Asian Journal of Advanced Research and Reports

15(7): 58-73, 2021;Article no.AJARR.75606
ISSN: 2582-3248

Seroprevalence and Molecular Detection of Rift Valley Fever in Sheep, Gezira state, Sudan (2017-2019)

Asia M. Elhaj[1*], Adam D. Abakar[2], Ahmed A. Mohamedani[3], Elamin A. Ahmed[4] and Tamador M. A. Elhassan[5]

[1]Blue Nile National Institute for Communicable Diseases, University of Gezira, Sudan.
[2]Faculty of Medical Laboratories Sciences, University of Gezira, Sudan.
[3]Faculty of Medicine, University of Gezira, Sudan.
[4]Faculty of Agricultural Sciences, University of Gezira, Sudan.
[5]Veterinary Research Institute, Khartoum, Sudan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJARR/2021/v15i730414
Editor(s):
(1) Dr. Hasan Aydogan , Selcuk University, Turkey.
Reviewers:
(1) Bülent Bayraktar, Bayburt University, India.
(2) Mohamed Azooz, Central Laboratory for Evaluation of Veterinary Biologics, Egypt.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75606

Original Research Article

Received 07 August 2021
Accepted 13 October 2021
Published 18 October 2021

ABSTRACT

Aims: Livestock is the largest subsector of the Sudanese domestic economy and is a growing contributor to exports. Rift Valley fever (RVF) is an economically important arthropod-borne virus disease in Africa, primarily affecting sheep, goats, and cattle. RVF causes paramount loss in sheep industry in Sudan. The objective of this study was to detect RVF in sheep.
Methodology: Five hundred and fifty-six sheep blood Serum from inoculated animals was collected from six study areas in the Gezira (Sudan)was evaluated for the presence of neutralizing antibodies using the diagnostic kit (ID. Vet.) for detecting antibodies directly against the RVFV nucleoprotein (NP) in serum and Reverse transcription (RT)-Polymerase Chain Reaction (PCR) protocol was used as standard test to diagnose and confirm RVFV among studied animals.
Results: The ELISA test indicated that the highest Seroprevalence was found at *Shikaira* (38%), while samples from *Albasabeir*, *Wad alnaem* and *Umalqura* tested positive for antibodies against

**Corresponding author: Email: Asyavet018@gmail.com;*

RVFV by 15%, 10% and 9% respectively. The lowest Seroprevalence found at *Wad anor* and *Shabona* (5% and 2%). The overall seroprevalence was 14.2% in the study sites, there is no significant difference among the six study sites with respect to seroprevalence. The Rt-PCR provided more sensitive and specific detection of RVFV in serum samples (sensitivity= 95% specificity = 80%).

Conclusion: The results from this study showed the presence of anti-RVFV antibodies in all study sites, suggesting that RVFV is actively circulating among sheep in Gezira state. The high seroprevalence of RVFV infection in Gezira state indicates its endemicity. Test validation using ROC analysis was able to distinguished between ELISA (actual class) and the PCR (predicted class) and the AUC = 0.79 which indicate that the PCR is gold standard test. Thus Rt-PCR used in the present study is reliable to detect precisely the M segment of RVFV and it is valuable for Wide area survey, among both sheep and Aedes spp. Targeting the prevailing genotypes of RVF-M segment, especially at high-risk areas and as a confirmatory test that should be considered by reference laboratories in the region.

Keywords: Rift valley fever; ELISA; Rt-PCR; Sudan.

1. INTRODUCTION

Livestock is the largest subsector of the Sudanese domestic economy and is a growing contributor to exports. In Sudan, the livestock export forms the basis for the foreign exchange earnings. Sudan is ranking 7th in sheep meat production among the top 10 producing countries in the world [1] Sudan exports live sheep and sheep mutton mainly to Saudi Arabia and other Gulf countries. According to FAO [2] the Sudan exported about 1568 tons of mutton, with the value of 8367 thousand USA dollars. Rift Valley fever (RVF) is an economically important arthropod-borne virus disease in Africa, primarily affecting sheep, goats, and cattle [3]. It is caused by Rift Valley Fever virus (RVFV), genus Phlebovirus, which is a member of Bunyaviridae. Sheep are extremely susceptible to RVFV infection with 70–100% mortalities [3]. RVF causes paramount loss in sheep industry in Sudan.

