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Research Article

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

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Abstract:
Rift Valley Fever Virus (RVFV) is the causative agent of Rift Valley Fever (RVF), transmitted either by hematophagous insects that infect animals and humans or by 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 animals 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: 3200 by Chessboard (CB) ELISA assay. However, out of 188 samples, 147 samples were positive for ELISA assay, of which 12 (8.2 %) were positive for anti-RVFV IgM and 135 (91.8 %) for anti-IgG antibodies. The antigen was successfully produced, and successfully adhered to ELISA plates. Simple and inexpensive methods give good results. The results 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
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Introduction

Rift Valley Fever (RVF) is a re-emerging viral vector-borne disease caused by Rift Valley Fever Virus (RVFV) with rapid global socio-economic impact, a member of family *Bunyaviridae* genus *Phlebovirus* that affects domestic ruminants and humans [1]. In ruminants, it induces almost 100% mortality among young animals, a high rate of abortion in pregnant females, and is responsible on listlessness, fetid, unsteady gait, reluctant to stand, bloody diarrhea, abortion, liver necrosis and death [2, 3]. 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 [2,4]. It is transmitted by mosquitoes, direct contact with infected animal tissues and products [5].

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 – 2001. In Saudi Arabia, reported 11,882 human cases with 164 deaths [6,7,8]. In Yemen, reported 1080 human cases and 141 deaths [7,8,9], over 20.000 abortion animals and 620 dead [7,10]. Several outbreaks were followed after heavy rainfall in Egypt, Kenya, Somalia, Tanzania, Sudan, Mayotte, and Mauritania from 2003 to 2012 [11].

There are several methods that are used in RVF diagnosis, Scott, et al. [12] determined that the accuracy of serological methods in detecting anti-RVFV 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 domestic animals, and establishment primary database of RVF prevalence in Yemen.

Materials and Methods

This study enrolled a total of 188 animals that suffered from sickish collected from stockyards and fields of Taiz Governorate, Southwestern Yemen, aged from 2 months to over than 3 years which included sheep, goats and cattle, during the period from January 2013 until August 2016. About 5 ml of blood were collected by venipuncture into sterile tube, allowed to clot, centrifuged (2000 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 [13,14].

Preparation of RVFV antigen

According to the information mentioned by Smithburn, et al. [15] 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 [16].

Lamb inoculation, two lambs aged 2 and 4 months, were inoculated intraperitoneally with 0.5 ml of infected human serum by RVFV containing 10^4 LD_{50} (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 1600 when it seemed unsteady gait. No any lamb died during the virus incubation period.

Procedure for lamb liver derived sucrose acetone extracted RVFV antigen, 10g 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 1800 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 thaiosulphate 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. [17]. 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 microtiter 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 1h 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 h in titer IgG or 2 h in IgM), washed 4 times. 50 µl of diluted horseradish peroxidase conjugated anti-species (anti-sheep, anti-cattle and anti-goat) IgG or IgM (Sigma Chemical Co.) was added to all wells, incubated (1 h in 37˚C), 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 1.2 after 20 minutes of substrate incubation with the strong positive serum antibodies IgG while done a value of 1.3 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 anti-RVFV IgM and IgG antibodies was done according to Voller et al. [18]. 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 1h 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-A12). Plates were incubated at 37 ˚C for (1 h in test IgG or 2 h in IgM). After being washed 3 times, 100 µl of horseradish peroxidase conjugate labeled anti-species (whole molecules of IgG or IgM diluted according to the manufacturer's recommendation, Sigma Chemical Co.) was added into each well, and incubated (1h in 37 ˚C). The plates were washed 3 times, and blotted on paper towels. 100 µl/well of orthophenylenediamine 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 (Cutt-off) were considered positive.

Cutt-off = $\bar{X}$ + 3SD

$\bar{X}$ = 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' farmers, stockyards' owners, or Shepherds.

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: 3200 by using CB titration ELISA assay for both IgM and IgG antibodies.

