



Epidemiological Study on Rift Valley Fever Virus among Humans in Taiz Governorate (Yemen)

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Abstract

Rift Valley Fever Virus (RVFV) is the etiologic agent of Rift Valley Fever (RVF), transmitted to humans by mosquitoes or direct contact with infected animals. This study was performed to preparing RVF antigen from liver of infected lamb to be used in detecting anti-RVFV antibodies by using Enzyme Linked Immunosorbent Assay (ELISA) and to study epidemiological prevalence of anti-RVFV IgM and IgG antibodies among humans which conducted in the Researches Center of Taiz University and Central Health Laboratory, Taiz Governorate, Southwestern Yemen. RVFV antigen was successfully prepared at a titer dilution of 1:3,200 by Chessboard (CB) ELISA assay. However, out of 234 samples, 154 samples were positive for ELISA assay, of which 49 (31.8%) were positive for anti-RVFV IgM and 105 (68.2%) for anti-IgG antibodies. The antigen was successfully produced, and successfully adhered to ELISA plates. Simple and inexpensive methods give good results. The result can be used to develop and refine predictive database for RVF transmission based on environmental and remote sensing data.

Keywords: Rift valley fever; Antigen; ELISA; Epidemiology; Yemen

Abbreviations

RVFV: Rift Valley Fever Virus; RVF: Rift Valley Fever; ELISA: Enzyme Linked Immunosorbent Assay; IgM: Immunoglobulin M; IgG: Immunoglobulin G; rpm: Round per Minute; CB: Chessboard; 3SD: Three Standard Deviation

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Introduction

RVFV is the causative agent of RVF, a zoonotic disease affecting both ruminants and humans [1]. In ruminants, it induces almost 100% mortality among young animals and a high rate of abortion in pregnant females [2]. In humans, severity of infection can vary from mild to very severe clinical symptoms, including fever, blindness, encephalitis, and hemorrhagic fever with a fatal outcome [2,3]. It is transmitted to humans by mosquitoes, direct contact with infected animal tissues and products [4].

RVF is an endemic disease in Africa and the Arabian Peninsula, the outbreak was appeared for the first time in the Saudi Arabia and Yemen in 2000 to 2001. In Saudi Arabia, reported 11,882 human cases with 164 deaths [5-7]. In Yemen, reported 1,080 human cases and 141 deaths [6-8], over 20,000 abortion animals and 620 dead [6,9]. Several outbreaks were followed after heavy rainfall in Egypt, Kenya, Somalia, Tanzania, Sudan, Mayotte, and Mauritania from 2003 to 2012 [10].

There are several methods that are used in RVF diagnosis Scott et al. [11] determined that the accuracy of serological methods in detecting anti-RVF antibodies was indicated that Enzyme Linked Immunosorbent Assay (ELISA) was the most precise of the other serological methods, so this study aimed to preparing RVF antigen from liver of infected lamb to be used in detecting anti-RVFV antibodies by using modern ELISA technique and to study epidemiological prevalence of anti-RVFV IgM and IgG antibodies among humans, and establishment primary database of RVF prevalence in Yemen.

Materials and Methods

A total of 234 persons who suffered from feverish, aged from 4 years to over than 50 years old,

Table 1: Epidemiological prevalence rate of anti-RVFV IgM and IgG among humans in relation to socio demographic characteristics.

Variables	ELISA assay of prepared antigen					
	Anti-IgM	Anti-IgG	Total	P-Value	df	Asymp. Sig.
	No. (%)	No. (%)	No. (%)			
Age Groups						
<10	0 (0.00)	2 (1.3)	2 (1.3)	0.396	1	0.529
10-20	2 (1.3)	13 (8.4)	15 (9.7)			
21-30	12 (7.8)	19 (12.3)	31 (20.1)			
31-40	20 (13.0)	36 (23.4)	56 (36.4)			
41-50	8 (5.2)	20 (13.0)	28 (18.2)			
>50	7 (4.5)	15 (9.7%)	22 (14.3)			
Total	49 (31.8)	105 (68.2)	154 (100)			
Localities						
Urban	25 (16.2)	52 (33.8)	77 (50.0)	0.03	1	0.863
Rural	24 (15.6)	53 (34.4)	77 (50.0)			
Sex						
Males	25 (16.2)	72 (46.8)	97 (63.0)	4.386	1	0.036
Females	24 (15.6)	33 (21.4)	57 (37.0)			
Occupation						
Shepherds	13 (8.4)	52 (33.8)	65 (42.2)	12.78	1	0.000
Employee	19 (12.3)	41 (26.6)	60 (39.0)			
Farmers	17 (11.0)	12 (7.8)	29 (18.8)			

