Serological evidence of Rift Valley fever virus among humans in Mersin province of Turkey

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ABSTRACT

Background & objectives: Rift Valley fever virus (RVFV) is a vector-borne pathogen that causes serious outbreaks among livestock, and severe symptoms and mortality in humans. The virus is known to be widespread throughout African countries and Arabian peninsula. The aim of the present study was to investigate the seroprevalence of RVFV infection among human populations of Mersin province, Turkey.

Methods: A region-wide serological survey was conducted on humans residing in rural and urban areas of Mersin province located in the subtropical mediterranean region of Turkey from July 2011– January 2014. Plasma samples were tested for the presence of anti-RVFV antibodies using commercially available indirect immunofluorescence assay.

Results: The overall past infections were detected in 48 (4.9%) of the 977 human blood samples. The RVF virusspecific IgG positivity was detected in 33 (4.9%) of the 677 blood samples obtained from the urban area and in 15 (5%) of the 300 samples obtained from the rural area. There was no statistically significant difference in the distribution of RVFV IgG positivity rates between urban and rural areas (p = 0.933); though difference was significant between the rural areas (p = 0.029).

Interpretation & conclusion: The study confirmed for the first time, the presence of the RVFV antibody in the urban and rural areas of mediterranean province of Mersin in Turkey, suggesting wide circulation of RVFV in the human population.

Key words Rift Valley fever virus; Mersin seroepidemiology; Turkey

INTRODUCTION

Rift Valley fever virus (RVFV) is an emerging vector-borne zoonotic pathogen which belongs to the *Bunyaviridae* family, *Phlebovirus* genus¹. The virus affects humans and ruminants, and hence the disease is important for public health. In ruminants, RVFV is characterized by an abortion or fetal malformation in pregnant animals and neonatal mortality². The majority of human cases can be asymptomatic but disease spectrum can range from a mild self-limiting febrile illness to a severe disease with retinitis, encephalitis and hemorrhagic fever^{3–} ⁴. In rare cases, death can also occur³. It has been known that RVFV has been responsible for extensive and severe destroying outbreaks throughout the Africa and Arabian Peninsula since many years ago⁵.

The environment, especially high rainfall seems to be an important risk factor for periodic RVF epidemics (escalating vector mosquito populations) in Africa and the Middle East^{6–7}. The virus was first described in early 1930s in Kenya¹ and is known to be widespread in many African countries. In particular, new outbreaks in the Middle East have revealed the potential for the disease to expand beyond the African continent⁸. The latest major outbreak occurred in 2016 in Republic of Niger⁹, which reported a total of 348 cases and 33 deaths due to RVF in humans.

The RVFV transmitted primarily by *Aedes* and *Culex* mosquitoes¹⁰, but could also be transmitted with infected tissues and body fluids of animals by direct contact. Therefore, veterinarians and farmers are particularly affected by the disease¹¹. Possible circulation of the RVFV is also reported among the endemic areas⁷.

Confirmative diagnosis of the disease is carried out by conventional virus isolation using cell culture, detection of IgG and IgM antibody with enzyme-linked immunosorbent assay (ELISA), immune fluorescent antibody (IFA) test or serum neutralization test; and by detection of virus RNA with reverse transcriptase polymerase chain reaction (RT-PCR) assay. Crossreactions at sero group level are likely to occur with other members of the *Phlebovirus* genus¹².

Despite the occurrence of several epidemics of RVF during the past decades below the Saharan barrier and in the countries of the Arabian Peninsula, RVFV is not present in the Mediterranean countries. However, RVFV

TURKEY

More

is representing an emerging health threat for the Mediterranean Basin and neighboring countries¹³. Increasing distribution range of the virus, presence of competent vector species in regions known to RVFV-free, such as Europe¹⁴, climatic changes, vector ecology, demographic characteristics, land-use variations, and globally extensive human travel have been allowing introduction of the disease to new geographical areas⁶.