Out of 188 samples (54 cattle, 97 sheep, and 37 goats), 147 (78.2%) were positive for ELISA assay, and 41 (21.8%) were negative. However, the overall positive results rate 135 (71.8%) were positive for IgG (38 cattle, 75 sheep, 22 goat) while 12 (8.2%) were positive for IgM (3 cattle, 7 sheep, 2 goat). The epidemiological prevalence rate of anti-RVFV IgM and IgG among animals is shown in Table 1. Results are revealed that RVFV was equally targeted the age groups < 1 year, 1-2 years and the prevalence rate of anti-IgM was 6 (50%) in both. On the other hand, the highest prevalence rate was 70 (51.9%) in age group 1-2 years of anti-RVFV IgG and decreased to 36 (26.1%) in age group < one year. However, the highest prevalence rate was 7 (58.3%) and 75 (55.6%) in sheep of anti-IgM and IgG respectively. However, the relationship among age groups and immunity response to IgM and IgG are not significant > 0.05. In general, rate of males was higher than females. So, 82 (60.7 %) and 7 (58.3 %) of cases were males more infected than females 5 (41.7%) and 53 (39.3%) of anti-IgM and IgG respectively. The relationship amongst sex, RVFV infection, and immunity response of the

animals is shown a statistical significant < 0.05.

Actually, the highest epidemiological distribution rate of anti-RVFV cases among animals according to clinical signs and species is shown in Table 2. The highest epidemiological distribution rate was found 82 (55.8%) in sheep, decreased to 41 (27.8%) in cattle and 24 (16.4%) in goats. However, the sheep were the most affected in RVFV, followed by cattle and goats. In additional, among the 147 cases, of which 54 (36.7%) were had listlessness, 42 (28.6%) unsteady gait, 18 (12.2%) blindness which recorded for the first time in Yemen, 21 (14.3%) reluctant to stand, and 12 (8.2%) of cases had abortion. The relationship between clinical signs, species of animal, and immunity response is significant < 0.05. However, the results revealed that the highest epidemiological distribution rate were demonstrably appeared 63 (42.9%) in autumn and decreased to 48 (32.7%) in summer. 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 animals, RVFV infection, and seasons of year is not occurred statistical significant > 0.05.

Table 1 Epidemiological prevalence rate of IgM and IgG among animals in relation to age groups and sex

Variables	ELISA assay of prepared antigen							
	IgM			**Total** No.(%)	**IgG**			**Total** No.(%)
	Cattle No.(%)	**Sheep** No.(%)	**Goat** No.(%)		**Cattle** No.(%)	**Sheep** No.(%)	**Goat** No.(%)	
Age Groups								
< 1	1 (8.3)	5 (41.7)	0 (0.00)	6 (50.0)	13 (9.4)	19 (13.8)	4 (2.9)	36 (26.1)
1-2	2 (16.7)	2 (16.7)	2 (16.7)	6 (50.0)	16 (11.9)	43 (31.9)	11 (8.1)	70 (51.9)
2-3	0	0	0	0	6 (4.4)	8 (5.9)	5 (3.7)	19 (14.1)
> 3	0	0	0	0	3 (2.2)	5 (3.7)	2 (1.5)	10 (7.4)
Total	3 (25.0)	7 (58.3)	2 (16.7)	12 (100)	38 (28.1)	75 (55.6)	22 (16.3)	135 (100)
P-Value	0.186				0.981			
df	1				1			
Asymp. Sig.	0.666				0.322			
Sex								
Males	2 (16.7)	4 (33.3)	1 (8.3)	7 (58.3)	12 (8.9)	56 (41.5)	14 (10.4)	82 (60.7)
Females	1 (8.3)	3 (25.0)	1 (8.3)	5 (41.7)	26 (19.3)	19 (14.1)	8 (5.9)	53 (39.3)
P-Value	0.133				9.838			
df	1				1			
Asymp. Sig.	0.715				0.002			

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

Table 2 Epidemiological distribution of RVFV among animals in relation to clinical signs and season