No.: Number of cases; Asymp. Sig.: Asymptotic Significance; df: Degree of Freedom; P-Value: Possibility Value.

admitted in some hospitals of Taiz City, southwestern Yemen, during the period from January 2014 until August 2016. About 5 ml of blood were collected by venepuncture into sterile tube, allowed to clot, centrifuged (2,000 rpm, 10 min) for separation of serum, transferred into new sterile tube, inactivated in water bath at 56°C for 30 minutes, and stored in refrigerator at -20°C until used by ELISA [12,13].

Preparation of RVFV antigen

According to the information mentioned by Smithburn et al. [14] the concentration of RVFV was highest in the liver of infected animal. Based on this data, RVFV antigen was prepared from liver of lamb by using sucrose acetone extraction method according to methods described by Clarke and Casals [15].

Lamb inoculation, two lambs aged 2 months and 4 months, were inoculated intraperitoneally with 0.5 ml of infected human serum by RVFV containing 104 LD₅₀ (it was kindly supplied by the Department of Virology, Central Researches laboratories, Taiz Governorate) for each under strict control measures. Sufficient virus was present in serum of lambs after 4 days of inoculated, and the titer of virus in the serum was 1,600 when it seemed unsteady gait. No any lamb died during the virus incubation period.

Procedure for lamb liver derived sucrose acetone extracted RVFV antigen, 10 g of infected lamb Liver was put in sterile mortar, and thoroughly minced. Four volumes of 8.5% aqueous solution of sucrose (after sterilized by filtration) were added to the liver tissue, and mixed well until homogenized. One volume of homogenate was added to 20 ml of chilled acetone after vigorous shaking. The tightly stoppered bottles were centrifuged at 1,800 rpm for 5 minutes at 4°C. The supernatant fluid was aspirated, and chilled acetone equal to the aspirated amount was added to the sediment. The bottles were placed in an ice bath for at least one hour to dehydrate the

gummy sediment. The centrifugation process was repeated two times, and the supernatant was aspirated and completed drying the sediment by attaching vacuum pump through a filter flask to the bottle containing the sediment. Normal saline was added to the dried sediment in a volume equal to 0.4 volume of the original volume of the homogenate. The sediment was dissolved within 2 hours, and the solution was left overnight in refrigerator. After complete dissolving, the solution was centrifuged in a refrigerated centrifuge at 20,000 rpm for at least 30 minutes. The supernatant containing the viral antigen was inactivated with binary (20% sodium thiosulphate solution sterilized by autoclaving, used to neutralize the 2-Bromoethylamine hydrobromide action), kept in a bottle and frozen at -70°C until used.

Serological examination

Chessboard (CB) titration ELISA procedures for the prepared antigen was done according to Rose et al. [16]. The prepared antigen was subjected to evaluation of the binding activity against specific antibody molecules IgM, IgG, and subjected to serial 2-fold dilution in coating buffer (carbonate-bicarbonate, pH 9.6) as is described in the Annex, to obtain dilutions of 1:50; 1:100; 1:200; 1:400... etc. Row H was left empty as blank. 50 µl of each dilution was dispensed into horizontal rows A-G wells in polystyrene micro titer plates (Maxisorp; Nunk, Copenhagen, Denmark, plates), incubated overnight at 4°C, washed 4 times by washing buffer. 50 µl/well of blocking buffer was dispensed to all wells, incubated for 1 hour at 37°C, washed 4 times. Two reference sera (strong positive and known negative) were diluted 2-fold 1:10; 1:20; 1:40 and so on in diluting buffer. 50 µl of each dilution was added to vertical columns (1-10) while the columns (11-12) received 50 µl of the known negative serum sample, incubated at 37°C for (1 hour in titer IgG or 2 hours in IgM), washed 4 times. 50 µl of diluted horseradish peroxidase conjugated goat anti-human IgG or swine anti-human IgM (Sigma Chemical Co.) was added to all wells,

Table 2: Epidemiological prevalence rate of anti-RVfV IgM and IgG among humans in relation to severity of disease and season.

