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Full Length Research paper

A seroepidemiological study of rift valley fever virus among slaughter houses' workers in Saudi Arabia

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The aim of this study was to determine the activity of the Rift Valley Fever Virus (RVFV) in non-exposed regions in Saudi Arabia following the outbreak that had occurred in the southwestern region of Saudi Arabia in 2000 - 2001. An Enzyme-Linked Immunosorbent Assay (ELISA) was used to detect anti-RVFV IgG antibodies among subjects working in abattoirs or adjacent livestock holding yards as a high risk group and healthy blood donors as a control group in seven non-epidemic regions of Saudi Arabia (Jeddah, Makkah, Al-Madina, Riyadh, Taif, Dammam, Yanbou). The serological tests were carried out at the Special Infectious Agents Unit, Biosafety Level 3, King Fahd Medical Research Centre, at King Abdulaziz University, Jeddah. A total of 1256 high-risk subjects (working in abattoirs or adjacent livestock holding yards) and 1216 blood donors were studied. Only 9 of 1256 (0.72%) high risk subjects and 2 of 1260 (0.16%) blood donors were RVFV IgG positive. All nine high risk RVFV-IgG positive subjects were of Bangladeshi nationality, whereas the two positive donors were of Saudi nationality from Dammam city. High risk practice that increases the risk of exposure to RVFV infection at work or at home was also studied. The reason for positive RVFV-IgG in the two supposedly-low risk blood donors was likely an old exposure to the virus in the epidemic regions (Jazan, Tihama of Asir, and Al-Qunfuda) back in 2000 - 2001. This could not be confirmed as it was not possible to contact these two donors to inquire about a past history of visiting any of the epidemic regions. The study confirms that the RVF epidemic in Saudi Arabia was confined to the epidemic regions (Jazan, Tihama of Asir, and Al-Qunfuda) with no serological evidence of spread of the infection among human subjects living outside the epicenters. The preventive measures undertaken at the time of the epidemic and thereafter by the concerned parties such as the Ministry of Health, Ministry of Agriculture, and Ministry of Municipality, clearly prevented the spread of RVFV to the rest of Saudi Arabia.

Key words: Rift valley fever virus, epidemic, Saudi Arabia.

INTRODUCTION

Rift valley fever (RVF) virus is an arthropod-borne virus that periodically causes epidemics and epizootics in the African continent (Vialat et al., 2000). It causes severe disease in both animals and humans leading to high mor-bidity and mortality. It also results in substantial economic losses due to death of RVF-infected livestock (WHO, 2000). The disease was first identified in sheep in Kenya during 1931 (Daubney et al., 1931), it is endemic almost everywhere in subtropical Africa (Garcia et al., 1989; Vialat et al., 2000). Epidemics of RVF were limited to the African continent until 2000, when an epidemic occurred simultaneously in southwestern Saudi Arabia (Jizan, Asir and Al-Qunfuda) and the neighboring north-western regions in Yemen (Madani et al., 2003). A total of 886 RVF human cases were reported in Saudi Arabia (Madani et al., 2003). The date of onset of the first case was 26 August 2000, and the last case was on 22 September 2001, after which no cases were reported till the writing of this paper in October 2009.

The objective of this study was to determine the extent of spread of the RVF virus outside the epicenters in

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Table 1. Demographic data of the study population.

Demographic variables	Slaughterhouse workers (%)	Blood donors (%)
Number	1256	1260
Age (mean ± SD), in years	34.07± 7.60	40 ± 13.71
Sex		
Male	1256 (100)	1260 (100)
Female	0	0
Nationality		
Saudi	9 (0.71)	1151 (91.3)
Non-Saudi	1247 (99.28)	109 (8.65)
Bangladesh	712 (56.68)	8 (0.63)
India	146 (11.62)	17 (1.34)
Egypt	121 (9.63)	0
Yemen	71 (5.65)	0
Pakistan	19 (1.51)	20 (1.58)
Sudan	29 (2.30)	0
Turkey	15 (1.19)	0
Srilanka	6 (0.47)	0
Other	137 (10.90)	64 (5.07)
Location		
Jeddah	361 (28.74)	205 (16.50)
Riyadh	240 (19.108)	161 (12.77)
Almadina	174 (13.8)	197 (15.6)
Makkah	165 (13.13)	158 (12.5)
Dammam	128 (10.19)	219 (17.38)
Yanbu	120 (9.5)	155 (12.30)
Altaif	56 (4.45)	163 (12.9)
Baha	12 (0.95)	0

Saudi Arabia following the RVF epidemic in southwestern region in2000 - 2001 (Franz et al., 1997; Garcia, 2001).

METHODS

From September 2004 to April 2006, blood specimens were collected from subjects working in slaughterhouses or adjacent livestock holding yards as a high risk group and healthy blood donors as a control group in seven non-epidemic regions of Saudi Arabia (Jeddah, Makkah, Al-Madina, Riyadh, Taif, Dammam, and Yanbou).