Variables	**Cattle** No.(%)	**Sheep** No.(%)	**Goat** No.(%)	**Total** No. (%)	**P-Value**	**df**	**Asymp. Sig.**
Clinical signs							
Listlessness	26 (17.7)	20 (13.6)	8 (4.5)	54 (36.7)			
Blindness	0 (0.00)	18 (12.2)	0 (0.00)	18 (12.2)			
Unsteady gait	9 (6.1)	21 (14.3)	12 (8.2)	42 (28.6)	5.007	1	0.025
Reluctant to stand	3 (2.0)	16 (10.9)	2 (1.4)	21 (14.3)			
Abortion	3 (2.0)	7 (4.8)	2 (1.4)	12 (8.2)			
Total	41 (27.9)	82 (55.8)	24 (16.3)	147 (100)			
Season							
Summer	13 (8.8)	25 (17.0)	10 (6.8)	48 (32.7)			
Autumn	18 (12.2)	35 (23.8)	10 (6.8)	63 (42.9)			
Winter	3 (2.0)	8 (5.4)	0 (0.00)	11 (7.5)	0.297	1	0.586
Spring	7 (4.8)	14 (9.5)	4 (2.7)	25 (17.0)			

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

Discussion

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

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: 3200. This is in consistent with those recorded by Said [19] who produced same antigen from the liver of sheep at a dilution of 1: 200. Similar antigen prepared by Paweska, et al. [20] from infected mouse liver at a dilution of 1: 400. Moreover, Maysa [21] prepared RVFV antigen from the liver of hamster at a dilution of 1:3200. 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 147 out of 188 cases were positive for ELISA assay their ages ranged from < one year to over than 3 years. However, the overall epidemiological prevalence rate of anti-RVFV IgM was 8.2% (12 out of 147). Nearly similar results were previously reported by Thonnon, et al. [22] who found of 12.5% during their survey in Senegal. Moreover, the highest epidemiological prevalence rates of 16.67%, 34.8% and 100% were recorded respectively by Zeller, et al. [23], Nabeth, et al. [24] and Pepin, et al. [25] whose collected their samples from affected herd after RFV outbreak, but the lowest prevalence rate was recorded by Paweska, et al. [20] who recorded 0.28% were seropositive against RVFV in both sheep and goats. On the other hand, the overall epidemiological prevalence rate of anti-RVFV IgG antibodies was 91.8% (135 out of 147). Lower prevalence rate of 18.7%, 50% (7 of 14), 40%, 14.5%, 20.95% and 14.9% were previously recorded by Zeller, et al. [23], Joshi, et al. [26], Thonnon, et al. [22], Youssef [27], Mysa, [21], and Byomi, et al. [28] whose collected their samples after epizootic outbreak respectively. The difference in results between this study and the comparable one are may attributed to difference in the health status of the animals, variance in sample size, specificity of test and low concentration of the antibodies in the serum samples.

The highest epidemiological prevalence rate of anti-IgM was equally recorded 6 (50%) in both age group ranging from 1-2 years and < 1 year. This is in disagreement with Zeller, et al. [23] and Qlaleye, et al. [29] whose recorded that the prevalence rate in the age group > 2 years and over than the 3 years was higher than that in the age group < 2 years and in the < 3 years old respectively, and may be related to lower in our chosen animals or samples than the other one. In contrary to this, the higher epidemiological prevalence rate of anti-IgG was 51.9% in age group ranging from 1-2 years then 26.7% in age group < one year, this is in disagreement with Zeller, et al. [23], Qlaleye, et al. [29] and Youssef [27] who recorded that animals over 2 years and over 3 years had a higher prevalence of antibodies to RVF. They may be collected most of their samples from animals aged two years or more. However, the difference in results may be related to that we collected our samples from animals met the case defined of acute RVF infection. Therefore, there was a significant difference between age groups and immunity response of animals > 0.05.

