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Virus isolations and high population density implicate *Culex antennatus* (Becker) (Diptera: Culicidae) as a vector of Rift Valley Fever virus during an outbreak in the Nile Delta of Egypt

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ABSTRACT

In June, 2003, Egypt's hospital-based electronic disease surveillance system began to record increased cases of acute febrile illness from governorates in the Nile Delta. In response to a request for assistance from the Egyptian Ministry of Health and the World Health Organization (WHO), the U.S. Naval Medical Research Unit No. 3 (NAMRU-3) provided assistance in identifying the cause and extent of this outbreak. Testing of human clinical samples ($n = 375$) from nine governorates in Egypt identified 29 cases of RVF viremia that spanned the period of June to October, and a particular focus of disease in Kafr el Sheikh governorate (7.7% RVF infection rate). Veterinary samples ($n = 101$) collected during this time in Kafr el Sheikh and screened by immunoassay for RVFV-specific IgM identified probable recent infections in cattle (10.4%) and sheep (5%). Entomologic investigations that focused in rural, rice growing villages in the Sidi Salim District of Kafr el Sheikh during August–September, 2003, collected, identified, and tested host-seeking female mosquitoes for the presence of pathogenic viruses. Three isolates of RVF virus (RVFV) were obtained from 297 tested pools of female mosquitoes and all three RVFV isolates came from *Cx. antennatus* (Becker). While *Cx. pipiens* has been considered the primary vector of RVF virus in Egypt and is often the most common man-biting species found, *Cx. antennatus* was the dominant species captured at the 2003 outbreak location in Kafr el Sheikh governorate. This is the first time that *Cx. antennatus* has been found naturally infected with RVFV in Egypt.

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1. Introduction

Rift Valley fever (RVF) is a viral disease of man and animals that was first recognized in 1931 in the Rift Valley of Kenya as an illness affecting sheep, cows, and humans (Daubney et al., 1931). The viral agent causing the disease is a single stranded RNA virus of the genus *Phlebovirus*, in the family Bunyaviridae. Periodic RVF outbreaks in livestock (goats, sheep, cattle, and camels) and acute febrile illness with hemorrhagic syndrome in humans have been reported widely throughout south and central Africa, from Kenya westward into Nigeria, Niger, Burkina Faso, Senegal, and Mauritania and northward into Egypt (Digoutte and Peters, 1989; Diallo et al., 2005). Rift Valley fever made its first appearance outside of southern Africa in 1977, and its first incursion into Saudi Arabia and Yemen during 1999 (Hoogstraal et al., 1979; Meegan et al., 1980; Arthur et al., 1993; Shoemaker et al., 2002). Egypt is the most northern, and populous nation to have suffered from RVF and the human illness and death experienced there during the 1977–1978 epizootic was of unprecedented severity (Laughlin et al., 1979). Since then, RVF outbreaks in Egypt have occurred in 1993, 1999, and most recently, 2003. In most cases these were believed to have begun as epizootics among sheep, goats, cattle, and camels, which serve as amplifying hosts of the virus. The outbreaks of RVF in Upper Egypt during 1977 were preceded by epizootics that occurred to the south of Egypt in Sudan, Kenya, and Uganda, and were thought to result from the movement of herd animals into Egypt from the south (Gad et al., 1986). The virus is especially lethal for young animals and causes abortions in older ruminants and camels. Horses, pigs, and birds are reportedly unaffected. The Rift Valley fever virus is capable of being spread by airborne contagion, through cuts in the skin during butchering or birthing, orally by drinking raw milk from infected animals and via biting arthropods. At least 33 species of mosquito, spanning six different genera, are capable of developing and transmitting RVFV (Turell et al.,

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2002). Certain species of East African floodwater mosquitoes with drought-resistant eggs, such as *Ae. mcintoshi* (Huang) [reported as *Ae. lineatopennis* (Ludlow)] are able to maintain the virus in a sylvatic, enzootic cycle by means of vertical transmission (Linthicum et al., 1985b).