The RVFV was first isolated in 1930 in the Rift Valley Province , Kenya, associated with large epidemic in domestic ruminants and human [4]. It has been widely distributed in sub-Saharan Africa, with epizootic activity affecting animals in Kenya, Tanzania, Zambia, and Uganda [5]. The first report of RVF occurrence In the Sudan was by [6] from human sera derived from Southern Sudan.

The RVFV is associated with mosquitoes. It is transmitted to live stock and human by the bites of infected mosquitoes of the genus Aedes and by expoure to tissues or blood of infected animals [7]. In East and South Africa, the main vector known to transmit RVF is the Aedes mosquito, including Aedescircumluteeolus, and Aedesmicintoshi. More recently, a group of entomologists have identified different vectors that have been responsible for RVF virus transmission in Western Africa, including Aedesvexans, Aedesochraceus [8] Aedesdalzieliand Culex mosquitoes [9] Infected mosquito eggs can survive for years in the soil, once proper environmental conditions exist, such as heavy rainfall, which fills natural depressions in the earth creating stagnant water (dambos) the hardy eggs hatch, and new, infected mosquitoes start a new epizootic cycle [10].

1.1 Virus Isolation

The virus could be isolated from blood, but that should be within the first 8 days of the infection to detect the virus, otherwise the virus will disappear from the blood. Some efforts have been done in this direction to identify fatal human cases of RVF during outbreak periods. Some efforts have been shown by [11] to develop a diagnostic tool to be used in a field lab setting, particularly in the remote rural areas. This trial was used during Kenyan outbreak in 2006-2007 and the results were promising. Virus could also be isolated in hamsters or adult mice intraperitoneally. Moreover, different cell culture types could be used to isolate the virus [12].

1.2 Viral Genetic Components Detection

Recently scientists have been able to detect the RVFV genetic material. Reverse Transcriptase PCR was used for this purpose and to analyze the virus phylogentic which is very useful to detect the viral RNA sequencing, to study the strain type of RVFV and could identify the source

or region where the virus came from [13]. The virus is well known as an enveloped tripartite single stranded RNA genome. According to [14] and [15] this tripartite genome named (due to its size) as large (L), Medium (M) and Small (S) segment. The L encodes the L protein and the RNA polymerase. The M encodes the virion glycoproteins GI and G2 which are involved in attachment to the host cell through unidentified receptors and elicit neutralizing antibodies. While the S encodes the nucleocapsid (N) protein, which encapsulates each of the genome RNA segments to form ribonucleoprotein complex (RNP). The RNP is the template for the viral RNA polymerase [16]. RVF virus may withstand environmental temperatures of 25 – 30 0C for approximately 80 minutes. Exposure to contaminated body fluids of infected animals stills the major mode of transmission. This is a particular hazard to those exposed to contaminated blood and abortion products in the abattoir, and in areas of food preparation in endemic regions [17] Compared with other viral haemorrhagic diseases, such as Ebola and Marburg disease, the RVF has a low mortality.

RVFV infects many animals;including sheep, goats, cattle, camels, and Asian water Buffaloes. The symptoms of the disease, in the different animal are similar, and include fever, hepatitis, and abortion. Additionally, sheep and cattle, once infected, become highly viraemic, thus allowing both early and highly effective infection of the vector and hence efficient disease transmission. In less severe cases, infected animals may present with injected conjunctiva, nasal discharge, weakness and decreased milk production. In humans, the virus is hepatotropic, and shows intrahepatic viral replication leads to massive and patchy hepatic necrosis [18].

1.3 Detection of the RVFV RNA in Mosquitoes

Investigation of RVFV in mosquitoes as the main carrier for the disease during the Madagascar epizootic outbreak in 1991 and Mauritanian in 1998 outbreak was unable to find the virus [19]. But this changed when [20] could detect RVFV RNA using Rt-PCR method in Egypt in two different areas. Similarly in Sudan the virus was isolated from the mosquitoes during 2007 outbreak [21] These results were promising and could help to identify the circulation of the virus during interepidemic or cryptic cycle as early as possible. This could aid in the allocation of the resources to the high-risk regions at the right time. The discrepancy in finding the virus in mosquitoes might be attributable to the diagnostic techniques that were used. RVFV can be detected by classical virological methods including histopathology, virus isolation [22] , detection of antigen and antibodies [23,24,25] and molecular assays [26,27] .The RVFV infection can be further confirmed by immune staining using specific antibodies [28,29] However, technical expertise is needed for this approach. Currently, a combination of serological and molecular methods is preferred in laboratory diagnosis. Enzyme-linked immune sorbent assay (ELISA) is commonly used to confirm RVFV infection. Several assays are available for the detection of both RVFV antigens and anti-RVFV antibodies [30,31]. Molecular methods are rapid to detect the viral RNA. Therefore, a number of highly sensitive nucleic acid based molecular tests have been developed and proven useful during RVF outbreaks [32]. In Areas of endemic disease winds will disseminate infected mosquitoes, as well as infected eggs, which go on to remain dormant in previously virgin soil for several years [33].