The results also revealed that the overall epidemiological prevalence rate of anti-RVFV IgM cases in sheep 58.3% was higher than that recorded by Paweska, et al. [20] and Nabeth, et al. [24] whose recorded 0.16% and 34.8% respectively. On the other hand, higher percentage than that recorded in this study was reported by Pepin, et al. [25] who obtained 99.75% of IgM antibodies in sheep. However, the overall prevalence rate of anit-IgG cases was 55.6% in sheep. Nearly similar to those recorded by Youssef [27] who recorded 33.5%. In contrary to this, lower prevalence rate of 21.1%, 26.74% and 21.4% was recorded by Youssef [27], Mysa, [21] and Byomi, et al. [28]. On the other hand, higher prevalence rate 97.3% was recorded by Pepin, et al. [25]. As mentioned above, the sheep are the most sensitive animals and susceptible to RVF.

Regarding sex. the overall epidemiological prevalence rate of anti-IgM in males and females was 58.3% and 41.7% respectively. The higher prevalence rate in males than females may be related to the breeding purpose of animals Yedloutschnig, et al. [30]. Moreover, the overall prevalence rate of anti-IgG 60.7% in males was higher than females 39.3%. In opposition to this, Youssef [27], Mysa, [21] and Byomi, et al. [28] who found that rate of males 12%, 13.54% and 8.6% was lower than females 15.6%, 50% and 17.7% respectively. This difference in the results may be related to the fact that lactating and breeding females are reared for older ages than males so they are more liable to contract the infection more than males. Incidentally, the males are more involved than females in this

study. Obviously, there was a statistical significant amongst gender, RVFV infection, and immunity response of animals < 0.05.

The epidemiological distribution rate of anti-RVFV cases according to clinical signs and species revealed that the higher distribution rate 36.7% accompanied with listlessness, 28.6% associated with unsteady gait and 14.3% reluctant to stand. On the other hand, the lower distribution rate 8.2% associated with abortion and 12.2% blindness in sheep. However, the blindness phenomenon is recently shown in Yemen, and is no recorded in any previous study. The abortion in sheep and goats is in a consistence with Byomi, et al. [28] who proved that RVF play an important role in abortions especially in sheep and goats. There was a statistical significant among clinical signs, RVFV infection, and immunity response of animals < 0.05.

The highest epidemiological distribution rate of anti-RVFV antibodies recorded 63 (42.9%) and 48 (32.7%) in autumn and summer respectively. These findings are in agreement with Qlaleye, et al. [29] 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 Mysa [21], who recorded lower prevalence rate 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 animals > 0.05.

Conclusion

The prepared antigen was successfully produced and successfully adhered to ELISA plates at a highest titer dilution of 1: 3200. Simple, inexpensive methods give good results. However, out of 188 samples, 147 samples were positive for ELISA assay, of which 12 (8.2 %) were positive for anti-RVFV IgM and 135 (91.8 %) for anti-IgG antibodies. Epidemiological prevalence of RVF among animals would be the highest in Autumn and in Summer, especially during rainfall and Greater Feast. The most affected animals by RVFV are the sheep, 12.2% of the sheep is blinded. On the other hand, the most infected animals are having age group below 2 years, and the males are more infected than females. Apparently healthy animals may be a source of infection, so care must be taken during contact with liver and spleen of any animals. 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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Conflicts of interest

There are no personal or professional conflicts of interest.