Egypt's 2003 RVF outbreak, which began in June, peaked in August, and ended in October, appeared to follow a different pattern, originating within, and remaining confined to the Nile Delta with no apparent involvement in Upper Egypt or neighboring nations. Unlike previous outbreaks in Egypt, which involved mainly domestic animals, and secondarily, the human population, the 2003 outbreak was largely a human epidemic with no reports of livestock disease (WHO, 2003). This report presents highlights of the 2003 outbreak investigation conducted jointly by NAMRU-3 and the Egyptian Ministries of Health and Agriculture, that identified RVFV as the viral etiology of infection in humans and livestock, Kafr el Sheikh governorate as a particular focus of the outbreak at that time, and *Cx. antennatus* as the only mosquito species from which RVFV was repeatedly isolated. This last result is especially significant as it marks the first confirmation of *Cx. antennatus* as a naturally infected carrier of RVFV in Egypt, and documents the striking population dominance of this species at the outbreak site.

2. Methods and materials

2.1. Analyses of human clinical samples

NAMRU-3, with its biosafety level 3 containment facility, experienced staff, and record of long-standing accomplishment serves as a WHO Regional Collaborating Center for Viral Disease Research, and was a collaborating partner with the Egyptian Ministries of Health and Agriculture in this outbreak investigation. From the start of the outbreak in June, 2003, the MOH Central Laboratory began sending samples of human sera or cerebrospinal fluid (CSF) from suspected cases of RVF to the NAMRU-3 Virology Department for immediate diagnostic testing. Samples were delivered and received in the BSL-3 containment unit where each was surface sterilized, given an accession number and logged into a Laboratory Information Tracking System (LITS) database that included the MOH identification number, collection date, place, time, age, and sex of the patient. Aliquots were drawn off for immediate processing and the remaining sample was given a second label with the new accession number and stored at −70 °C.

Virus isolation was attempted by inoculating aliquots (0.2 ml each) of sera or CSF into each of three different cell lines (Vero [=African green monkey kidney], BHK [=baby hamster kidney] and C6/36 [*Aedes albopictus* cells]) growing as monolayers in 12-well culture plates and incubated at 37 °C with 5% CO_2. Daily observation for cytopathic effects (CPE) in the cell monolayers extended to 10 days post inoculation. Samples producing CPE were re-passaged for confirmation and those with confirmed CPE were processed for virus identification by indirect fluorescent antibody test (IFAT) using RVFV-specific monoclonal antibodies.

Molecular-based screening was performed on RNA that was extracted from 50 μL samples of sera or CSF using the Qiagen Viral RNA mini kit (Qiagen, CA, USA). Extracted RNA was tested for RVFV using a nested reverse transcriptase-polymerase chain reaction (RT-PCR) that targets the NSs coding region of the small (S) segment (~750 bp) according to the procedure and primer sequences published by Sall et al. (2001). Amplified products were loaded onto 2% agarose gel, electrophoresed, and visualized by ethidium bromide staining against a molecular weight reference ladder. The internal primers amplified a DNA fragment of 662 bp in samples that contained RVFV.

2.2. Analyses of veterinary samples

The Egyptian Ministry of Agriculture collected sera during the summer of 2003 from livestock in areas of the Nile Delta where the majority of human febrile illness was occurring. Except for an identification number, location, date, and identification as either cow, sheep, goat, or buffalo, samples were provided without data on the animal's sex, health condition, vaccination history, specific village, and relation to human cases of illness. Livestock sera delivered to NAMRU-3 Virology Department was given an accession number and logged into the LITS database. Aliquots of sera were heat inactivated, screened by nested RT-PCR as described for human clinical samples, and tested by immunoassay for RVFV-specific IgM according to the published method of Niklasson et al. (1984). Remaining sera with the new accession number was frozen at −70 °C.