1.4 Rift Valley Fever Virus in Sudan

Before 1977 RVF was geographically limited to sub-Saharan Africa causing sporadic epizootics in animals and accidental contact infection in humans, which was rarely fatal. However, in 1977 and again in 1978 extensive epizootics of RVF with unprecedented human disease and fatalities occurred in Egypt [34-36]. It was following its dramatic appearance in Egypt that RVF came into prominence as a potential international disease problem.

In the Sudan serological evidence of the existence of RVF was available in 1936, when a limited survey including parts of East and Central Africa revealed neutralizing antibodies in 6-7 % of 164 human sera derived from Southern Sudan [37]. The disease was of little or no concern until 1973, when RVF virus was identified for the first time as the cause of an extensive epizootic involving sheep, cattle and less severely goats in Kosti District on the White Nile, some 200 km south of Khartoum [37]. The disease emerged again in 1976 after rainy summer in a farm in north of Khartoum where new cattle from White Nile state added to the existed herd suggested that the virus was carried to Khartoum from the central of Sudan where the virus was enzootic [37]. In addition, after the two epizootic outbreaks of RVF in Sudan 1973 and 1976, the human

illness that associated with outbreaks promoted some researchers to conduct serological study among Sudanese human population to show to what extent the RVFV circulated in the country. In 1980, 846 human sera collected from Hospital of Khartoum and military were tested to the antibodies towards RVFV. The result showed that 3.2% of them had antibody of RVF but unfortunately the geographic and age cluster were not available for those samples but in general the sera of 138 male and females belong to patients from Khartoum and Gazira states and the rest of the samples were from military recruits who belong to many regions in Sudan [38] During an unusual heavy rainfall season, in October 2007, a serological diagnosis was conducted using Rift Valley Fever (RVF) IgM Enzyme Linked- Immuno Sorbent Assay (ELISA) for detection IgM immunoglobulin in suspected outbreak of hemorrhagic fever in The White Nile, Gezira, Sinnar and Blue Nile States of the Sudan. A total of 323 blood samples were collected from cattle, sheep and goats and analysed. The overall percentages of IgM antibodies in the three species of animals in the study areas were;53.6% for caprine, 48.3% for ovine and 21.3% for bovine. Gazira State showed highest morbidity (50%) followed by The White Nile (24.6%) and then Sinnar and Blue Nile states, (21%). Bovine showed low infection whereas caprine morbidity was high followed by the ovine. The overall positive percentage of all animal examined (cattle, sheep and goats) from the study areas was 35.6% [39]. RVF becomes a central issue in a new global economy of world trade [40] It has hard impact on the African countries whose economies are based on animal resources. It will be difficult for them to compete. Sudan is a good example for this severe economic impact as it is a big producer of animal resources as well as one of the main exporters of live animals to many of the Middle East Countries and mainly Saudi Arabia [41] In 2007 [42] reported that there is a new outbreak of RVF in animals and humans lasted approximately five months from September 2007 to January 2008. It seems that the virus has circulated endemically in Sudan since the first outbreaks before 30 years as reported in 1973 [43], this gave evidence that the virus could persist for many decades without sign of outbreak;however, it could emerge when the conditions are appropriate [44]. The main objectives of this study are to diagnose and characterize the Rift Valley Fever Virus in sheep in high-risk area and to estimate prevalence of RVFV in sera of sheep by using ELISA and to confirm RVFV by Rt-PCR methods.

2. MATERIALS AND METHODS

2.1 Study Design

A prospective descriptive study was conducted in six different sites in Gezira State, in the period 2017-2019.

2.2 Study Area

This study was conducted in Gezira State (Fig. 1) which is located in the central region of Sudan and has an area of 23,373 km² and an estimated population of approximately 3.2 million people according to the 2008 national census (CBS, 2008). The State is dissected by the Blue Nile River with Wad Madani town as its capital. The average daily temperature is 32°C during summer (April, May, June to middle of July) and 22°C during winter (November to the end of January). The rainy season starts in July and ends by October, with an estimated annual rainfall of 140 – 225 mm. The relative humidity is 38% in autumn (end of July to the middle of October) and 30% during winter.