Variables	ELISA assay of prepared antigen					
	Anti-IgM	Anti-IgG	Total	P-Value	df	Asymp. Sig.
	No. (%)	No. (%)	No. (%)			
Severity of Disease						
Headache	19 (12.3)	86 (55.8)	105(68.2)	3.819	1	0.051
Vomiting	16 (10.4)	3 (1.9)	19 (12.3)			
Hemorrhagic fever	14 (9.1)	2(1.3)	16 (10.4)			
Ocular diseases	0 (00.0)	8 (5.2)	8 (5.2)			
Encephalitis	0 (00.0)	6 (3.9)	6 (3.9)			
Season						
Summer	23 (14.9)	41 (26.6)	64 (41.6)	1.973	1	0.16
Autumn	19 (12.3)	37 (24.0)	56 (36.4)			
Winter	0 (00.0)	4 (2.6)	4 (2.6)			
Spring	7 (4.5)	23 (14.9)	30 (19.5)			

No.: Number of cases; Asymp. Sig.: Asymptotic Significance; df: Degree of Freedom; P-Value: Possibility Value.

incubated (1 hour in 37°C), and washed 4 times. The substrate buffer was added to all test wells (50 µl/well), incubated at 37°C in dark place for 20 minutes. 50 µl of stopping buffer was added to all wells. The optical density was measured at 492 nm using plate readers. The highest dilution of antigen was done a value of 1.1 after 20 minutes of substrate incubation with the strong positive serum antibodies IgG while done a value of 1.2 with the strong positive serum antibodies IgM, and under 0.1 with negative serum was considered the optimal dilution of the antigen.

ELISA procedures for detection of RVFV IgM and IgG antibodies were done according to Voller et al. [17]. The serum samples were analyzed for detecting anti-RVfV IgM and IgG antibodies by using indirect ELISA assay. Ninety-six well polystyrene microtiter plates (Cooke M 29 AR; Dynatech plates) were coated with 100 µl/well (Row H wells were left empty as blank) of RVFV antigen diluted in coating buffer, covered, incubated at 4°C overnight, washed 3 times using washing buffer. 100 µl/well of blocking buffer was dispensed to all wells, incubated for 1 hour in moist chamber at 37°C for 30 minutes, washed 3 times. Serum samples were diluted (1:10 for test IgG or 1:40 for IgM) in diluting buffer, and 100 µl of each serum sample was added in duplicate. Each plate included a positive control serum (wells A1-A6) and a negative control serum (wells A7 to A12). Plates were incubated at 37°C for (1 h in test IgG or 2 hours in IgM). After being washed 3 times, 100 µl of horseradish peroxidase conjugate labeled goat anti-Human IgG or swine anti-human IgM (whole molecules of IgG and IgM diluted according to the manufacturer's recommendation, Sigma Chemical Co.) was added into each well, and incubated (1 hour in 37°C). The plates were washed 3 times, and blotted on paper towels. 100 µl/well of ortho-phenylenediamine hydrochloride substrate was added, and plates leaved for 20 minutes at 37°C in dark. The reaction was stopped through 10 minutes after adding 50 µl /well of stopping buffer. The plates were read by ELISA using dynatech plate reader at 492 nm. The samples with optical density values greater than three standard deviation (3SD) above the mean of the negative control serum samples (Cut-off) were considered positive.

$$\text{Cut-off} = X + 3\text{SD}$$

X=mean of negative control serum samples.

SD=Standard Deviation of negative control serum samples.

Statistical analysis

Statistical analyses of the data were performed using statistical software package SPSS version 16. The categorical variables were done using Chi-square test at a 99% confidence level, and a significance level of 0.05 was used to determine the relationships between the data collection and epidemiological prevalence rates. Asymptotic Significance ≤ 0.05 was considered to be significant.

Ethical approval

Ethical approval for this study was granted by the Microbiology Division, Biology Department, Faculty of Science, Sana'a University Ethical Committee. Permission to conduct the study was sought from Sana'a to Taiz Governorate authorities. Oral and written consent was obtained from all participants.

Results

The prepared antigen from liver of lamb was successfully produced and titrated by using specific IgM and IgG antibodies through CB ELISA titration assay. However, the highest titer dilution was investigated 1:3,200 by using CB titration ELISA assay for both IgM and IgG antibodies.