2.3. Entomologic investigations

Owing to the number of samples and RVFV isolates originating from Kafr el Sheikh Governorate, Ministry of Health personnel requested that mosquito surveillance efforts be focused there in an effort to identify vector mosquito species. Collections during August and September 2003 were made in three villages of Sidi Salim District (Sad Khamis, Okla El Bariyat, and El Sehmawy), in northern Kafr el Sheikh (N31°5′23.243″ E31°0′13.236″), about 150 km north of Cairo (Fig. 1). This low, flat district is watered primarily by irrigation canals drawing off the western (Rosetta) branch of the Nile and supports a dense human population of ~760/km^2. It is a productive farming area for rice (summer), wheat (winter), cotton, and corn south of brackish Lake Burullus. High resolution satellite images of this area do not identify our three small study villages, but show how intensively the entire Delta region south of Lake Burullus is farmed. At 460 km^2, Burullus is Egypt's second largest lake and is designated a protected natural wetland area. It has lost 20% of its size in the last century due to blowing sand from dunes in the north, and silt from agricultural drainage in the south. Inflow of fresh water from agriculture, along with fertilizers, pesticides, and sewage has reduced fish species, and fishing, and the southern shoreline has been progressively lost to dense reed swamps of *Phragmites australis*. Increasing soil salinity is another problem in the northernmost parts of the Delta, but rice cultivation helps to leach salt from the soil and render it suitable for other crops. Aquaculture of *Tilapia,* shrimp, and waterfowl has become an important alternative industry in low areas of Sidi Salim and other districts bordering the lake that are too saline for rice production. Sidi Salim District has a human population of ~332,000 and abundant domestic animals including buffalo, cattle, donkeys, horses, sheep, goats, chickens, ducks, cats, and dogs. The climate and meteorology of Sidi Salim District may be typified by that of Alexandria, 100 km west, where records have been kept since 1945 (Fig. 2). The dry summer season runs from May to October with highest day and night temperatures in August. Winter months of November to April are characterized by mean night time temperatures of 10–14 °C with most rainfall during the coldest months of December to February. Total rainfall averages 200 mm/yr.

Adult female mosquitoes were collected from the study sites using CDC light traps baited with dry ice to generate carbon dioxide (CO_2) as an attractant. Traps were placed around human dwellings and animal shelters and operated from late afternoon to early the next morning. In the laboratory cold-anesthetized field-collected mosquitoes were sorted and identified on a chill table using the taxonomic keys of Edwards (1941), Harbach (1985) and Glick (1992). Identified female mosquitoes were pooled (10–50 per pool) according to species, blood fed status, location, and date of collection then frozen in liquid nitrogen for later virus isolation attempts. For virus screening and isolation, mosquito pools

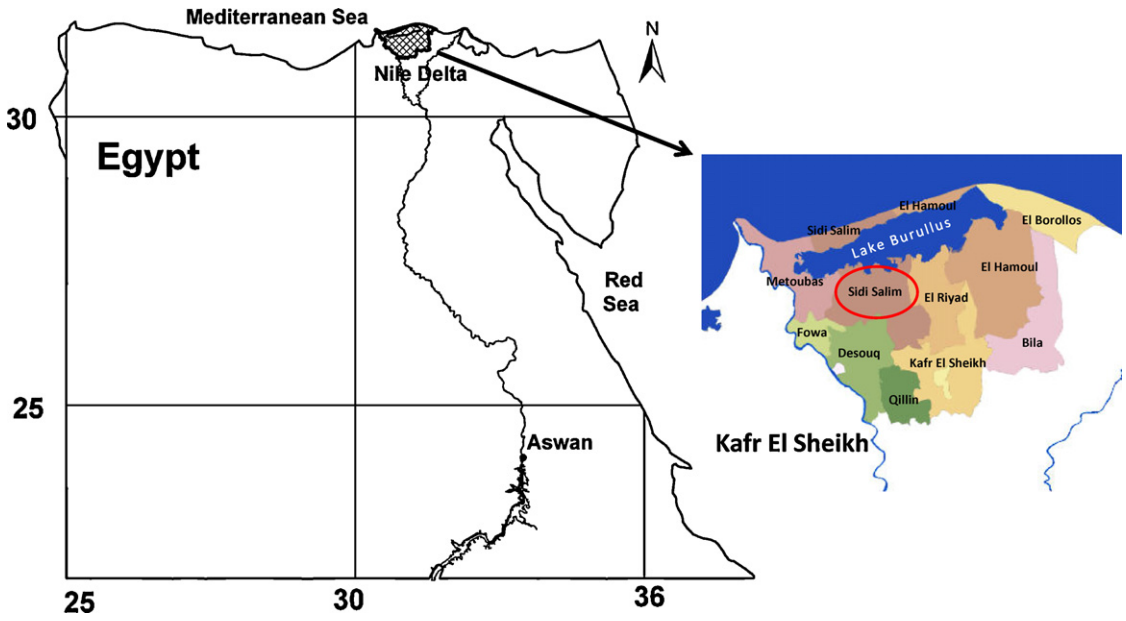

Fig. 1. Map of Egypt showing location of Kafr el Sheikh Governorate in the Nile Delta and outset showing Sidi Salim District as the site for mosquito collections during the 2003 RVF outbreak.

were briefly thawed, washed with phosphate buffered saline (pH 7.5) containing penicillin (500 units/ml), streptomycin (500 mg/ml) and fungizone (Amphotericin B; 10 μg/ml), ground in cold PBS (as above), then centrifuged at 3000 rpm for 30 min at 4 °C. Aliquots (0.2 ml each) of the mosquito supernatants were screened by inoculation into cell cultures (Vero, BHK and C6/36) in the manner previously described for screening human sera and CSF. The RNA was extracted from 50 μL aliquots of mosquito pool supernatant and tested by RT-PCR in the same manner described for screening human sera and CSF. A minimum infection rate (MIR) was calculated for mosquito species and collection sites yielding virus as the ratio of RVFV-positive pools to the total number of mosquitoes tested.

