production). Forceps and other instruments used during the dissections were dipped in 70% ethanol and flame sterilized after dissections of a pool to eliminate transfer

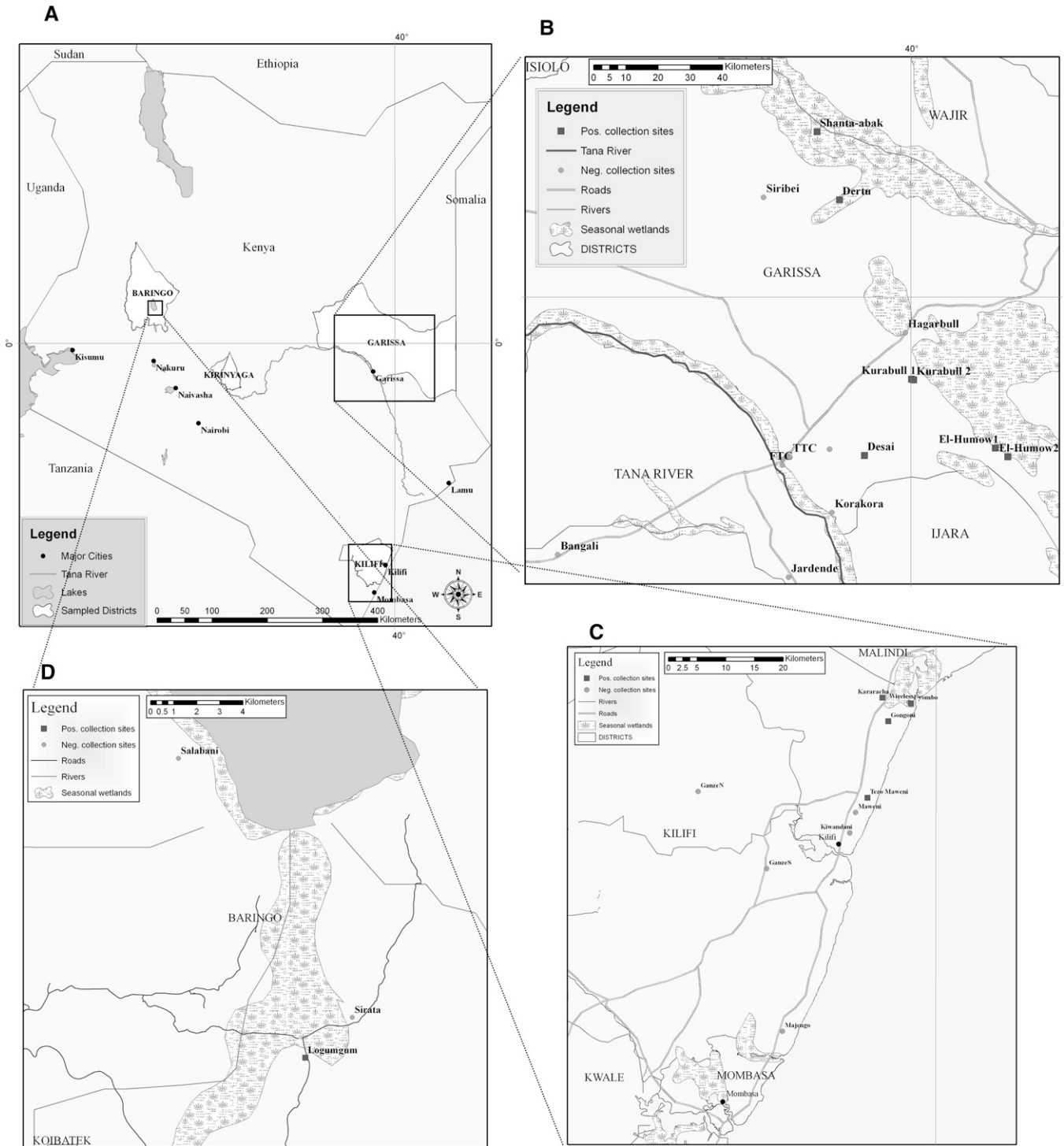


FIGURE 1. (A) Entomological investigation study sites for the Kenyan Rift Valley fever (RVF) outbreak, 2006–2007. Positive collection site = collection sites with human RVF case reports. Negative collection sites = collections sites without human RVF case reports. (B) Detail of collection sites in and around Garissa District. (C) Detail of collection sites in Kilifi District. (D) Detail of collection sites in Baringo District.

of virus between pools. Following the dissections, the remaining mosquito bodies were re-pooled for virus testing. Differences in parous rates were analyzed by  $\chi^2$  using the Vassar Stats software (<http://faculty.vassar.edu/lowry/VassarStats.html>).

**Arthropod processing.** Mosquito pools ( $\leq 25$  mosquitoes per pool) were homogenized in 1.5 mL microcentrifuge tubes containing one 4.5 mm copper bead and 0.75 mL minimum

essential medium (MEM) supplemented with 2% fetal calf serum, 2 mM glutamine, antibiotic mixture (fungizone, 100 U/mL penicillin, and 100 U/mL streptomycin) and vortexed for 2 minutes, or until all the mosquitoes were homogenized. The homogenates were clarified by centrifugation at 4°C and 12,000 rpm for 10 minutes, and the resulting supernatants were immediately processed further or stored at  $-80^{\circ}\text{C}$ . Trituration

and manipulation of arthropod homogenates was carried out in a biosafety level 3 (BSL-3) containment laboratory. Ticks were pooled in groups of five to 10, depending on their size and were homogenized in MEM using pre-chilled mortars and pestles, and the resulting homogenates were clarified as described previously.