2.3 Study Population

Sudan's vast multitudes of domestic animals represent a large proportion of all African Livestock. The country ranks first to third among all African countries in the number of cattle, sheep, goats and camels, third in the number of poultry and fifth in the number of donkeys [45]. In 2009 Sudan official sources estimated cattle numbers at 41.653 million, sheep 51.555 million, goats 43.270 million and camels 4.521 million. In addition to these there were also 7.515 million donkeys and 784 thousand horses [46,47].

2.4 Blood and Serum Samples

Five hundred and fifty-six sheep blood samples were collected from six sites across the study area;these sites represented the south, east, west and north of Gezira state. Each blood sample was obtained with a separate needle and vacuum tube containing anticoagulant. One set of samples was immediately mixed with AVL (Qiagen, Valencia, CA), a lysis buffer for nucleic acid purification from the Qiagen QiaAmp viral RNA extraction kit (Qiagen), so as to inactivate RNA viruses in samples [48] and resolving the issue of infectious samples. All samples were

carried out in ice bags in the field and then stored at -80°C.

2.5 Serological Test by ELISA

Serum from sheep blood samples was evaluated for the presence of neutralizing antibodies using the diagnostic kit (ID. Vet.) for detecting antibodies directly against the RVFV nucleoprotein (NP) in serum.

2.6 Sample Preparation

A 96-plate containing the test and control samples was prepared before transferring them into an ELISA microplate using a multi – channel pipette in order to avoid differences in incubation times between samples.

2.6.1 Wash solution preparation

The wash concentrate (20x) was brought to room temperature (21±50C) and was mixed thoroughly to ensure that the Wash concentrate is completely solubilized. Then the Wash solution (1x) was diluted by the Wash concentrate (20x) in distilled/deionized water.

2.7 Description and Principles

The Well was coated with a recombinant Rift Valley Fever nucleoprotein. Samples were tested and the controls were added to the micro wells. Anti-nucleoprotein antibodies, if present, form an antibody- antigen complex which masks the nucleoprotein epitopes, then an antinucleoprotein peroxidase (HRP) conjugate was added to the micro wells to fixe the remaining nucleoprotein epitopes, forming an antigen- conjugate- HRP complex. After one hr. of incubation after washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added. The resulting coloration depends on the quantity of specific antibodies presenting the sample to the test:

- In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution.
- In the presence of antibodies, no coloration appears.

Fig. 1. Map of Sudan showing Gezira states and study sites

2.8 Testing Procedure

The following reagents were added to the plate;50 µlof Dilution Buffer-19 to each well, 50 ul of the Positive control to wells A1and B1, 50 ul of the Negative control to the well C1and D1, 50 ul of each sample to be tested to the remaining wells. Then the plate was Incubated for 1 hour ± 4 min at 37(±2 0C) and the plate was washed 3 times with approximately 300 ul of the washing solution. Drying of the wells was avoided between washings the anti-RVF-NP Conjugate 1x was prepared by diluting the Anti-RVF- NP Po Conjugate 10x to1/10 in Dilution Buffer 19. 100 ul of the Conjugate 1X was added to each well using the micropipette. Then the plate was incubated for 30 min ± 3 min at 21 0C (± 5 0C). Each well was washed 3times with approximately 300 ul of the wash solution. Drying of the wells was avoided between washings, then 100 ul of the Substrate Solution was added to each well, then the plate was incubated for 15 min ± 2min at 21 0C (±5 0C) in the dark. 100 ul of the stop solution was added to each well in order to stop the reaction. The result read using the reader and optical density was recorded at 450nm.

2.9 Virus Isolation and RNA Extraction

2.9.1 RNA extraction

Viral RNAs were extracted from the serum samples using QIAamp viral RNA kit. About 140 ul of serum was added to 560 µl AVL buffer containing carrier RNA into 1.5 ml micro-centrifuge tube and was mixed by pulse-vortexing for 15 seconds and then followed by incubation at room temperature for 10 minutes. About 560 µl of About 630 µl of the mixture was transferred to QIAamp spin column mounted on 2 ml collection tube and was centrifuged at 6000 x (8000rpm) for 1 minute. The column was transferred to another collection tube and the 630µl of the mixture was passed through it. The column was washed twice with 500 µl of washing buffers WB1, WB2, respectively. RNA was eluted by 60 µl buffer AVE equilibrated to room temperature. Viral RNAs and total nucleic acids extracted from serum samples were quantified using a spectrophotometer at 260 nm wavelength.