3. Results

3.1. Human clinical samples

Human samples of sera ($n = 265$) and CSF ($n = 110$) from suspected cases of RVF were received for diagnostic testing between June and October, 2003 and originated from hospitals in nine of Egypt's 29 governorates (Aswan, Beni Suef, Beheira, Cairo, Dakahlia, Kafr el Sheikh, Qalyubia, Qena, and Sharqia). Accessioned samples from 375 patients were screened for viral pathogens by cell culture and RT-PCR. The majority of cases originated from Kafr el Sheikh (55%), followed by the Abbassia Fever Hospital in Cairo (23%), and Behira governorate (19%). Sample collection dates spanned the period from June 4, 2003 to October 15, 2003 with most from August (42%) and September (43%). A total of 15 isolates of RVFV were obtained for an overall isolation rate of 4%. Samples that yielded isolates were collected in June (2/42), August (12/158), and September (1/161); these originated from Kafr el Sheikh (11/207) and Cairo's Abbassia Fever Hospital (4/87). Screening by nested RT-PCR identified 19 cases of RVF viremia in samples from August (13/158), September (5/161), and October (1/8). These PCR positives originated from Kafr el Sheikh (11/207), Behira (7/72), and Cairo (1/87) governorates.

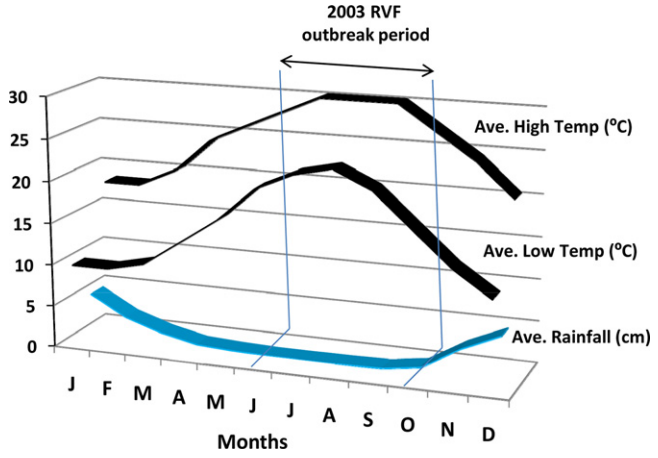

Fig. 2. Mean monthly high and low air temperatures and rainfall, Alexandria Egypt, 1945–2007.

3.2. Veterinary samples

Livestock sera collected by the Ministry of Agriculture in the Sidi Salim District of Kafr el Sheikh governorate during August, 2003 yielded a single sample (Ovid) that was RVFV-positive by RT-PCR. Screening by ELISA identified RVFV-specific IgM responses in 10% of cows (5/48) and 5% of sheep (2/36). No IgM was detected in sera from 9 goats and 8 buffalos. It is suspected, but not known with certainty, that these livestock samples were taken from animals that aborted or showed signs of illness.

3.3. Mosquito collections and analyses

Guided by RVFV isolations and positive screening tests on human and veterinary samples, mosquito light trapping focused within the Sidi Salim District of Kafr el Sheikh. A total of 27 nights of light trapping was conducted during the period 26 August–9 September 2003 with 26 nights focused on three villages in an area of ~2400 km^2. Catches for one trap ranged from 98 to 2027 mosquitoes/trap-night, and six species were identified from a total of 9179 females (Table 1). *Culex antennatus* was most abundant (95.8%) followed, distantly, by *Anopheles tenebrosus* (2.7%). *Culex*

Table 1
Mosquito species captured by CO_2-baited light trap and pooled for virus isolation. Sidi Salim District, Kafr el Sheikh Governorate, Egypt, 26 August–9 September, 2003.

