ORIGINAL ARTICLE

Serologic evidence of exposure to Rift Valley fever virus detected in Tunisia

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Abstract

Rift Valley fever virus (RVFv) is capable of causing dramatic outbreaks amongst economically important animal species and is capable of causing severe symptoms and mortality in humans. RVFv is known to circulate widely throughout East Africa; serologic evidence of exposure has also been found in some northern African countries, including Mauritania. This study aimed to ascertain whether RVFv is circulating in regions beyond its known geographic range. Samples from febrile patients ($n = 181$) and nonfebrile healthy agricultural and slaughterhouse workers ($n = 38$) were collected during the summer of 2014 and surveyed for exposure to RVFv by both serologic tests and PCR. Of the 219 samples tested, 7.8% of nonfebrile participants showed immunoglobulin G reactivity to RVFv nucleoprotein and 8.3% of febrile patients showed immunoglobulin M reactivity, with the latter samples indicating recent exposure to the virus. Our results suggest an active circulation of RVFv and evidence of human exposure in the population of Tunisia.

Keywords: Arbovirus, seroprevalence, Tunisia, unexplained acute fever, vectors
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Introduction

Rift Valley fever virus (RVFv) is an arthropod-borne *Phlebovirus* in the family *Bunyaviridae,* with widespread epidemiology throughout sub-Saharan African regions first reported in the early 1900s by a veterinary surgeon in Kenya [1]. The virus is maintained and transmitted by mosquitoes, mainly *Aedes* and *Culex* species [1,2]. Though the virus has been shown to be maintained in mosquito populations through vertical and horizontal transmission [3], there has also been evidence of its maintenance in mammalian reservoirs, and there remains the possibility that there are other uncharacterized wildlife reservoirs which allow transmission at the wildlife/domesticated livestock interface *via* mosquito [4].

The epidemiologic range of the virus has been increasing, with reported outbreaks in West Africa, Saudi Arabia, North Africa and southern Africa [5–9]. There is evidence that RVFv is capable of transmission through a varied repertoire of diverse anthropophilic mosquito species, primarily *Aedes* and *Culex* species [10]. Indications of increasing diversity of vector competence may lead to rapid expansions in the future, which may pose a risk to countries geographically farther from traditional areas considered endemic [11].

Increasing circulation of RVFv in competent mosquito populations is a common observation in endemic areas after increased rainfall; it is usually followed by increased transmission to domesticated animals, where it is capable of causing severe animal health problems such as abortion [11–13]. This

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can result in a significant economic burden on the affected communities, particularly in arid areas that rely on geographically limited agriculture. An increased prevalence in human disease is also observed, particularly amongst agricultural workers, who are at a higher risk of exposure *via* mosquito bite, and abattoir workers or butchers, who are at risk of exposure *via* contaminated blood when preparing meat from infected animals [13]. Exposure *via* the bodily fluids of infected animals and also *via* aerosolization of the virus during butchering has been previously suggested as a major route of exposure for high-risk groups [14] and may put an individual at higher risk of developing more severe clinical symptoms, such as haemorrhagic manifestations due to exposure to higher viral titres.

Rift Valley fever outbreaks are regularly reported in East Africa and have also been reported in Mauritania [8]. One recent study identified Tunisia as a high-risk country; the authors inferred that environmental conditions and the presence of the vector meant that an epizootic occurrence was possible if the virus was present—a constant and considerable risk throughout the year, with a particularly high probability in July, after the wettest months [15]. Previous studies looking for serologic evidence of exposure to Rift Valley fever in 2006–2007 found no positivity in the studied population [16]. Indeed, as a result of anthropogenic influences such as irrigation and well drilling, surface water levels in several arid regions have increased over the years, allowing the emergence of vector-borne diseases due to the wider distribution of competent mosquitos throughout the country [17], thus increasing potential risk since previous studies were performed in both human and animal populations. On the basis of mathematical modelling, Tunisia is considered at risk for RVFv [15]. In this study, we performed a seroepidemiologic survey to demonstrate circulation of RVFv in Tunisia.

Materials and Methods

Study sites

Tunisia covers a wide climatic range, from a Mediterranean climate, with its rainy winter, in the north to a Saharan climate in the south (Fig. 1). The northern part of the country is separated from the south by the Tunisian Ridge, a range of hills running northeast to southwest for approximately 220 km, marking the climatic boundary between the Mediterranean Sea to the north and the dry steppes of central Tunisia. Between the northern slopes of the Tunisian Ridge and the chains of hills bounding it on the south are extensive plateaus, called the High Tell. The Sahara is separated from the central steppe land by a series of salted areas called chotts.

Sample collection

Samples were collected from patients seeking care at hospitals who had reported unexplained acute fever ($n = 181$). Samples were also actively taken from abattoir workers ($n = 38$) in order to survey evidence of previous exposure amongst groups designated to be at high risk of infection. Most participants originated from the governorates of Sousse, Sfax and Mahdia (Fig. 1), and samples were collected during the summer of 2014. All participants were asked for history of tick and/or mosquito bites. Samples were collected by Vacutainer, and plasma was separated, frozen at −20°C and transported to Public Health England Porton Down, UK, for analysis. The study was conducted after receipt of ethical approval from the ethical committee (HHS-IRB 00008931; Farhat Hached University Hospital, protocol reference date 8 April 2013).

RNA extraction and PCR

Nucleic acid extraction was performed using a QiaAmp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions. Purified RNA was stored at −20°C until required. Amplification was performed using a previously published assay [18] as follows: forward: 5′-AAAGGAACAATGGACTCT GGTCA-3′; reverse: 5′-CACTTCTTACTACCATGTCCTC-CAAT-3′, probe 5′-6FAM AAAGCTTTGATATCTCTCAGTG CCCCAA BHQ1-3′. Reverse transcriptase (RT)-PCR assays were conducted in one step using the Superscript III quantitative real-time PCR (qRT-PCR) kit (Life Technologies) according to the manufacturer's instructions with cycling conditions of 50°C for 10 minutes and 95°C for 2 minutes, followed by 40 cycles of 95°C for 10 seconds and 60°C for 40 seconds, followed by holding at 40°C for 2 minutes. Data acquisition was performed using the ABI 7500 Real-Time PCR machine (Applied Biosystems) with 45 analysed cycles with a threshold of 0.05 and data analysed using the ABI 7500 on-board software.

Indirect immunofluorescence assay

Sera were tested for the presence of antibodies reactive to RVFv using commercially available indirect immunofluorescence testing kits (Euroimmun). All serum samples were tested for the presence of RVFv by qRT-PCR before serologic testing under Containment Level 2 conditions. In brief, sera samples were diluted 1/100 and incubated on irradiated/fixed RVFv infected and noninfected Vero cells for 60 minutes at room temperature before washing five times for 5 minutes in sample buffer including 0.1% Tween 20. Antibodies binding to the infected cells were detected and measured through a secondary antibody labelled with fluorescein isothiocyanate; 1/100 was set as the minimum cutoff. For immunoglobulin (Ig) M measurement, the process was modified by initial dilution of sera in EUROSORB IgG removal