The abundance and diversity of mosquito species in various regions of Turkey, in and around residenctial areas, and livestock, makes Turkey ideal for circulation of vector-borne viruses^{15–16}. No seroepidemiological study has been carried out on humans about the presence and distribution of RVFV in the subtropical southern regions of the country where the research was conducted. There is a large unawareness in the region regarding the understanding of the circulation of the virus. Hence, this study was carried out to investigate RVFV infection serologically in human populations residing in the urban and rural areas of Mersin province located in the subtropical Mediterranean region of Turkey.

MATERIAL & METHODS

Study samples

In this prospectively planned study, selection of the cases according to the regions was made by stratified sampling method. Between July 2011 and January 2014, in total 977 blood samples were collected, which included 677 people living in urban areas or dealing with animal husbandry or farm animals; and 300 people living in rural areas of Mersin province, Turkey. In the province, four localities in the urban area and five localities in the rural area were selected.

The blood samples were collected from these localities according to the districts as—Urban districts: Akdeniz–214, Toroslar–186, Yenisehir–162, Mezitli–115; and Rural districts: Tomuk–175, Kulak–48, Bahsis–38, Elvanli–34 and Kosebalci–5 (Fig. 1). Blood samples were drawn into EDTA-containing tubes and were centrifuged at $4.000 \times \text{g}$ for 15 min for plasma separation. The plasma at the top of the tube was transferred into a clean micro centrifuge tube and was stored at –80 °C until serological examination.

Detection of IgG antibodies against RVF virus

Plasma samples were examined for the presence of anti-RVFV IgG antibodies using commercially available indirect immunofluorescence test (Anti-Rift Valley fever virus IIFT [IgG], catalog no. FI 280a-1010 G, EUROIMMUN, Germany). Test and evaluation proce-

Elvanli Tomuk

Fig. 1: Geographic representation of the areas from where blood samples were collected in Mersin province, Turkey.

dure were carried out in line with the recommendations of the manufacturer. In brief, plasma samples were diluted at a rate of 1/100; and incubated with irradiated/ fixed RVFV infected and non-infected Vero cells for 30 min at room temperature. The slides were then washed five times for 5 min in washing buffer (0.1% Tween 20). Antibodies binding to the infected cells were detected and measured through a secondary antibody labeled with fluorescein isothiocyanate. Slides were mounted in mounting media and viewed under a fluorescent microscope at 495/517 nm. Positive and negative controls provided with the commercial kit were included in each slide.

Statistical analysis

The statistical analysis of the results obtained in the study was performed with statistical package for social sciences (SPSS) package for Windows ver. 11.5.0. In the statistical evaluation of the study data, categorical data were calculated as frequency and percentage, and continuous data as mean \pm standard deviation. Statistical evaluation was performed with Chi-square and Likelihood Ratio test from the cross-table statistics. Because the age did not show normal distribution, they were summarized with median, first and third quartile values and also with values of minimum–maximum mean and standard deviation. Statistical significance was defined by a *p*-value of <0.05.

Ethical statement

The study was reviewed and approved by the local ethics commission of Mersin University Clinical Research Ethics Committee (Approval No. 2013/320; dated 26 september 2013). Written informed consent was obtained from participants prior to their enrolment in the study.

374

RESULTS

Anti-RVF virus IIFT (IgG)

IgG class antibodies were found positive in 48 (4.9%) of the 977 blood samples and negative in the remaining 929 (95.1%) samples. The RVF virus-specific IgG positivity was 4.9% (33/677) in the urban samples and 5% (15/300) in the rural samples. There was no statistically significant difference in the distribution of RVFV IgG positivity rates between urban and rural areas (p = 0.933) (Table 1).

The median age of study samples was 35 yr (minmax: 15–81 yr, Q_1-Q_3 : 28–45 yr), 811 of them were males, and 166 were females. The samples from urban area consisted of 619 (91.4%) males and 58 (8.6%) females with a median age of 33 yr (min-max: 18–71 yr, Q_1-Q_3 : 27–42 yr); and the samples from rural area consisted of 193 (64.3%) males and 107 (35.7%) females with a median age of 40.50 yr (min-max: 15–81 yr, Q_1-Q_3 : 28–52 yr).