2.9.2 Reverse transcription (RT)-Polymerase Chain Reaction (PCR) protocol

Two primers specific for amplification of RVFV M segment were designed and they were produced a 342 bp Rt- PCR product. Below are the primers used:

Primer	Sequence
RVF2	′- CTG TCT GGC ACA GCA TTG AT-3′.
RVF	5′- CAC ATT GAA ACA CCC ACA CC-3′.

A single tube RT-PCR amplification was carried out using One step Access RT-PCR system (QIAGEN, USA), stander of 50 µl reaction mixture contained in a final concentration 1x enzyme mix reaction buffer, 0.5 µl of 10Mm dNTPs mix 5.0 µl of 25 Mm MgCL, 1.0 µl of 5.0 Units enzyme mix, 2.0 µl of 20 picomole of each primer, 5.0 µl of target RNA was used. The total volume was completed to 50.0 µl using RNase free water.

The cycling program was consisted of a reverse transcription step at 50° C for 30 min, a pre denaturation step at 95° C for 15 min, this was followed by 40 cycles of denaturation at 94° 0C for 1 min, annealing temperature at 56 0C for 30 seconds each, extension at 72 0C for 45 seconds. The reaction mixture in each Rt-PCR tube was then subjected to a final extension step at 72 0C for 10 minutes. All Rt-PCR amplifications were carried out at 50 ul volume per tube.

2.9.3 Preparation of agarose gel

The agarose gel was prepared by adding 1 gram of agarose powder to 100ul of Tris acetate buffer mixed together and dissolved by microwave, then ethidium bromide was added to the gel for staining, after cooling down the gel was poured in rack.

Following amplification, 12 ul from each Rt-PCR tube containing amplified product was loaded onto gels of 1.5% agarose and electrophoresed. The Rt-PCR products were visualized under UV light.

2.10 Data Analysis

Seroprevalence, with 95% confidence intervals, was calculated for each area and overall, taking into account the different sampling weights in each area. Seroprevalence was compared between areas, using the ANOVA test, also it was used to estimate the association of age, sex and locality with the outcome (seropositive to RVFV) while controlling for possible confounding.

For this purpose, age was categorized into Four group, >1, 1< 3, 3-5, >5.

2.10.1 Screening test

Screening test will used to identify animals more likely to have a RVFV infection. There are two measures that are commonly used to evaluate the performance of screening tests: the sensitivity and specificity of the test. Sensitivity tests the ability to correctly identify animal carrying the virus (true positive rate), whereas specificity tests the ability of the test to correctly identify the animals without having the virus (true negative rate). Sensitivity and specificity test was calculated by the following equations:

Sensitivity% = No. of true positives
No. of true positive+ No. of false negative

Specificity%= No. of true negative
No. of true negatives+No. of false negatives

Likelihood ratio = No. of true negative
No. of true negative + No. of false negative

2.10.2 Receiver Operating Characteristic (ROC) curve analysis

To evaluated performance and accuracy of ELISA and Rt-PCR to discriminate infected cases from normal cases ROC curve analysis [49] was used.

3. RESULTS AND DISCUSSION

3.1 Characteristics of Study Animals

Serum samples were randomly collected in a cross-sectional survey in 2017 from six sites namely;Shikaira, Wad alnor, Wad alnaem, Shabona, Albasabeir and Umalquora which represent a number of sites reported as area infected by virus in 2007 and 2010 (Fig. 1). As shown in Table 1, total number of 556 animals were sampled and the animals were distributed according to gender, females represent 479 (86.2%) and males 77 (13.8%). The age was grouped in four categories about 62% of animal aged between 1 to 3 years, while 22.1% in age 3 to 5 years (Table 2). These animals represent four ecotypes of sheep that are normally raised by nomads in Savannah and semi-arid zones in Sudan;they are of *Dobasi, Ashgar, Whatsich* and *Baladi* ecotypes.

3.2 Detection of RVFV Using ELISA

ELISA is reported as the most sensitive test for the detection of border line levels of antibody to RVF, total of 556 serum samples from non-vaccinated sheep were subjected for detection of the antibodies against the virus infection using IgG ELISA-kit. The ELISA test indicated that the highest Seroprevalence was found at Shikaira (38%), while samples from Albasabeir, Wad alnaem and Umalqura tested positive for antibodies against RVFV by 15%, 10% and 9%respectively (Fig. 2). The lowest Seroprevalence found at Wad anor and Shabona (5% and 2%). The overall seroprevalence was 14.2% in the study sites. However, there is no significant difference among the six study sites with respect to seroprevalence. A similar result obtained by [50] who reported no significant difference among five districts in Mozambique with respect to RVFV seroprevalence.