Mosquito species	Number (%)	No. and type of pools tested	RVFV positive pools
Culex antennatus	8798 (95.8%)	218 (182 unfed; 36 bloodfed)	3 (2 unfed; 1 bloodfed)
Culex pipiens	102 (1.1%)	27 (25 unfed; 2 bloodfed)	0
Culex perexiguus	6 (0.1%)	3 (3 unfed)	0
Aedes detritus	1 (0.01%)	1 (1 unfed)	0
Anopheles tennebrosus	248 (2.7%)	41 (34 unfed; 7 bloodfed)	0
Anopheles pharoensis	24 (0.3%)	17 (16 unfed; 1 bloodfed)	0
Total	9179	297 (261 unfed; 36 bloodfed)	3 (2 unfed; 1 bloodfed)

antennatus accounted for 92–97% of each trap's nightly collection with median trap rates of 168–312/trap-night (Table 2). Blood-engorged mosquitoes (4.2% of the light trap catch) represented four species and accounted for similar proportions (*An. pharoensis*, 4.2%; *An. tenebrosus*, 4.0%; *Cx. antennatus*, 4.2%; and *Cx. pipiens* 6.9%). *Culex pipiens,* the accepted primary vector of RVFV in Egypt, represented only 1% of the total collection. Virus was isolated by cell culture in 3 of 297 mosquito pools (261 unfed + 36 blood-engorged) tested. All three isolates were from *Cx. antennatus* (two pools of 50 unfed and one pool of 13 blood-engorged mosquitoes) and IFAT identified these isolates as RVFV. Virus isolation results and their identifications as RVFV were subsequently confirmed by nested RT-PCR screening of the supernatants from all 297 mosquito pools. The site-specific MIRs calculated for RVFV in unfed *Cx. antennatus* during this outbreak period represent towns separated by a distance of ~75 km.

4. Discussion

An internet search for information on the 2003 RVF outbreak in Egypt turns up just one item: A World Health Organization report, dated 2nd September 2003, reporting 45 cases of RVF, including 17 deaths, that occurred in "Seedy Salim" District of "Kafr Al-Sheikh" Governorate and crediting NAMRU-3 with confirmation of the RVF diagnosis in clinical samples (WHO 2003). Despite stating that continued close monitoring of the situation would continue, no further reports or summaries of this incident have been publicly disclosed. This abrupt and complete closure of the incident might seem unusual, but consideration should be given to the broad and possibly devastating economic impact that even a rumored outbreak of RVF may trigger. Unstated in the WHO report is that detection of this outbreak was achieved through Egypt's newly implemented National Electronic Disease Surveillance System (NEDSS). This hospital- and clinical laboratory-based network was a collaboration between the Egyptian Ministry of Health and Population, the CDC, USAID, NAMRU-3, and the WHO. While the report cited RVF cases and deaths in just one of the 10 districts that make up Kafr el Sheikh Governorate, the number of suspected RVF cases reported through NEDSS totalled 651 for the June 6 to September 9, 2003 period, and involved the Nile Delta governorates of Dakahlia ($n = 131$), Kafr el Sheikh ($n = 241$), and Behira ($n = 276$). We received no clinical information or outcome data on any of the 375 cases that were tested in our laboratory, but assume a presentation of encephalitis in those cases where CSF was the sample provided (110/375 = 29%). It is intriguing that children ≤5 years old accounted for 17.4% of the CSF samples, compared to 5.5% of the sera, but this may be due to chance. We are puzzled that Kafr el Sheikh, which yielded 21 confirmed RVF cases, was the source of 65% of the sera and 32% of the CSF samples whereas neighboring Behira governorate, with 7 confirmed RVF cases, was the source of only 2% of the sera and 61% of the CSF samples. Although Cairo's Abbassia Fever Hospital was the source for many clinical specimens of acute febrile illness (70 sera and 8 CSF), and five confirmed cases of RVF, this specialized research hospital draws patients from all of Egypt's 29 governorates and it seems unlikely that these cases actually originated from the urban Cairo governorate. Rift Valley fever in Egypt occurs at the rural village level and is associated with livestock contact and irrigation practices that promote mosquito breeding. It is speculated that Egypt's national production of live, attenuated RVF vaccine for veterinary use, coupled with a responsive program for vaccination of livestock since 1994 (CDC, 1994), may have dampened the 2003 epizootic and limited it to mainly a human outbreak.