**Viral RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR).** Rift Valley fever virus has the potential to cause serious to fatal hemorrhagic disease in humans and to cause laboratory infections through aerosolized infectious material. To minimize the potential of laboratory exposure at the KEMRI laboratory, samples were inactivated by the addition of Trizol-LS (Invitrogen Inc., Carlsbad, CA) reagent at biosafety level 3 (BSL3) before RNA extraction and RT-PCR screening at biosafety level 2 (BSL2).

Viral RNA was extracted from mosquito homogenates using Trizol-LS reagent according to the manufacturer's instructions and as modified by O'Guinn and colleagues.<sup>40</sup> Briefly, 0.25 mL of mosquito homogenate was combined with 0.75 mL Trizol-LS reagent and processed to yield purified total RNA. The final RNA pellet was resuspended in 12 µL of nuclease-free water and then stored on ice or frozen at -80°C.

The RNA was converted into complementary DNA (cDNA) as previously described<sup>40</sup> with minor modifications. Briefly, 10 µL of RNA was combined with 2 µL of random hexamer (100 nmol) in a dome-topped PCR tube and placed in a thermal cycler programmed as follows: 70°C for 10 minutes to denature the sample then 4°C for 5 minutes. Next, 4 µL of 5X buffer, 2 µL of 0.1 M DTT, 0 µL or 0.25 µL of RNase Inhibitor (40U/µL), 1 µL of Superscript II or III reverse transcriptase (Invitrogen, Inc.), and 1 µL of 10 mM dNTP (Invitrogen, Inc.) was added, the sample spun down, and then heated at 25°C for 15 minutes, 42°C for 50 minutes, 70°C for 15 minutes, then held at 4°C or stored at -20°C.

The PCR amplification of targeted viral sequences was accomplished either as described by O'Guinn and colleagues<sup>40</sup> or as follows. Each 25 µL reaction contained, 2.5 µL of 10X PCR buffer, 1 µL forward primer (50 pmole), 1 µL of reverse primer (50 pmole), 0.5 µL of 10 mM dNTP, 18.7 µL of water, 0.3 µL of Taq polymerase, and 2 µL of cDNA. Amplification conditions were 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 45 seconds, and then 72°C for 7 minutes, followed by a final hold at 4°C. Positive control cDNA and a no template negative control were also included in each set of PCR reactions. Two sets of primers targeting either a 735 or a 551 base pair fragment of the M segment of RVFV were used during the PCR testing (Table 1).

TABLE 1

Rift Valley fever virus (RVFV)-specific primers used for reverse transcription-polymerase chain reaction (RT-PCR) screening of vector samples\*

Name	Primer sequence	Target size
RVF 1 FWD	Forward: 5'-GAC TAC CAG TCA GCT CATT ACC-3'	551 bp
RVF 2 REV	Reverse: 5'-TGT GAA CAA TAG GCA TTG G-3'	
RVFFOR1	Forward: 5'-GTC TTG CTT GAA AAG GGA AAA-3'	735 bp
RVFREVE	Reverse: 5'-CCT GAC CCA TTA GCA TG-3'	

\*Ibrahim and others, 1997.

Electrophoresis of the PCR products was conducted using either 2% pre-cast E-gels (Invitrogen, Inc.) by using 1% agarose gels and Tris-acetate EDTA buffer containing ethidium bromide. The PCR product bands were visualized using a UV transilluminator and recorded using a Polaroid camera (Polaroid, Waltham, MA) and 667 Polaroid film.

Laboratory sequencing of the PCR amplification product from a subset of processed mosquito pools that tested positive for RVFV was conducted as previously described,<sup>40</sup> briefly as follows. Purification of the PCR product was done using QIAquick PCR purification kit (Qiagen, Valencia, CA) following manufacturer's instructions. Sequencing was accomplished using ABI PRISM Dye terminator cycle sequencing kits (according to the manufacturer's directions) and using the ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). Sequences were compared using the MegaAlign program (Lasergene analysis software, DNASTAR, Inc., Madison, WI).

The pooled infection rate (IR) program was used to estimate infection rates and 95% confidence intervals (CI) for species from which RVFV was identified (PooledInfRate, Centers for Disease Control and Prevention, Fort Collins, CO: <http://www.cdc.gov/ncidod/dvbid/westnile/software.htm>).

**Virus isolation.** Aliquots of RVFV RT-PCR negative mosquito pools were shipped to CDC, Fort Collins where they were tested by virus isolation. Clarified mosquito pool homogenates were tested by plaque assay in Vero (African green monkey kidney) cells as previously described using 50 µL of each mosquito pool supernatant.<sup>22</sup> The second overlay was applied 4 days post infection and plates observed for viral plaques through 11 days post infection. Plate wells in which plaques were observed were harvested by removing the agarose overlay, and resuspending the cells in 1 mL Dulbecco's minimal essential medium (DMEM) supplemented with 15% fetal bovine serum (FBS; Invitrogen Inc.). Viruses were amplified by infecting T-25 flasks of Vero cells with 25 µL of each plaque isolate in DMEM with 2% FBS. Flasks were observed and supernatants were harvested when cytopathic effects were evident. Virus isolates were identified by RT-PCR and sequencing.