References

1. Sasita Shabani S, Mangi JE, Mohamed M and Candida SM. Knowledge, attitudes and practices on Rift Valley fever among agro pastoral communities in Kongwa and Kilombero districts, Tanzania. ***BMC Infectious Diseases***. 2015, 15 (1): 356
2. Laughlin LW, Meegan JM, Strausbaugh LW, Morens DM and Watten RH. Epidemic Rift Valley fever in Egypt, observation of the spectrum of human illness. ***Trans R Soc Trop Med Hyg***. 1979, 73(6):630-633
3. Office International des Epizooties/World organization for Animal Health. Rift valley fever., Jowa State University, College of veterinary Medical, RVF_H1106_0507. OIE, 2007
4. Tariq A, Madani YY, Al-Mazrou MH, Al-Jeffri AA, Mishkhas AM, *et al*. Rift Valley Fever Epidemic in Saudi Arabia: Epidemiological, Clinical, and Laboratory Characteristics. ***Clinical Infectious Diseases***, 2003, 37(8):1084-1092
5. Elizabeth Cook, Grossi-Soystter EN, de Glanville WA, Thomas LF, Kariuki S, *et al*. The sero-epidemiology of Rift Valley Fever in people in the lake Victoria basin of western kenya. ***PLoS Negl Trop Dis***. 2017, 11(7): e0005731
6. Pepin M, Bouloy M, Brian HB, Kemp A and Janusz Paweska J. Rift valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. ***Vet Res***. 2010, 41 (6):125-128
7. Chaniotis B. Arthropod-borne viral zoonoses in the Mediterranean area (An overview). Information circular-WHO Mediterranean Zoonoses Control Center, 2003, 56:1-16
8. Seleem MN, Asmaa Hussein, Ismail AA and Nafie EK. Serological studies on RVF in some patients of fever hospital in Upper Egypt. ***J Egypt Vet Med Assoc***. 2002, 62(6a):261-271
9. World Health Organization. Rift Valley fever outbreak., Disease Outbreak Reported, ***Global Alert and Response*** *(GAR)*, 2000
10. Abdo-Salem AS, Waret-Szkuta A, Roger F, Olive MM, Saeed K and Chevalier V. Rift valley fever in yemen. ***Trop Anim Health Prod***. 2011, 43 (2):471-480
11. Boushab MB, Fatima Zahra, Sidi WOB, Mohamed LOS, Marie Roseline DB, *et al*. Sever human illness caused by Rift Valley Fever Virus in Mauritania. Oxford University Press. ***Open Forum Infect Dis***. 2016, 3 (4):ofw200
12. Scott RM, Feinsod FM, Allam IH, Ksiazek TG, Peters CJ, *et aL*. Serological tests for detecting Rift Valley fever viral antibodies in sheep from the Nile Delta. ***J Clin Microbiol***. 1986, 24(4): 612-614
13. Burleson FG, Chambers TM and Wiedbrauk DL. Virology: a laboratory manual. 2nd edition, Academic Press San Diego, Sydney, 1992
14. Provenzano M, Rossi CR, Mocelline S. The usefulness of quantitative Real time PCR in immunogenetics. ASHI quarterly, Third quarter 2001
15. Smithburn KC, Haddow AJ and Lumsden WHR. Rift Valley Fever; Transmission of the virus by mosquitoes. ***Br J Exp Pathol.*** 1948, 30 (1):35-47