Interestingly, RVFV was isolated in cell culture from only 2 (1.8%) of the CSF samples whereas RT-PCR amplified RVFV-specific RNA from 15 (13.6%) of these CSF samples. The reverse applied to sera where cell culture isolations of RVFV were successful in 13 (5%) cases and RT-PCR identified RVFV in just 4 (1.5%) of the cases. The nested RT-PCR procedure we employed targeting the S segment was reportedly more sensitive than virus isolation by cell culture for detection of RVFV in the sera of infected mice and lambs (Sall et al., 2001) but was certainly less sensitive than cell culture for detecting a single RVFV-infected mosquito in a pool of 50 (Ibrahim et al., 1997). Sang et al. (2010) reported 18 isolations of RVFV from mosquito pools of 25 that had tested negative by a nested RT-PCR screening.

The entomologic results we report for the 2003 RVF outbreak are in contrast to Egypt's first experience with RVFV in 1977. At that time *Cx. pipiens* accounted for 94% of all Culicines collected and frequent isolations of RVF virus were made from blood-engorged *Cx. pipiens*, but 783 pools of unfed *Cx. pipiens* yielded only a single isolate of RVFV (Hoogstraal et al., 1979). *Culex antennatus* and three

Table 2
Mosquito species trap rates, proportions, and RVF virus isolation results at three sites in Sidi Salim District, Kafr el Sheikh Governorate, Egypt, during the 2003 Rift Valley fever outbreak.

AA	Sad Khamis	Okla El Bariyat	El Sehmawy	Combined
GPS coordinates	N31°5′23″ × E31°0′13″	N30°45′7″ × E31°19′4″	N30°49′51″ × E31°15′23″	–
Trap Nights (26 Aug.–09 Sep. 2003)	8	8	10	26
Mean Mosquitoes/trap-night (Median)	562 (324)	316 (287)	193 (174)	344 (251)
Mean *Cx. antennatus*/trap-night (Median)	536 (312)	305 (279)	184 (168)	330 (238)
Cx. antennatus % of total	92–97%	92–99%	92–98%	8578/8953 (96%)
An. tenebrosus % of total	0–4%	0–4%	1–7%	248/8953 (2.7%)
Cx. pipiens % of total	0–6%	0–3%	0–2%	98/8953 (1.1%)
RVF virus isolates/unfed *Cx. antennatus*	1/4178 (90 pools)	1/2302 (50 pools)	0/1727 (37 pools)	2/8207(177 pools)
RVF virus isolates/fed *Cx. antennatus*	1/113 (11 pools)	0/142 (7 pools)	0/116 (8 pools)	1/258 (26 pools)
Cx. antennatus MIR (RVF virus) unfed	0.24	0.43	0	0.24

other species made up just 6% of the mosquitoes collected during that epidemic. During Egypt's 1993 RVF outbreak, screening of more than 36,000 Culicines trapped at the epicenter of infection, in Aswan governorate, failed to yield any RVFV isolates (Turell et al., 2002). *Culex antennatus* accounted for just 7.5% of these mosquitoes (Gad et al., 1999).

Laboratory studies have found that in addition to *Cx. pipiens*, *Cx. antennatus* and four other Egyptian mosquito species (*Ae. caspius*, *An. multicolor*, and *An. pharoensis*) were capable of acquiring and transmitting RVFV (Gad et al., 1995). *Culex antennatus*, with the highest overall RVFV transmission rate in these experiments, was long-lived, widely distributed, very common during the summer and fall, and demonstrated a broad host feeding pattern that argued strongly for its potential as an important secondary vector of RVFV in Egypt (Gad et al., 1987a). Further laboratory studies found that *Ae. caspius* and *Cx. antennatus* were most susceptible to infection and dissemination of RVFV (Turell et al., 1996).

Four different families of virus (Bunyaviridae [genus *Phlebovirus*: Rift Valley fever virus, Arumowot virus]; Togaviridae [subfamily Alphavirus: Sindbis virus]; Flaviviridae [genus *Flavivirus*: West Nile virus; Reoviridae [genus *Orbivirus*: Acado virus] most of whose members are known to be pathogenic to humans and/or animals, have been isolated from naturally infected *Cx antennatus* (Hurlbut, 1956; Berge, 1975; Lee, 1977; Knudson, 2006). This mosquito is also a confirmed vector of *Wuchereria bancrofti* in Egypt (Rifaat et al., 1968).