## RESULTS

**Entomological collections.** More than 297,000 mosquitoes were collected, and 164,626 were identified as belonging to 36 species in nine genera. A total of 72,058 of these were sorted into 3,003 pools and tested for RVFV (Table 2). Mosquito densities differed dramatically among the ecologically distinct districts where the mosquitoes were collected. In Garissa and Baringo, peak abundance exceeded 5,000 mosquitoes per trap per night (Table 3). In contrast, mosquito counts in Kilifi and Kirinyaga never exceed 100 mosquitoes per trap-night. Species diversity also differed greatly among the different districts (Table 2). In Garissa, 72.3% of the mosquitoes identified were floodwater *Aedes* species in contrast to the predominance of *Mansonia* spp. (87.0%) in Baringo. Notably, *Culex poicilipes*, an important RVFV vector in West Africa, was abundant in Garissa (10.0%) and Kilifi (38.6%), but scarce in Baringo (1.1%) and Kirinyaga (0.2%) (Table 2). In Kilifi, the most abundant species were *Cx. poicilipes* and *Aedes pemaensis* (23.2%), whereas *Culex quinquefasciatus* (29.5%), *Culex annulioris* (15.3%), and a large proportion

TABLE 2  
Summary of mosquito collection data and Rift Valley fever virus (RVFV) reverse transcription-polymerase chain reaction (RT-PCR) results from four districts of Kenya, December 2006–June 2007