An Enzyme-Linked Immuno-sorbent Assay (ELISA) (Biological Diagnostic Supplies Limited, UK) was used to detect anti-RVFV IgG antibodies in the collected specimens (Ibrahim et al., 1997; Sail et al., 2002). The tests were carried out at the Special Infectious Agents Unit, Biosafety Level 3, King Fahd Medical Research Centre, at King Abdul-Aziz University, Jeddah. The test was performed according to the manufacturer's instructions. Briefly, mouse (mouse anti-RVFV antibody) was adsorbed onto ELISA plates (Nunc, Denmark) overnight at +4°C. After washing, plates were blocked with 10% skimmed milk in PBS for 1 h at 37°C. Test and control sera were mixed with antigen. This serum-antigen mixture was then added to the wells and incubated, followed by mouse antivirus antibody incubation. Incubation (it should be anti-mouse IgG HPRO-conjugate: reviewer) was performed before the addition of ABTS and stop solution. Optical densities (OD) were measured at 405 nm. The result was determined once the run was accepted according to the specified criteria by manufacturer's after defining the net optical density values for each serum as the value determined with RVFV Ag minus the value of the control Ag, which was used in subsequent calculations of percentage positivity (PP) of the controls and tested serum using the equation:

PP= Net OD (C+, or C-, or test serum/ net mean OD of C++) × 100.

GraphPad software (by GraphPad Software Inc, 2002 - 2005) was used for data analysis. The unpaired t-test was used to compare means for continuous data. Comparison of proportions (categorical data) was by Fisher's exact test.

RESULTS

A total of 2516 samples were collected during the study period; 1256 samples from slaughterhouses' workers in eight cities and 1260 samples from blood donors in seven cities within Saudi Arabia. Table 1 shows the demographic features of the study population.

Table 2. Slaughterhouse workers and blood donors who were positive for RVF-IgG.

Variables	Slaughterhouse workers (%)	Blood donors (%)
Total number screened	1256	1260
Total no. positive for RVF-IgG	9/1256 workers (0.72)	2/1260 donors (0.16)
Location of subjects with positive RVF-IgG		
Riyadh	4 (0.31)	0
Jeddah	2 (0.15)	0
Makkah	1 (0.079)	0
Almadina	1 (0.079)	0
Altayef	1 (0.079)	0
Dammam	0	2 (0.15)
Nationality		
Bangladesh	9/9 (100)	0

Of the 1256 samples collected from slaughterhouses' workers, 165 samples were from Makkah, (from two slaughterhouses), 174 samples from Madinah (from five slaughterhouses), 240 samples from Riyadh (from five slaughterhouses), 361 samples from Jeddah (from five slaughterhouses), 128 samples from Dammam (from one slaughterhouse), 56 samples from Taif (from four slaughterhouses), 12 samples from Baha (from one slaughterhouse), and 120 samples from Yanbu (from two slaughterhouses). Of the 1256 workers, 1247 (99.3%) were non-Saudi and the remaining 9 workers were Saudis who worked only in post-butchering preparation of meat-foods and meat marketing. Only 9 out of 1256 (0.72%) specimens tested from slaughterhouses' workers were positive for specific RVFV IgG; four workers from Riyadh, two workers from Jeddah, and one worker each from Makkah, Almadina, and Altaif. None of the workers in Dammam, Baha and Yanbu was positive for RVFV IgG.

A total of 1260 samples were collected from blood donors in seven cities within Saudi Arabia, namely, Makkah (158 donors), Madinah (197 donors), Riyadh (161 donors), Jeddah (205 donors), Dammam (221 donors), Taif (163 donors), and Yanbu (155 donors). Of the 1260 blood donors tested, 1151 (91.3%) were Saudi citizens and 109 donors were non-Saudis (Table 1). All collected samples showed no specific IgG antibodies against RVFV, except for two samples from two Saudi blood donors in Dammam. Unfortunately, information regarding whether they were previously exposed to RVF or vaccinated against RVF and whether they resided in or visited an epidemic area (e.g. Jizan city) was not available.

Table 2 shows the demographic features of the nine slaughterhouses' workers and the two blood donors who were positive for RVFV IgG. Table 3 shows various risk factors associated with RVFV positive serology among slaughterhouses' workers.

DISCUSSION

It was speculated that the RVF virus was introduced into the Arabian Peninsula in 1997 - 1998 during the RVF epidemic in east Africa via introduction of infected imported livestock or via windborne infected mosquitoes and that climatic conditions had promoted sufficient vector populations to support transmission in Saudi Arabia and Yemen (Madani et al., 2003). Two lines of evidence supported this speculation. First, the epidemic began spontaneously in geographically diverse areas in Saudi Arabia and Yemen, suggesting that dissemination of the virus probably occurred before the epidemic period (Madani et al., 2003). Second, the genetic sequence of the virus isolated in Saudi Arabia and Yemen was closely related to that of the virus isolated in the 1997 - 1998 outbreaks in east Africa (Shoemaker et al., 2002). This virgin-soil epidemic in the Arabian Peninsula emphasized the threat of the introduction of the virus into other parts of Saudi Arabia or the rest of the world (Madani et al., 2003; Niklassonet al., 1984; Shoemaker, et al, 2002).