The geographic range of *Cx. antennatus* is pan-African, extending from Botswana and Angola in the south, to Senegal and Mauritania in the west, up through the Rift Valley to Egypt, and eastward into Israel, Jordan, and Iran (Gad et al., 1987b; Harbach et al., 1988). In Kenya, three years of mosquito collection during an inter-epizootic period found *Culex antennatus* to be the third most abundant Culicine with the second highest MIR (0.61) for RVFV (Linthicum et al., 1985b). The complete absence of *Cx. antennatus* from extensive mosquito collections made during Kenya's 2006/2007 RVF epidemic is puzzling (Sang et al., 2010).

In Egypt, detailed study of the distribution and bionomics of *Cx. antennatus* found this species breeding in every governorate, primarily in flooded areas, rice fields, and irrigation channels, and in mainly fresh water; tolerant of brackish conditions, but relatively intolerant of pollution (Gad et al., 1987b; Harbach et al., 1988). In Sharkiya governorate in the Nile Delta, *Cx. antennatus* and *Cx. pipiens* were essentially the only mosquito species collected from inside bedrooms and animal sheds during a year of intensive study (Gad et al., 1995). The majority of *Cx. antennatus* were taken in cattle sheds and in exit traps, suggesting a bridging role in transmission and a potential for underestimating this species when collecting is limited to the inside of houses. More than half of *Cx. antennatus* caught in bedrooms had taken human blood while most of those caught in animal sheds had taken blood from sheep and goats (Gad et al., 1995). Parous rates of *Cx. antennatus* in the Nile Delta ranging from 70% to 93% in man-biting collections and from 60% to 89% in exit traps attest to the longevity of this mosquito (Gad et al., 1987b). Indoor house surveys of mosquitoes from sites throughout Egypt typically observe *Cx. pipiens* and *Cx. antennatus* as the two most common species, but with *Cx. pipiens* more abundant than *Cx. antennatus* by a factor of 2–80 (Mostafa et al., 2002; Abdel-Hamid et al., 2009). Outdoor CO_2-baited light traps in the center of the Nile Delta (Gharbiya governorate) found *Cx. antennatus* to be the dominant mosquito species with humans accounting for <10% of blood sources, cow/buffalo 37–76%, horse/donkey 14–28%, and sheep/goat 19%. By comparison, human blood was found in 34–48% of the engorged *Cx. pipiens* that were captured (Zimmerman et al., 1985). Collectively, *Cx. antennatus*, with typically fewer numbers and preference for feeding on cows and sheep throughout its range would seem to make it an important vector for the rural, epizootic cycle of RVF, with a key role in carrying the infection over into humans. Greater numbers, and keener anthropophilic tendencies of *Cx. pipiens* make it the vector with capacity for protracted human outbreaks and epidemics of RVF in more urban settings.

The site-specific MIR of 0.43 that we calculated for *Cx. antennatus* is an order of magnitude higher than that of *Cx. pipiens* during Egypt's 1977 RVF epidemic, but we consider this result to be exceptional. The density of *Cx. antennatus* that we document, its dominance over every other species of mosquito collected, and the insignificant numbers of *Cx. pipiens* captured were also unusual, as we had expected *Cx. pipiens* to be dominant in our collections. In consequence, we believe that our 26-night mosquito profile, based on outdoor CO_2 light traps, is site-specific and reflects the unique situation in Egyptian rice fields when optimal temperature, water volume, quality, and nutrients have combined to produce the summer's peak population of *Cx. antennatus* adults.

In summary, this investigation identified RVFV in cases of human illness, livestock, and female mosquitoes during a notable outbreak of febrile illness and encephalitis that occurred in the Nile Delta of Egypt during the summer of 2003 (June–October). The results of serology performed on a limited number of livestock sera taken from Sidi Salim, and the detection of RVFV in one of these samples provide evidence of animal infections that accompanied, or preceded, the human outbreak. Notably, this work marks Egypt's first record of RVFV occurring naturally in *Cx. antennatus* and fulfills predictions made by Gad et al. (1987a,b) regarding the vector potential of this species. Multiple natural infections of RVFV in *Cx. antennatus* at the time and place of this RVF outbreak, and the clear dominance of this mosquito over all other species captured now provide evidence that it does serve as an important vector of RVFV in Egypt. The prevention and control of RVF outbreaks should therefore include measures that assess, target, and reduce larval and adult populations of *Cx. antennatus*.

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