Taxa*	Baringo				Garissa				Kilifi				Kisumu				Total	
	Number (%)†	Pools‡	+ve§	+ve¶	Number (%)	Pools	+ve	+ve	Number (%)	Pools	+ve	+ve	Number (%)	Pools	+ve	Number	Pools	
																		+
<i>Ae. Aedimorphus</i> spp.	0	–	–	–	7 (0.0%)	0	0	0	0	–	–	–	0	–	–	7	0	
<i>Ae. aegypti</i>	0	–	–	–	90 (0.1%)	0	0	0	0	–	–	–	0	–	–	94	0	
<i>Ae. circumluteolus</i>	49	(0.1%)	0	0	124 (0.1%)	0	0	0	0	–	–	–	27 (0.8%)	2	0	200	2	
<i>Ae. mcintoshi</i>	21	(0.0%)	2	0	0 (0.0%)	0	0	0	4 (10.0%)	–	–	–	–	2	0	25	2	
<i>Ae. mcintoshi/circumluteolus</i>	0	(0.0%)	0	0	35772 (42.3%)	500	26	0	0	0	0	0	0	–	–	35772	500	
<i>Ae. Neomelanimon</i> spp.	11	(0.0%)	0	0	93 (0.1%)	3	0	0	0	–	–	–	8 (0.2%)	0	0	112	3	
<i>Ae. ochraceus</i>	26	(0.0%)	0	0	25353 (30.0%)	450	23	0	0	0	0	0	0	–	–	25380	450	
<i>Ae. pennaensis</i>	0	–	–	–	0	–	–	–	1541 (23.2%)	65	1	0	0	–	–	1541	65	
<i>Ae. simpsoni</i>	25	(0.0%)	1	0	17 (0.0%)	0	0	0	4 (0.1%)	0	0	0	1 (0.0%)	0	0	47	1	
<i>Ae. Stegomyia</i> spp.	24	(0.0%)	1	0	64 (0.1%)	1	0	0	6 (0.1%)	0	0	0	0	–	–	94	2	
<i>Ae. sudanensis</i>	17	(0.0%)	1	0	1705 (2.0%)	36	0	0	5 (0.1%)	0	0	0	0	–	–	1727	37	
<i>Ae. tricholabis</i>	7	(0.0%)	0	0	223 (0.3%)	1	0	0	0	–	–	–	0	–	–	230	1	
<i>Ae. vittatus</i>	0	–	–	–	2 (0.0%)	0	0	0	0	–	–	–	0	–	–	2	0	
<i>Aedeomyia fufurea</i>	3	(0.0%)	0	0	0	–	–	–	7 (0.1%)	0	0	0	0	–	–	10	0	
<i>Aedes</i> spp.	698	(1.0%)	22	0	744 (0.9%)	5	0	0	220 (3.3%)	8	0	0	20 (0.6%)	1	0	1682	36	
<i>An. pharoensis</i>	25	(0.0%)	0	0	0	–	–	–	0	–	–	–	0	–	–	25	0	
<i>An. coustani</i>	692	(1.0%)	5	0	582 (0.7%)	10	0	0	33 (0.5%)	1	0	0	8 (0.2%)	0	0	1315	16	
<i>An. funestus</i>	7	(0.0%)	1	0	3 (0.0%)	0	0	0	0	–	–	–	49 (6.8%)	1	0	59	2	
<i>An. gambiae</i>	34	(0.0%)	0	0	1286 (1.5%)	17	0	0	49 (0.7%)	0	0	0	243 (6.8%)	10	0	1612	27	
<i>An. maculipalpis</i>	0	–	–	–	0	–	–	–	0	–	–	–	54 (1.5%)	3	0	54	3	
<i>An. pharoensis</i>	35	(0.1%)	1	0	32 (0.0%)	0	0	0	0	–	–	–	22 (0.6%)	1	0	89	2	
<i>An. squamosus</i>	51	(0.1%)	0	0	4983 (5.9%)	88	2	0	13 (0.2%)	1	0	0	32 (0.9%)	2	0	5079	91	
<i>Anopheles</i> spp.	44	(0.1%)	0	0	440 (0.5%)	0	0	0	2 (0.0%)	0	0	0	26 (0.7%)	1	0	512	1	
<i>Coquillettidia</i> spp.	0	–	–	–	0	–	–	–	1 (0.0%)	0	0	0	1 (0.0%)	0	0	2	0	
<i>Cq. aurites</i>	83	(0.1%)	0	0	0	–	–	–	0	–	–	–	0	–	–	83	0	
<i>Cq. fuscopennata</i>	0	–	–	–	0	–	–	–	0	–	–	–	28 (0.8%)	1	0	28	1	
<i>Culex</i> spp.	261	(0.4%)	2	0	814 (1.0%)	1	0	0	663 (10.0%)	23	0	0	773 (21.8%)	32	0	2511	58	
<i>Cx. annulitoris</i>	27	(0.0%)	0	0	0	–	–	–	0	–	–	–	543 (15.3%)	22	0	570	22	
<i>Cx. bitaeniorhynchus</i>	221	(0.3%)	5	0	545 (0.6%)	16	0	0	533 (8.0%)	22	3	0	91 (2.6%)	5	0	1390	48	
<i>Cx. duttoni</i>	0	–	–	–	0	–	–	–	0	–	–	–	82 (2.3%)	2	0	82	2	
<i>Cx. ethiopicus</i>	0	–	–	–	0	–	–	–	0	–	–	–	202 (5.7%)	7	0	202	7	
<i>Cx. macfiei</i>	0	–	–	–	0	–	–	–	5 (0.1%)	0	0	0	38 (1.1%)	0	0	43	0	
<i>Cx. poicilipes</i>	759	(1.1%)	17	0	8461 (10.0%)	135	0	0	2565 (38.6%)	100	3	0	8 (0.2%)	0	0	11793	252	
<i>Cx. quinquefasciatus</i>	2787	(4.0%)	75	1	1848 (2.2%)	24	0	0	362 (5.5%)	8	0	0	1048 (29.5%)	42	0	6045	149	
<i>Cx. simpsoni</i>	0	–	–	–	172 (0.2%)	1	0	0	111 (1.7%)	4	0	0	53 (1.5%)	3	0	336	8	
<i>Cx. tigripes</i>	4	(0.0%)	0	0	4 (0.0%)	0	0	0	0	–	–	–	48 (1.4%)	1	0	56	1	
<i>Cx. univittatus</i>	219	(0.3%)	8	1	1128 (1.3%)	26	0	0	52 (0.8%)	1	0	0	140 (3.9%)	7	0	1539	42	
<i>Cx. vansomeri</i>	0	–	–	–	63 (0.1%)	0	0	0	–	–	–	–	3 (0.1%)	0	0	66	0	
<i>Ex. chrysogaster</i>	0	–	–	–	0	–	–	–	9 (0.1%)	0	0	0	0	–	–	9	0	
<i>Eretmapodites</i> spp.	0	–	–	–	2 (0.0%)	0	0	0	0	–	–	–	0	–	–	2	0	
<i>Ficalbia hispida</i>	11	(0.0%)	0	0	0	–	–	–	0	–	–	–	0	–	–	11	0	
<i>Ficalbia</i> spp.	4	(0.0%)	0	0	0	–	–	–	0	–	–	–	1 (0.0%)	0	0	5	0	
<i>Hodgesia</i> spp.	149	(0.2%)	4	0	0	–	–	–	0	–	–	–	0	–	–	149	4	
<i>Ma. africana</i>	14134	(20.2%)	327	2	2 (0.0%)	0	0	0	278 (4.2%)	12	0	0	0	–	–	14414	339	
<i>Ma. uniformis</i>	46703	(66.8%)	804	15	0	–	–	–	174 (2.6%)	8	0	0	0	–	–	46877	812	
<i>Mansonia</i> spp.	2703	(3.9%)	14	0	2 (0.0%)	0	0	0	0	–	–	–	0	–	–	2705	14	
<i>Orthopodomyia</i> spp.	40	(0.1%)	3	0	0	–	–	–	0	–	–	–	0	–	–	40	3	
Grand Total	69874	(100%)	1293	19	84561 (100%)	1314	51	0	6642 (100%)	253	7	0	3549 (100%)	143	0	164626	3003	
Species positive	4	–	–	–	3	–	–	–	0	–	–	–	0	–	–	10	0	

\* Genus: *Ae.* = *Aedes*; *An.* = *Anopheles*; *Cq.* = *Coquillettidia*; *Cx.* = *Culex*; *Ex.* = *Eretmapodites*; *Ma.* = *Mansonia*.

† Number of mosquitoes of given species identified and relative abundance (%) for that district.

‡ Number of pools tested.

§ Number of pools positive for RVFV by RT-PCR.