A total of 234 samples, 154 (65.8%) of cases were positive for ELISA assay, and 80 (34.2%) were negative. However, the overall positive results rate was 49 (31.8%) and 105 (68.2%) for antibodies IgM and IgG respectively as it is shown in Table 1, regarding Table 1, the epidemiological prevalence rate of anti-RVfV IgM and IgG among humans. Results are revealed that the highest prevalence rate 56 (36.4%) in age group 31 years to 40 years was 20 (13.0%) and 36 (23.4%) of anti-RVfV IgM and IgG respectively. However, the overall prevalence rate of RVF cases was 77 (50.0 %) equally recorded in urban and rural patients. Noteworthy, the urban patients were equivalent in infection to the rural. The relationship among age groups, localities and immunity response to IgM and IgG are not significant >0.05. In general, rate of males was higher than females. So, 97 (63.0%) and 57 (37.0%) of patients were males more infected than females respectively. However, the highest epidemiological prevalence rate of patients' occupations was 65 (42.2%) in shepherds, so they were more contact with animals. The relationship amongst sex, occupations, RVFV infection and immunity response of the patients is shown a statistical significant ≤ 0.05.

Actually, the severity of disease is depended on viremia levels. Table 2 is shown that the headache was had the highest distribution rate 105 (68.2%) among IgM and IgG patients, decreased to 19 (12.3%) Vomiting and 16 (10.4%) Hemorrhagic fever among anti-IgM patients while Ocular diseases and Encephalitis had lowest distribution rate 8 (5.2%) and 6 (3.9%) among anti-IgG patients respectively. The relationship between Severity of Disease and immunity response is significant ≤ 0.05 . However, the results revealed that the highest epidemiological distribution rate were demonstrably appeared 64 (41.6%) in summer and, decreased to 56 (36.4%) in autumn. Therefore, the prevalence rate was decreasingly started at end of autumn and dramatically increased at end of spring. The relationship among immunity response for the patients, RVFV infection, seasons of year is not occurred statistical significant >0.05 .

Discussion

This study was performed to preparation RVF antigen to be used in detecting anti-RVFV antibodies in humans or animal's serum samples by using modern ELISA technique and to study epidemiological prevalence of anti-RVFV IgM and IgG among humans.

However, the prepared antigen from lamb liver was successfully prepared using sucrose acetone extraction technique, and also assessed successfully by IgM and IgG antibodies. This antigen was successfully adhered to CB ELISA plates directly at a highest titer dilution of 1:3,200. This result is in consistent with those recorded by said who produced same antigen from the liver of sheep at a dilution of 1:200 [18]. Similar antigen prepared by Paweska et al. [19] from infected mouse liver at a dilution of 1:400. Moreover, Maysa prepared RVFV antigen from the liver of hamster at a dilution of 1:3,200 [20]. From the mentioned above, the difference in titration results may be related to variance in methods of test, accuracy and sensitivity of test.

The present study showed that of those (154 out of 234) cases were positive for ELISA assay their ages ranged from 4 years to over than 50 years. Nearly similar to those obtained in Egypt, Saudi Arabia Kingdom, Nigeria, Kenya, Somalia, Mauritania by Madani et al. [3], Seleem et al. [7], Qlaleye et al. [21], Nabeth et al. [22], Woods et al. [23] and Youssef [24].

However, the overall epidemiological prevalence rate of anti-RVFV IgM was 31.8%. This is similar to those obtained by MMWR recorded 33.4% in Egypt [25], and is nearly similar to those reported by Sarthou et al. [26] and Qlaleye et al. [21] during their survey in Mauritania and Nigeria who's obtained of 24.3% and 23.2% respectively. The highest epidemiological prevalence rate 13.0% was in the age group ranging from 31 years to 40 years. This is in agreement with Madani et al. [3], Qlaleye et al. [21], MMWR [25] and Memish et al. [27] whose results were similar or slightly lower percentages, and may be referred to individuals with ruminant exposure, or patients with specific age groups, variance in sample size and specificity of test and low concentration of the antibodies in the serum samples.