The median age of the IgG-positive population (n = 47) was determined as 38.50 yr (min–max: 18–81 yr, Q_1-Q_3 : 29.75–50.75 yr). Anti-RVFV IgG positivity was 84.8% (28/33) in males and 15.2% (5/33) in females in urban samples. Anti-RVFV IgG positivity was 66.7% (10/15) in males and 33.3% (5/15) in females in rural areas. There was no statistically significant difference in RVFV IgG positivity according to gender between urban (p = 0.581) and rural area (p = 0.321) (Table 1).

Anti-RVFV IgG positivity was found in 9 of the 115 (7.8%) samples collected from Mezitli district, 13 of the 186 (7%) samples from Toroslar, 8 of the 214 (3.7%) samples from Akdeniz, and in 3 of the 162 (1.9%) samples from Yenisehir in urban areas (Table 2). There was no statistically significant difference between the RVFV IgG positivity according to the urban areas (p = 0.141). In the urban area, 16 (2.4%) cases were evaluated as 1-positive, 9 (1.3%) were 2-positive, 6 (0.9%) were weak positive and 1 (0.1%) was 3-positive in IgG-positive 33 cases.

Table 2. Distribution of RVFV seropositivity in urban and rural areas of Mersin, Turkey (2009–2014)

Region	Anti-RVFV IgG				
	Negative	Positive	Total		
Urban areas					
Akdeniz	206 (96.3)	8 (3.7)	214		
Mezitli	106 (92.2)	9 (7.8)	115		
Toroslar	173 (93)	13 (7)	186		
Yenisehir	159 (98.1)	3 (1.9)	162		
Total	644 (95.1)	33 (4.9)	677		
Rural areas					
Elvanli	31 (91.2)	3 (8.8)	34		
Tomuk	172 (98.3)	3 (1.7)	175		
Bahsis	34 (89.5)	4 (10.5)	38		
Kulak	43 (89.6)	5 (10.4)	48		
Kosebalci	5 (100)	0 (0)	5		
Total	285 (95)	15 (5)	300		

Figures in parentheses indicate percentages.

In rural area, anti-RVFV IgG positivity was found in 4 of the 38 (10.5%) samples collected from Bahsis district, 5 of the 48 (10.4%) samples from Kulak, 3 of the 34 (8.8%) samples from Elvanli, and 3 of the 175 (1.7%) samples from Tomuk. IgG positivity was not found in 5 blood samples collected from Kosebalci (Table 2). In rural area, 3 (1.0%) cases were evaluated as 1-positive, 3 (1.0%) were 2-positive and 9 (3.0%) were weak positive in IgG-positive 15 cases. According to these results, there was a statistically significant difference in RVFV IgG positivity between to the rural areas (p = 0.029). Specifically, the percentages of positives in the Bahsis (p = 0.023) and Kulak (p = 0.014) were found significantly higher than the Tomuk.

DISCUSSION

Rift Valley fever disease has significant impact on public health and economy both in medical and veterinary terms in the regions where it occurs. Due to the pres-

Table 1. Comparison of RVFV seroprevalence for persons living in urban and rural areas of Mersin province, Turkey during 2009–2014

Samples, n = 977		Anti-RVFV IgG				
	Gender	Negative	Positive	Total		
Urban area, n = 677	Male Female	591 (91.5) 53 (8.5)	28 (84.8) 5 (15.2)	619 (91.1) 58 (8.9)		
	Total	644 (100)	33 (100)	677 (100)	0.933	
Rural area, n = 300	Male Female	183 (64.2) 102 (35.8)	10 (66.7) 5 (33.3)	193 (64.3) 107 (35.7)		
	Total	285 (100)	15 (100)	300 (100)		

Figures in parentheses indicate percentages.

ence of a wide range of host and vector species, it is spreading from commonly reported areas to non-endemic newer regions; and therefore, it should be monitored carefully in both endemic and in previously unaffected regions¹⁷. In Turkey, researches related to vector-borne viral infections are very limited. To our knowledge, this is the first serological investigation of RVFV among human populations in urban and rural regions of Mersin province. In this study, IgG class antibodies were found positive in 4.9% (48/977) blood samples. There was no statistically significant difference in the distribution of RVFV IgG positivity rates between urban and rural areas [4.9% (33/677) in urban region vs. 5% (15/300) in rural region, p = 0.933]. This may be explained by the urbanization of the selected rural areas. Also, it could be attributed to the presence of the infected vector thought to be responsible for the spread of the virus.