TABLE 3

Site descriptions, mosquito densities, and Rift Valley fever virus (RVFV) mosquito infection rates at sites where RVFV-infected mosquitoes were detected

District	Site*	Site description	Mosquito density†	Positive species	IR‡	Lower limit	Upper limit	No. pools tested	No. pos. pools	No. mosq. tested
Garissa	El-Humow	Temporary human settlement near flooded wetlands.	6,192	<i>Ae. ochraceus</i>	2.54	1.53	3.98	282	17	6,884
				<i>Ae. mcintoshi/circumluteolus</i>	2.38	1.48	3.64	332	19	8,206
				<i>An. squamosus</i>	1.11	0.20	3.64	78	2	1,815
	Korabull	Temporary livestock holding area with > 3,000 cattle and 12 herdsmen.	178	<i>Ae. mcintoshi/circumluteolus</i>	2.00	0.53	5.41	61	3	1,525
				<i>Ae. ochraceus</i>	1.97	0.52	5.33	62	3	1,550
	Shanta-abak and Dertu combined	Permanent (Shanta-abak) and temporary (Dertu) human settlements near flooded wetlands.	307 (Shanta-abak); 30 (Dertu)	<i>Ae. mcintoshi/circumluteolus</i>	10.65	1.97	36.11	8	2	200
<i>Ae. ochraceus</i>				1.25	0.07	6.08	32	1	800	
Desai	Temporary human settlement with recently flooded wetland areas.	516	<i>Ae. ochraceus</i>	1.11	0.20	3.63	74	2	1,815	
			<i>Ae. mcintoshi/circumluteolus</i>	0.83	0.15	2.71	99	2	2,425	
Baringo	Logumgum	Wetlands on flooded shores of the Molo river, a Lake Baringo tributary.	5,269	<i>Cx. univittatus</i>	18.01	1.32	118.01	2	1	50
				<i>Ma. uniformis</i>	0.89	0.52	1.44	681	15	16,991
				<i>Cx. quinquefasciatus</i>	0.71	0.04	3.42	63	1	1,419
				<i>Ma. africana</i>	0.33	0.06	1.08	248	2	6,076
				<i>Cx. bitaeniorhynchus</i>	6.92	1.84	18.94	20	3	459
Kilifi	Gongoni, Tezo, and Uyombo combined	Permanent homesteads among mixed forest within 1 km of coastal mangrove swamps.	38 (Gongoni); 15 (Tezo); 63 (Uyombo)	<i>Cx. poicilipes</i>	1.28	0.34	3.46	97	3	2,374
				<i>Ae. pempaensis</i>	0.65	0.04	3.17	65	1	1,532

\* Adjacent sites were combined for infection rate calculations.

† Mosquito densities for all species collected from a given area reported as the mean number of mosquitoes per trap per night. For sites sampled on multiple nights, only data from the night with the highest density is reported.

‡ IR = estimated infection rates per 1,000 mosquitoes.‡ The pooled infection rate program (PooledInfRate, CDC, Fort Collins, CO) was used to estimate the bias corrected, maximum likelihood estimate infection rate with a scale of 1,000. Upper and lower limits are the 95% confidence intervals calculated by the program.

of unidentified *Culex* spp. (21.8%), were the most common species in Kirinyaga (Table 2). *Mansonia uniformis* and *Mansonia africana* were found in overwhelming numbers (> 5,000 mosquitoes/trap night) in Logumgum, one of the RVF affected villages in Baringo. Other mosquito species collected that were previously associated with RVFV, included *Anopheles pharoensis* and *Anopheles coustani*, both trapped in Garissa and Baringo (Table 2). One mosquito species, *Ae. pempaensis*, the crab hole breeding mosquito, was only collected near mangrove swamps in Kilifi.

Initial attempts to systematically collect other hematophagous arthropods like sand flies and ticks, quickly exceeded the resources of the team. In Garissa, 910 ticks representing 10 species from four genera were from cattle, sheep, and camels. Rift Valley fever virus was not detected in any of the tick pools. Only a limited number of sand flies and biting midges were collected and none were tested.

**Parity determination.** A total of 803 mosquitoes from El-Humow, Garissa District, were dissected for parity determinations (Table 4). These mosquitoes were from the four most abundant species collected in Garissa District during three trapping periods, December 19 and December 29–31,

TABLE 4

Parity status of four mosquito species collected during three trapping sessions in El-Humow, Garissa District, between December 19, 2006 and January 8, 2007

Species	Parity rates		
	19 December	29–31 December	8 January
<i>Aedes luridus</i>	71% (34/48)*	91% (114/125)	95% (21/22)
<i>Aedes ochraceus</i>	100% (28/28)	96% (123/128)	100% (22/22)
<i>Anopheles squamosus</i>	67% (22/33)	58% (60/104)	53% (35/66)
<i>Culex poicilipes</i>	35% (7/20)	42% (56/135)	69% (50/72)

\* Percent parous (no. parous/no. examined).

2006, and January 8, 2007, and included *Aedes ochraceus* (178 individuals dissected), *Aedes mcintoshi/circumluteolus* (195), *Cx. poicilipes* (227), and *An. squamosus* (203). The highest parous rates (> 96%) were observed for *Ae. Ochraceus* (from each trapping period), and for *Ae. mcintoshi/circumluteolus* during the latter two trapping periods (91% and 95% parous, respectively). Parity rates for *Ae. mcintoshi/circumluteolus* increased significantly between the December 19 and December 29–31 trapping periods ( $X^2_{1df} = 10.05$ ,  $P = 0.002$ ), but not between the December 29–31 and January 8 trapping periods ( $X^2_{1df} = 0.06$ ,  $P = 0.807$ ). *Culex poicilipes* had the lowest parous rates of the four species tested. The rate increased from 35% on December 19 to 69% on January 8, increasing significantly between the December 29–31 (42%) and January 8 trapping periods ( $X^2_{1df} = 13.6$ ,  $P = 0.0002$ ). Parity rates for *An. squamosus* were intermediate between those of the two *Aedes* species and *Cx. poicilipes*, and did not change significantly over the three trapping periods ( $X^2_{2df} = 1.68$ ,  $P = 0.432$ ).