16. Clarke DH and Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. ***Am J Trop Med Hyg***. 1958, 7(5):561-573
17. Rose NR, Friedman H and Fahey JL. Manual clinical laboratory immunology, 3rd edition. *Am Soc For Microbiology*, Washington, DC, 1986, 107-109
18. Voller A, Bidwell D and Bartlett A. Microplate enzyme immunoassays for the immunodiagnosis of virus infection, ***Am Soc for Microbiology***. 1976, 506-512
19. Said, Taradi Abd El fatah. Production of some diagnostic reagents for Rift Valley fever. PhD thesis, Cairo University, Faculty of Vet. Medicine, Department of Virology, 2003.
20. Paweska JT, Barnard BJ and Williams R. The use of sucrose acetone extracted RVFV antigen derived from cell culture in an indirect Elisa and HI. Onderstepoort, *J Vet Res*., Dec., 1995, 62(4): 227-233.
21. Maysa Awadallah. Some epidemiological studies on Rift Valley Fever. PhD thesis, Zagazig Uni., Faculty of Vet. Med. Zoonoses
22. Thonnon J, Picquet M, Thiongane Y, Mustapha LO, Sylla R, *et al*. RVF surveillence in the lower Senegal River basin: update 10 years after the epidemic. ***Trop Med International Health***. 1999, 4(8):580-585
23. Zeller HG, Akapko AJ and Ba MM. RVF epizootic in small ruminants in Southern Mauritania (October 1993): risk of extensive outbreaks. *Ann Soc Belg Med Trop*., 1995 June., 75(2): 135-140.
24. Nabeth P, Kane Y, Abdalahi Mo, Diallo M, Ndiaye K, *et al*. Rift Valley Fever outbreak, Mauritania, 1998: Seroepidemiologica, Virologica, Entomologic and Zoologic investigation. ***Emerg.Infect.Dis***. 2001, 7 (6):1052-1054
25. Pepin M, Paweska J and Bouloy M. Diagnostic specificity of ELISA-based tests for the detection of antibodies to Rift Valley Fever virus in French ruminants. ***Revue Méd Vét***. 2010, 161(3):104-107
26. Joshi MV, Elankumaran S, Joshi GD, Albert A, Padbidri VS, *et al***.** A post-epizootic survey of RVF-like illness among sheep at Veerapuram, Chennai, Tamil Nadu. *Indian J of Virology*, 1998, 14(2): 1-15; 10 ref.
27. Youssef AI. An epidemiological studies on RVF in Ismailia province. M.V.Sc. thesis (master degree), Department of Animal Hygiene, Behaviour and Zoonoses, Faculty of Veterinary Medicine, Suez Canal University, Egypt, 2004
28. Byomi AM, Samaha HA, Zidan, SA and Hadad GA. Some associated risk factors with the ocurrence of Rift Valley fever in animals and man in certain localities of Nile Delta, Egypt. ***Assiut Vet Med J***. 2015;16 (61):144
29. Qlaleye OD, Tomori O, Ladipo MA and Schmitz H. Rift Valley fever in Nigeria: infection in humans. ***Rev Sci Tech***. 1996, 15(3):923-935
30. Yedloutschnig RJ, Dardiri AH and Walker JS. Persistence of RVFV in spleen, liver, and brain of sheep after experimental infection. ***Contrib Epidemiol Biostat***. 1981, 3:2-7

Annex

ELISA reagents:

1. Phosphate buffer saline (PBS):

It was composed according to **Voller, *et al*., 1976** as follows:

Sodium chloride NaCl …….……....	8.00 g
Potassium chloride KCl ……..…….....	0.20 g
Potassium dihydrogen phosphate KH_2PO_4…….……..	0.20 g
Disodium hydrogen phosphate $Na_2HPO_4.12H_20$……	2.90 g
Distilled water ……..…….	1000 ml
pH…….…....	7.4

2. Coating buffer (carbonate-bicarbonate buffer, pH 9.6):

Sodium bicarbonate ($NaHCO_3$)…….……..…..	2 .93g
Disodium carbonate (Na_2CO_2)……..……..….....	1.59g
Distilled water.……...…….....	1000ml
pH……...…..	9.6

3. **Diluting buffer**, it was composed of 5g of bovine serum albumin, 50 µl tween 20, in 1-liter BPS.

4. **Blocking buffer**, it was prepared of 10g bovine serum albumin in 1-liter BPS.

5. **Washing buffer**, it was composed of 0.5 ml tween 20 in 1-liter BPS.

6. **Conjugate**, it was horse reddish peroxidase labeled anti-species whole molecules IgG and IgM (Sigma Chemical Co.).

7. Substrate buffer (Phosphate citrate buffer):

0.1M Citric acid (2.1 g in 100 ml distilled water)……...…	24.3 ml
Na_2HPO_4 (7.1 g in 100 ml Distilled water ……...……..	25.7 ml
Distilled water ……...…….....	50 ml
Orthophenylenediamine …….……..	40 mg
H_2O_2.....	20 µl

8. **Stopping buffer**, it was composed of 2.5M sulfuric acid (H_2SO_4), pH 3.3 and used for stopping the enzymatic reaction on ELISA substrate.