376

This finding is not unexpected with RVFV, because sandfly fever viruses belong to the *Phlebovirus* genus, and have been reported previously in this region. Frequent exposure to sandfly fever sicilian virus (SFSV) / sandfly fever cyprus virus (SFCV) or antigenically similar *Phlebovirus* strains and viruses of the sandfly fever naples virus (SFNV) species were reported previously from healthy blood donors in Mersin province¹⁸.

The RVFV infections are considered as a major threat to the human and animal health in many of the world nations where competent mosquito vectors are endemic¹⁹. Several vector surveillance studies were conducted for monitoring the circulation of arthropod-borne diseases important for human or animal health in Turkey. Outbreaks of West Nile virus transmitted by the same vector species are repeatedly reported in the Mediterranean basin and also in Turkey²⁰. Vectors that are responsible for the transport of the RVFV or other arbovirus had been abundantly reported in Turkey. In one of those, Culex pipiens made up 56% of the total species and Aedes spp. accounts for 20% of the total species collected from the southeastern Anatolia region of country¹⁶. The species composition was described in the south of Turkey in 1997 and the most abundant species was Cx. pipiens (26.7% of total catch) followed by Cx. tritaeniorhynchus (23.8%), Ae. caspius (23.4%), Ae. cretinus (10.7%), Ae. dorsalis (8.7%), Cs. annulata (6.2%), and Ae. vexans $(0.1\%)^{21}$.

There are only two reports of RVFV on animals in Turkey. The evidence of RVFV antibodies in humans obtained from a cohort of children with fever and/or arthritis was firstly revealed as 3.64% using indirect IgG ELISA in Turkey; it was 4.5% in cattle and 3.75% in sheep²². In one of the animal studies that was conducted

earlier, aborted fetuses (cattle, sheep and goat) in northern region of Turkey were investigated for the presence of RVFV RNA by RT-PCR; wherein none of them were found positive for RVFV²³. In an another study in the northern Turkey, RVFV antibodies were investigated with ELISA in different mammalian species (cattle, horse, goat, sheep and water buffalo) and none of the animals were reported to have antibodies to RVFV²⁴. Though, RVFV antibodies was reported in one (1.3%) of 72 camels in the Aegean region and 35 (8.5%) of 410 buffalo samples in central, northern, western regions of Turkey²⁵.

Human cases of RVFV were never reported in Morocco, Algeria, Tunisia and Libya (the Maghreb region); so RVF is considered as a disease of great concern in this Mediterranean basin of Africa. Therefore, establishing the epidemiological situation of this disease because of the potential for the future emergence has become a public health priority of management of surveillance activities and prevention of the expansion into new geographic areas²⁰. Arsevska *et al*²⁶ reported that the northern borders of the Maghreb were moderately suitable for RVF enzootics; however, they were highly suitable for RVF epizootics according to a multicriteria decision analysis. Moreover, the evidence of active circulation of RVFV and human exposure was demonstrated in the febrile patients and non-febrile healthy agricultural and slaughterhouse workers in Tunisia, northern Africa²⁷. Therefore, Turkey is remarkable for its close proximity to African Mediterranean countries and constitutes a bridge with Europe.

In Egypt, the primary RVFV vector was noted as *Culex* mosquito and RVFV has been identified in human cases, livestock, and vector mosquitoes in the Nile Delta of Egypt during the summer of 2003 at the outbreak which developed from febrile illness and encephalitis²⁸. It was also reported that the Middle East and North Africa region that encompasses Jordan, Syria, and Israel are also under continuous threat of the WNV and RVFV on account of its ubiquitous vector *Culex* spp²⁹.