**RT-PCR detection of Rift Valley fever virus in mosquito pools.** Rift Valley fever virus was detected in 77 of the 3,003 mosquito pools tested by RT-PCR. Direct sequencing of the PCR amplicons from a subset of the pools that tested positive for RVFV was used to confirm the results. The virus was detected in mosquitoes from Garissa, Kilifi, and Baringo districts but not from Kirinyaga district. Each district was found to contain a unique set of RVFV-infected mosquito species: *Ae. mcintoshi/circumluteolus*, *Ae. ochraceus*, and *An. squamosus* in Garissa; *Cx. poicilipes*, *Cx. bitaeniorhynchus*, and *Ae. pempaensis* in Kilifi; and *Ma. uniformis*, *Ma. africana*, *Cx. quinquefasciatus*, and *Cx. univittatus* in Baringo (Table 2). These results represent the first time RVFV has been detected in *Ma. uniformis*, *Ae. ochraceus*, *Cx. poicilipes*, *Cx. quinquefasciatus*, *An. squamosus*, and *Cx. univittatus* in Kenya and the first ever isolations from *Ae. pempaensis*, *Cx. univittatus*, and *Cx. bitaeniorhynchus*.

Eighteen RVFV isolates were identified by virus isolation from RT-PCR-negative pools of *Ae. mcintoshi/circumluteolus* (8 isolates), *Ae. ochraceus* (7 isolates), *Ma. africana* (1 isolate), and *Ma. uniformis* (2 isolates) collected at the Desai (6 isolates) and El-humow (9 isolates) sites in the Garissa district and the Logumgum site in the Baringo district (3 isolates) (BLAST identities > 99%).

Estimated infection rates (bias corrected, maximum likelihood) of RT-PCR positive pools for a subset of locations are presented in Table 3. Infection rates per thousand mosquitoes ranged from 0.83 to 10.65 (for both *Ae. mcintoshi/circumluteolus* and *Ae. ochraceus*) in Garissa District, with most IR being < 3.00. In Baringo District, the IR varied from 0.33 in *Ma. africana* to 18.1 in *Cx. univittatus*. However, this high rate for *Cx. univittatus* is probably misleading because of the small sample size (one of two pools tested positive with an estimated one infected mosquito of the 50 mosquitoes tested). In Kilifi District IR ranged from 0.65 in *Ae. pempaensis* to 6.92 in *Cx. bitaeniorhynchus*, and again, the higher IR may be biased by the relatively small sample size.

## DISCUSSION

Previous epizootics of RVF in Kenya have been correlated with the flooding of *dambos* after unusually heavy and persistent rainfall.<sup>20,23</sup> Such flooding then leads to the hatching of immense numbers of floodwater *Aedes* species, which are considered to be the reservoirs of the virus.<sup>41</sup> Colonization of the flooded sites by *Culex*, *Anopheles*, *Mansonia*, and other genera, are thought to contribute to further virus transmission and spread. Based on remotely-sensed rainfall and sea surface temperature anomalies, and on reports of extensive flooding in North Eastern Kenya, mosquito surveillance was initiated in Garissa District in mid December 2006. Laboratory confirmation of RVF affecting humans and livestock in Garissa in December was followed by case confirmation in seven other Kenyan districts (and in Somalia and Tanzania) over the ensuing four months. Entomologic investigations were carried out in four of the RVF-affected districts in Kenya, and RVFV was detected in 77 mosquito pools encompassing 10 mosquito species from three of the four districts. This report represents the first comprehensive entomologic investigation to be carried out during a RVF epidemic in Kenya.

The RVFV was detected in multiple pools of *Ae. mcintoshi/circumluteolus* and *Ae. ochraceus* collected in Garissa by RT-PCR and additionally by virus isolation from some of the RT-PCR negative pools. This, coupled with their high abundance in the area during the outbreak, suggests that these species may have played an important role in the epidemic/epizootic transmission of RVFV during this outbreak, and may have also contributed to the transmission of this virus during the 1997–1998 outbreak of RVF in Garissa. Identification of infected *Ae. ochraceus* in Garissa represents a new RVFV-vector association in East Africa. It is noteworthy that although *Ae. ochraceus* is a known vector of RVFV in West Africa, along with *Ae. vexans arabiensis* and *Ae. dalzieli*. *Aedes vexans arabiensis* is also a vector of RVFV in Saudi Arabia<sup>14,42–45</sup> and although the species has not been documented in Kenya, it has been found in neighboring Somalia and Sudan.<sup>33,46</sup> *Aedes mcintoshi/circumluteolus* are members of the *Neomelanicion* subgenus while *mcintoshi* was originally identified as *lineatopennis* by Edwards<sup>33</sup> and both were later

speciated by Huang<sup>47</sup> and documented as reservoir and vector of RVFV in Kenya.<sup>20</sup> In Kenya, RVFV was previously detected in *Ae. mcintoshi* reared from field-collected larvae.<sup>20</sup> However, because little is known about the ability of *Ae. ochraceus* to transovarially transmit RVFV, investigations on the role of this species in the maintenance of the virus between epidemics in Garissa are critically important. Other members of the subgenera *Neomelanicion* and *Aedimorphus* that have previously been found infected with RVFV, such as *Ae. circumluteolus*, *Ae. dentatus*, and *Ae. cumminsi*<sup>20</sup> should likewise be assessed for transovarial transmission of RVFV as a mechanism for virus maintenance between epidemics.

