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


U.S. Naval Medical Research Unit Number Three, Cairo, Egypt

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 were screened by immunoblotting 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 207 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.

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 different genera, are capable of developing and transmitting RVFV (Turell et al.,...
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, Kafir el Sheikh governorate as a particular focus of the outbreak at that time, and *Cx. antenatus* 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. antenatus* 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% CO2. 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 LITIS database. Aliquots of sera were heat inactivated, screened by nested RT-PCR as described for human clinical samples, and tested by immunosassay 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 Kafir 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 Kafir 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/km2. 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 km², 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 (CO2) 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