The seroprevalence of RVFV was reported as 44.2% in sheep and 25.1% in goats in inter-epidemic period from Zambezia, a part of Mozambique and no noticeable clinical signs of RVFV in the investigated herds were reported³. A serosurvey, which was conducted in non-vaccinated livestock in Egypt during inter-epidemic periods, suggested that the overall seroprevalence was 2.29% ranging from 0% in goats, 0.46% in sheep, 3.17% in camels, and up to 5.85% in buffalos³⁰. Swai and Sindato³¹ showed that in the northern Tanzania, the prevalence of

RVFV was 27.5% from apparently healthy, nonvaccinated camels. Therefore, for the disease surveillance and prevention of transmission to humans, it is very important to monitor the RVFV circulation in interepidemic period and in livestock.

Pourrut et al³² showed an overall RVFV prevalence of 3.3% in the forested zones 2.9% in savannas, 2.2% and in lakes region, and 8.3% in Gabon rural populations. These results show that lake region has a potential public health threat about virus circulation by creating convenient sites for mosquito vectors. In a study, Kenya anti-RVFV IgM prevalence was reported as 18% of the persons enrolled. The most important risk factor in a cross-sectional study in for RVFV infection was proposed direct contact with sheep blood or body fluids³³. Seufi and Galal³⁴ reported that housewives followed by farmers, and students were more vulnerable to the infection during RVF outbreak in 2007 in Sudan. Arum et al35 demonstrated a relation with variation in RVF vector abundance and ecological zones, which indicates potential risk areas for RVF transmission and circulation. Especially the abundance of the specific vectors is attributed to the nature of the land, soil types (wet or dry), and amount of rainfall³⁶.

In the present study, the distribution of anti-RVFV IgG antibody was found considerably high in Mezitli (7.8%) and Toroslar districts (7%), followed by Akdeniz (3.7%) and Yenisehir (1.9%); however, no statistically significant difference was detected in the urban areas (p = 0.141). In the rural areas, the distribution of anti-RVFV IgG positivity showed variations. The highest antibody positivity was detected in Bahsis (10.5%) and Kulak districts (10.4%) followed by Elvanli (8.8%), Tomuk (1.7%) and Kosebalci (0%); which were found to be statistically significant (p = 0.029). Rural areas are important for serologic investigation of RVF, because the ecological conditions are suitable for mosquito vector species. In this study also, the blood samples were collected, from the areas localized near the wetlands from human rural population living in contact with livestock. The findings of this study highlight the risk of RVFV for seronegative human rural population.

The detection of antibodies in RVFV with serological techniques has been widely used for RVFV surveillance. But, it is necessary to exclude cross-reactive antibodies between RVFV and genetically related other phleboviruses in order to establish actual exposure with virus neutralization test, which is approved as the gold standard serological method. However, because of the required live virus, this test can only be carried out in laboratories with appropriate biosafety level^{2, 37}. The kit used to detect the RVFV-specific IgG antibodies (EUROIMMUN Anti-Rift Valley fever virus IIFT [IgG], Germany) identifies antibodies against the RVFV; and the sensitivity and specificity for IgG are 90 and 99%, respectively. The IgG positivity rates determined in this study reflect the first epidemiological data from Mersin region. Although the data were not corrected in terms of cross-reactive antibodies, the results of this study show that exposure with a virus belongs to the *Phlebovirus* genus.

The limitation of our study was the anti-IgM RVFV antibodies not being performed in all the cases. However, the demonstrated seropositivity for anti-IgG RVFV antibodies should be accepted as evidence for exposure to RVFV. To understand the RVFV transmission in Turkey, additional studies involving different vector mosquito species, livestock and humans with larger study samples and area using more sensitive methods like PCR need to be explored.

CONCLUSION

In summary, established RVFV seroprevalence in human population suggests viral circulation and exposure in Mersin, Mediterranean basin of Turkey, in spite of any report of concern to clinical disease in susceptible hosts. It is important to monitor the RVFV circulation in the animals and humans in Turkey for the early detection of epidemic occurrence of the disease and prevention of transmission with the virus. Therefore, our results could be supported with animal survey, vector dynamics and cross-reactive antibodies of other phleboviruses to elucidate the fact exposure.

Conflict of interest

All the authors declare that they have no conflict of interest.

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378

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