The detection of RVFV from two pools of *An. squamosus* from Garissa adds to the list of species that are potentially involved in the transmission of RVFV in Kenya, especially because this species is widespread in Africa. Because *An. squamosus* uses a large variety of larval habitats, including standing pools of water, sluggish streams, and rice fields,<sup>48</sup> and is known to feed on livestock and humans, its status as a RVFV vector deserves further evaluation.

Dissection of mosquitoes to determine parity for four mosquito species from Garissa revealed that the highest parity rates were associated with *Ae. mcintoshi/circumluteolus* and *Ae. ochraceus* (71–95% and 96–100%, respectively); the parity rates for *Cx. poicilipes* and *An. squamosus* were substantially lower. This pattern is consistent with previous studies in Kenya showing that when *dambos* are flooded after heavy rains, the first species to emerge are floodwater *Aedes*, including *Ae. mcintoshi/circumluteolus* caused by hatching of eggs deposited during previous flooding cycles.<sup>49,50</sup> *Culex* and *Anopheles* larvae appear later, as gravid females use the newly-flooded sites for oviposition. Thus, the lower parity rates observed in *Cx. poicilipes* and *An. squamosus* may be caused by a later appearance of these species at the flooded sites. Studies of mosquito species succession in irrigated rice fields in East and West Africa have shown that *Cx. poicilipes* was most commonly found 6 or more weeks after flooding of the fields,<sup>51,52</sup> and that adult abundance remained high for an extended period.<sup>51</sup> Information on *An. squamosus* larval development and adult emergence is sparse. Although the IR for *Ae. mcintoshi/circumluteolus* and *Ae. ochraceus* were slightly higher than that determined for *An. squamosus* and *Cx. poicilipes*, the overlapping confidence intervals renders these differences non-significant.

In Baringo, *Ma. africana* and *Ma. uniformis* were collected in abundance. These species are known to breed around flooded areas or around the edges of water bodies containing emergent vegetation, and to feed predominantly on livestock. The RVFV was previously isolated from *Ma. africana* during the 1989 RVF outbreak in Naivasha, Kenya, Uganda, and the Central African Republic.<sup>53,54</sup> The detection of RVFV from multiple pools of *Ma. uniformis* in this survey and additional isolation from two RT-PCR negative pools by cell culture provided the first evidence that this species may have a role in RVF epizootic transmission in Kenya. This too calls for further experimental evaluation of the vectorial capacity of the species.

The RVFV was also detected in pools of *Cx. pipiens* and *Cx. univittatus* collected in Baringo. *Culex pipiens* was previously implicated as a vector during the RVF epidemic that occurred in Egypt in 1977<sup>3</sup> and a number of vector competence studies have also showed the efficiency of this and other

*Culex* species to act as a vector for RVFV.<sup>16</sup> Even though *Cx. univittatus* has not previously been found infected with RVFV, it is a known vector for West Nile virus in Africa,<sup>55</sup> belongs to a complex composed of several species one of which, *Cx. perexiguus*, has been found through experimental studies to efficiently vector RVFV.<sup>19,46</sup> This observation calls for vector competence evaluation of this species as well.

In response to human cases of RVF in Kilifi, traps were set around homes and near the coastal mangrove swamps. *Culex poicilipes*, *Cx. bitaeniorhynchus*, and *Ae. pempaensis* were the most abundantly collected species, and yielded three, three, and one RVFV isolates, respectively. *Aedes pempaensis* is not known to be a vector of RVF and has never been associated with the virus in nature. *Aedes pempaensis* breeds along the seashore in association with crabs that live in the mangrove swamps. Female *Ae. pempaensis* mosquitoes lay their eggs on the crabs and the larvae develop in saline pools near the shore. Lumbo virus (a member of the California serogroup) is the only documented virus that has been isolated from this species.<sup>56</sup> In comparison, high IR of 6.92 for *Cx. bitaeniorhynchus* and the relatively high IR of 1.28 for *Cx. Poicilipes* was recorded. Previous field collections of *Cx. poicilipes* in Mauritania after the 1998 RVF outbreak showed that this species was naturally infected with RVFV, whereas there is no documented association of *Cx. bitaeniorhynchus* with RVFV. There was no cell culture virus isolation obtained from the RT-PCR negative samples of these species. These high IRs would justify initiation of vector competence studies to evaluate the role of these species in RVFV transmission.