Moreover, the overall epidemiological prevalence rate of anti-RVFV IgG was 68.2%. In contrary to this, lower findings were reported by Seleem et al. [7], Maysa [20], Nabeth et al. [22], Woods et al. [23], Youssef [24], MMWR [25], Memish et al. [27], Thonnon et al. [28] and Tigoi et al. [29] whose recorded 13%, 15.3%, 24.4%, 10.6%, 15%, 10.3%, 10.53%, 11.1% and 19.5% respectively. The difference in results may be related to that we collected our samples from patients met the case defined of acute RVF infection. The results also

revealed that the highest prevalence rate 23.4% was in the age group ranging from 31 years to 40 years. This finding is in disagreement with previous studies in Egypt by Youssef [24] and Maysa, [20] who recorded lower rate of 13.7% in the age group 31 years to 40 years. Therefore, there was a significant difference between age groups and immunity response of humans >0.05 .

Regarding Localities, the overall distribution rate of RVF cases 77 (50.0%) in urban areas was similar to those in rural areas 77 (50.0%). On the other hand, lower percentages of 13.6%, 11.8% in rural areas and 5.3%, 8.7% in urban one were previously recorded by Seleem et al. [7] and Maysa [20] respectively. From the mentioned above we found that the distribution rate 25 (16.2%) and 24 (15.6%) of anti-RVFV IgM is similar to 52 (33.8%) and 53 (34.4%) of anti-RVFV IgG in rural and urban areas respectively. This may be attributed to many predisposing factors as presence of animal reservoir, agricultural activities, irrigation and those sleeping on surfaces of buildings in summer. There was also a significant difference between Localities and immunity response of humans >0.05 , but there was shown important with P-value 0.030.

Obviously, the overall epidemiological prevalence rate in males and females was 97 (63.0) and 57 (37.0%) respectively. The highest percentages in males than females may be attributed to those males are more repeated mosquito exposure than females during their sleeping outdoors. The obtained results are in agreement with Woods et al. [23]. The prevalence rate of anti-IgM cases was similar 25 (16.2) and 24 (15.6) in the males and females respectively while the prevalence rate of anti-IgG was higher 72 (46.8%) in the males than the females 33 (21.4%). These results may be attributed to the fact that males are more involved than females in occupations that require direct contact with susceptible animals and the infectious agents Turell et al. [30]. These results are in agreement with Seleem et al. [7], Maysa [20], Woods et al. [23], Youssef [24] and Byomi et al. [31]. There was a statistical significant amongst gender, RVFV infection and immunity response of humans <0.05 .

The highest prevalence rate 65 (42.2%) was in shepherds while it was decreased to 29 (18.8%) in Farmers. These results may be attributed to that shepherds were more contact with susceptible animals while the farmers were only contacted with breeding habitats of mosquitoes. These results are in consistent with those reported in Mauritania by Boushab et al. [10] who recorded lower prevalence rate was 26% in shepherds and 3% in farmers. There was also a statistical significant between occupations and immunity response of humans <0.05 .

The most common symptoms were 12.3%, 10.4% and 9.1% of anti-IgM accompanied with headache, vomiting and Hemorrhagic fever while the lowest prevalence was 5.2% and 3.9% of anti-IgG accompanied with Ocular diseases and encephalitis respectively. This is in agreement with Madani et al. [3], Woods et al. [23] and Arthur et al. [32]. Who recorded similar prevalence rate. There was a statistical significant among severity of disease, RVFV infection and immunity response of humans <0.05 .

The highest epidemiological prevalence rate recorded 14.9% and 26.6% in summer and followed by 12.3% and 24.0% in autumn of anti-RVFV IgM and IgG respectively. These findings are in agreement with Qlaleye et al. [21] who was mentioned that the infection rate was significantly higher during the wet season than during the dry season of the same year. This is in consistent with Maysa [20], who

recorded lower prevalence rate of anti-IgG was 20% in summer, 7.14% in autumn. The difference in results may be attributed to the environmental factors, vegetation, rainfall, slope degree, abundance of vectors and selected specimens. There was a significant difference between seasons of year and immunity response of humans >0.05 .

Conclusion

The prepared antigen was successfully produced and successfully adhered to ELISA plates at a highest titer dilution of 1:3,200. Simple, inexpensive methods give good results. The overall positive results rate 154 (65.8%) was positive for antibodies IgM and IgG. Epidemiological prevalence of RVF among humans would be the highest in autumn and in summer, especially during rainfall and Greater Feast. The people of 31 years to 40 years are the most infected, and the males are more infected than females. The result can be used to develop and refine predictive database for RVF transmission based on environmental and remote sensing data.

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Competing Interests

There are neither existed financial competing interests nor personal or professional conflicts of interest. The views expressed are those of the authors and not necessary those of the funding bodies.

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