Extensive measures to prevent its spread outside the epicenters in Saudi Arabia were undertaken by the concerned sectors such as the Ministry of Health, the Ministry of Agriculture (responsible for animal health), and Ministry of Municipality. As part of these preventive measures, movement of animals in and out of the epicenters was prohibited for almost 3 years from the onset of the epidemic, and livestock animals were vaccinated with a live attenuated vaccine, (Paweska et al., 2003). Even though, no clinical cases of RVF among humans were reported from regions outside the epicenters in Saudi Arabia since the last case was reported in 22 Septemebr 2001, there was always a concern of the possibility of spread of this infection to other regions in Saudi Arabia where the environment and climate was conducive for establishment of transmission.

This study showed that only 9 of 1256 (0.72%) high risk

Table 3. Risk factors for RVF-IgG positivity among slaughterhouses' workers.

Variables	RVF-IgG positive workers (%)	RVF-IgG negative workers (%)	P value (two tailed)
Number	9	1247	
Mean duration of work in the slaughterhouse ± SD (years)	4.22 ± 2.98	3.56 ± 2.53	0.4362
Number of animals contacted by the worker per day			
3 - 5 animals	7 (77.8)	762 (61.1)	0.4954
6 - 10 animals	2 (22.2)	462 (37)	0.4982
>10 animals	0	25 (2)	1.0000
Type of contact with animals			
Slaughtering & butchering	8 (88.9)	1122 (89.9)	1.0000
Skinning	8 (88.9)	1139 (91.3)	0.1800
Veterinary inspection	6 (66.7)	955 (76.5)	0.4459
Cleaning of meat	7 (77.8)	967 (77.5)	1.0000
Cleaning of workplace	9 (100)	1247 (100)	1.0000
Milking of animals	0	16 (1.2)	1.0000
Delivery of meat	0	20 (1.6)	1.0000
Work safety and hygiene reported by the workers			
Performing hand washing	9 (100)	1111 (89)	0.2537
Wearing gloves	1 (11)	328 (26.3)	0.4596
Wearing gowns	8 (88.9)	1176 (94.3)	0.4132
Wearing boots	9 (100)	1231(98.7)	1.0000
Not placing knife in the mouth	1 (11.1)	154 (12.3)	1.0000
Average number of wounds per day			
1 - 3	7 (77.8)	748 (59.9)	0.1681
4 - 6	1 (11.1)	459 (36.8)	0.1671
> 6	1 (11.1)	49 (3.9)	0.3071
No. of workers reporting mosquitoes at workplace	7 (77.7)	901 (72.2)	0.7140
No. of workers reporting ticks at workplace	0	182 (14.5)	0.3727
No. of workers reporting contact with animals at home	2 (22.2)	303 (24.2)	1.0000
Health status of workers			
Healthy	9 (100)	1177 (94.3)	1.0000
DM	0	19 (1.5)	1.0000
Chronic liver disease	0	12 (0.96)	1.0000
Chronic heart disease	0	13 (1.04)	1.0000
Chronic renal disease	0	14 (1.1)	1.0000
Location			
Riyadh	4 (44.4)	236 (18.9)	0.0736
Jeddah	2 (22.2)	359 (28.7)	1.0000
Makkah	1 (11.1)	164 (13.1)	1.0000
Almadina	1 (11.1)	173 (13.8)	1.0000
Altayef	1 (11.1)	55 (4.4)	0.3328
Dammam	0	128 (10.2)	0.6103
Baha	0	12 (0.96)	1.0000
Yanbu	0	120 (9.6)	1.0000

subjects and 2 of 1260 (0.16%) blood donors were RVFV IgG positive. All nine high risk RVFV-IgG positive subjects were of Bangladeshi nationality. High risk practice that usually increases the risk of exposure to RVFV infection at work or at home has no significant difference between the RVFV IgG-positive and IgG-negative workers. The extremely low positivity among slaughterhouses' workers suggested that the epidemic was contained in the epicenters. The reasons for the IgG positive serology in nine workers was not clear and may include exposure to animals illegally brought from epicenters or undisclosed visit to the epicenters. The reasons for positive RVFV-IgG in the two supposedly-low risk blood donors was likely an old exposure to the virus in the epidemic regions (Jazan, Tihama of Asir, and Al-Qunfuda) back in 2000 -20001. This could not be confirmed as it was not possible to contact these two donors to inquire about a past history of visiting any of the epicenters.

This study confirms that the RVF epidemic in Saudi Arabia was confined to the epidemic regions (Jazan, Tihama of Asir, and Al-Qunfuda) with no serological evidence of spread of the infection among human subjects living outside the epicenters. The preventive measures undertaken at the time of the epidemic and thereafter by the concerned parties such as the Ministry of Health, Ministry of Agriculture, and Ministry of Municipality, clearly prevented the spread of RVFV to the rest of Saudi Arabia.

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