More human cases of RVF were reported from Garissa and Baringo than from the other nine affected districts. Although previous RVF epizootics have been associated with periods of excessive seasonal rainfall, it has been suggested that RVF primarily affects inhabitants of the dry lands of the Rift Valley and the outlying semiarid and arid grazing lands, to include the Garissa and Baringo districts.<sup>57</sup> These districts are home to predominantly pastoral communities and about two-thirds of the RVFV positive pools were from mosquitoes collected in Garissa, and all except two of these were from floodwater *Aedes* species. The flood plains in northeastern Kenya provided an ideal habitat for the tremendous blooms of floodwater *Aedes*, which presumably initiate the RVF outbreak. With several years elapsing between major flooding events, the immunologically naïve and unvaccinated livestock populations<sup>6</sup> would presumably have developed high-titered viremias after being bitten by an infected floodwater mosquito. Those infected animals could then infect secondary and inefficient mosquito vectors that could then sustain and contribute to the spread of the virus to more livestock and humans.<sup>58</sup> Sheltering in close proximity to livestock during epizootics has also been previously observed as one predisposing factor relating to RVFV infections in humans.<sup>11</sup> About 24% of the RVFV positive mosquito pools were obtained from Baringo, and were mostly *Mansonia* species, which are known to feed preferentially on livestock and humans. The large herds maintained in Garissa and Baringo may have provided abundant blood meal sources for the initial floodwater *Aedes* mosquitoes, thus setting up a situation for transmission between the livestock and the *Aedes* and *Mansonia* species mosquitoes, and subsequent high human exposure. A similar situation may have existed in the other districts, except that different mosquitoes species may have been involved. The infection rates for *Ae. ochraceus* and

*Ae. mcintoshi/circumluteolus* recorded in El-Humow (1.94 and 1.96, respectively) and Kurabull (2.5 and 2.19, respectively), Garissa district, indicate that the level of infected vectors present where the humans and livestock lived in close proximity would have contributed to an increased risk for human infection by RVFV. It was anticipated that exclusion of blood fed mosquito specimens during sample screening would reduced the chance of detecting virus contained in host blood.

In Kirinyaga, unlike the other sites, mosquito numbers were much lower (27 mosquitoes per trap per night) (Table 3) and less than 1% of the collected mosquitoes consisted of flood water *Aedes* species. *Culex quinquefasciatus*, a known vector of RVFV, was the most abundantly collected mosquito, followed by *Cx. annulioris*, a mosquito species linked to RVFV transmission in Madagascar.<sup>58</sup> Although RVF was reported in the Kirinyaga area during this outbreak, RVFV was not detected in any of the mosquitoes collected from that area; an observation attributable partly to the timing of vector collection and partly to the prevailing climatic conditions and agricultural practices in the district. Kirinyaga is a humid highland area that is not prone to the extensive flooding such as that seen in Garissa or parts of Baringo, and hence is not suited for the emergence of abundant floodwater *Aedes* mosquitoes. Furthermore, it has been suggested that the hatching of floodwater *Aedes* in the highland areas occurs more frequently, but at reduced levels, thus allowing for low level enzootic transmission of the virus during the frequent rains.<sup>59</sup> Finally, the susceptible livestock populations in Kirinyaga were smaller and most may have some level of immunity to RVFV caused by previous enzootic transmission, thus avoiding the explosive outbreaks as those observed in Garissa and Baringo.

Our entomological surveillance during the 2006–2007 outbreak of RVF in Kenya has shown that RVFV transmission occurred in a variety of distinct ecological regions, and that vector species composition and abundance was different for each of these regions. This, and the newly observed RVFV – mosquito associations, underscores the need for further studies to understand the ecological factors that influence the distribution of mosquitoes and to determine the vectorial capacity of these mosquitoes for RVFV in Kenya. The distribution and abundance of floodwater *Aedes* that serve as the primary vectors and suspected reservoirs of RVFV in virus emergence zones, together with the secondary vectors that may participate in virus transmission must be mapped out in Kenya and other epidemic prone countries in the region if relevant and effective control measure are to be formulated aimed at combating the spread of this virus. Monitoring of the El Nino/Southern Oscillation (ENSO) events will also help in predicting above normal rainfall in East Africa,<sup>57</sup> and may help relevant authorities to more effectively prioritize and optimize their resources by targeting control efforts at appropriate vector habitats ahead of outbreaks in an effort to minimize livestock and human exposure in the future.

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Authors' addresses: Rosemary Sang, Joel Lutomia, and Marion Warigia, Arbovirology/Hemorrhagic Fevers Laboratory, Centre for Virus Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, E-mails: rsang@kemri.org, jlutomiah@kemri.org, and mwarigia@kemri.org. Elizabeth Kioko, Caroline Ochieng, Hellen Koka, and David Schnabel, US Army Medical Research Institute, Nairobi, Kenya, E-mails: ekioko@wrp-ksm.org, COchieng@wrp-nbo.org, hkoka@wrp-nbo.org, and DSchnabel@wrp-nbo.org. Monica O'Guinn and John S. Lee, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, E-mails: monica.oguinn@us.army.mil and john.s.lee@us.army.mil. Marvin Godsey and Barry Miller, Division of Vector-borne Infections Diseases, National Center for Disease Control and Prevention, Fort Collins, CO, E-mails: mjg9@cdc.gov and brm4@cdc.gov. David Hoel, Navy Marine Corps Public Health Center Detachment, Center for Medical Agricultural, and Veterinary Entomology, Gainesville, FL, E-mail: HoelD@NAMRU3.med.navy.mil. Hanafi Hanafi, US Naval Medical Research Unit Number Three, Abbassia, Cairo, Egypt, E-mail: Hanafi.Hanafi.eg@med.navy.mil. Robert F. Breiman, International Emerging Infections Program, CDC-Kenya, Nairobi, Kenya, E-mail: Rbreiman@ke.cdc.gov. Jason Richardson, Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand, E-mail: Jason.Richardson@afirms.org.

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