As shown in Table 3, the females showed increased numbers of animals with respect to anti- RVFV IgG positivity than the males (Table 4). This trend of female susceptibility was supported by [50] and [51] in both goat and sheep.

Table 1. The distribution of animals according to sex)

Area	No. of samples	Gender	
		Female	Male
Shikaira	37	26 (4.7%)	11(1.97%)
Wad anor	100	83(14.9%)	17(3%)
Wad alnaem	101	85(15.3%)	16(2.9%)
Shabona	99	86(15.5%)	13(2.3%)
Albasabeir	119	111(20%)	8(1.4%)
Umalquora	100	88(15.8%)	12(2.2%)
Total	**556**	**479**	**77**
Percentage		**86.2**	**13.8**

Table 2. The distribution of animals according to age

Area	No. of samples	Age Group			
		<1	1 < 3	3 - 5	>5
Shikaira	37	6(1.07%)	13(2.33%)	12(2.16%)	6(1.07%)
Wad anor	100	12(2.15%)	62(11.15%)	24(4.31%)	2(0.36%)
Wad alnaem	101	14(2.51%)	64(11.51%)	21(3.77%)	2(0.36%)
Shabona	99	10(1.79%)	63(11.33%)	23(4.13%)	3(0.54%)
Albasabeir	119	21(3.77%)	79(14.21%)	17(3.05%)	2(0.36%)
Umalquora	100	10(1.79%)	61(10.97%)	26(4.67%)	3(0.54%)
Total	**556**	73	342	123	18
Percentage		13.1	61.5	22.1	3.2

The high infection rate at Shikaira site reported from this study, was due to many factors which include sand dunes in the area coupled with permanent water sources (stagnant water) and these create suitable conditions for vector (Aedes mosquitoes) for breading, survival and development. These findings are strongly supported by other works done elsewhere [52,53].

High percentage of infection (33.5%) in old age (> 12 month) was reported [50] this result is in agreement with findings of this study (61.5%), usually the old females that constitute the bulk of our sample size are kept for breeding purposes thus could serve as reservoir for the virus and their movement increased the wide spread of the disease. Generally, the seroprevalence increased with age in this study similar to the result reported by [51] in sheep while [54] reported the same trends of age effect in camels. It is worth mentioning that in Sudan the husbandry of camel is usually accompanied by sheep breeding, for purpose of maximizing the profit from camel investment, a situation that may assist maintaining the transmission of RVFV between these highly susceptible two animal bread.

In our study the non-vaccinated samples recorded 14.2% this result in accordance with the findings of [51] who reported 14.9% however they reported 19.2% in vaccinated samples. There is no significant difference in prevalence between male and female sheep ($\chi 2$= 0.699, P = 0.4031), this result in accordance with that of [54] (who reported no significant difference between gender in sheep and camel as well as the study of [55] in cattle.

Table 3. Anti-RVF IgG seroprevalence among female sheep according to ages

	Female (+VE)				%+ve by area
area	<1	1 < 3	3 - 5	>5	
Shikaira	0	5(19.23%)	5(19.23%)	1(3.84%)	11(42%)
Wad anor	0	0	5(7.58%)	0	5(6%)
Wad alnaem	1(2.78%)	7(19.44%)	0	0	8(9.4%)
Shabona	0	2(2.53%)	0	0	2(2.3%)
Albasabeir	5(5.10%)	11(11.22%)	2(2.04%)	0	18(16.21%)
Umalquora	2(2.29%)	5(5.74%)	2(2.29%)	0	9(10.22%)
Overall					11.01%

Table 4. Anti-RVF IgG seroprevalence among male sheep according to ages

	Age group				%+ve
Area	>1	1 - 3	3 - 5	<5	
Shikaira	3(27.27%)	1(9.09%)	1(9.09%)	0	5(45.45%)
Wad anor	0	0	0	0	0
Wad alnaem	1(5.88%)	1(5.88%)	0	0	2(12.5%)
Shabona	0	0	0	0	0
Albasabeir	0	0	0	0	0
Umalquora	0	0	0	0	0
Overall					9.01

Table 5. *Anti-RVF IgG seroprevalence* according to sheep gender

	+ve	-ve	χ2	*P. value*
Male	7	63	0.699	0.4031
Female	51	341		

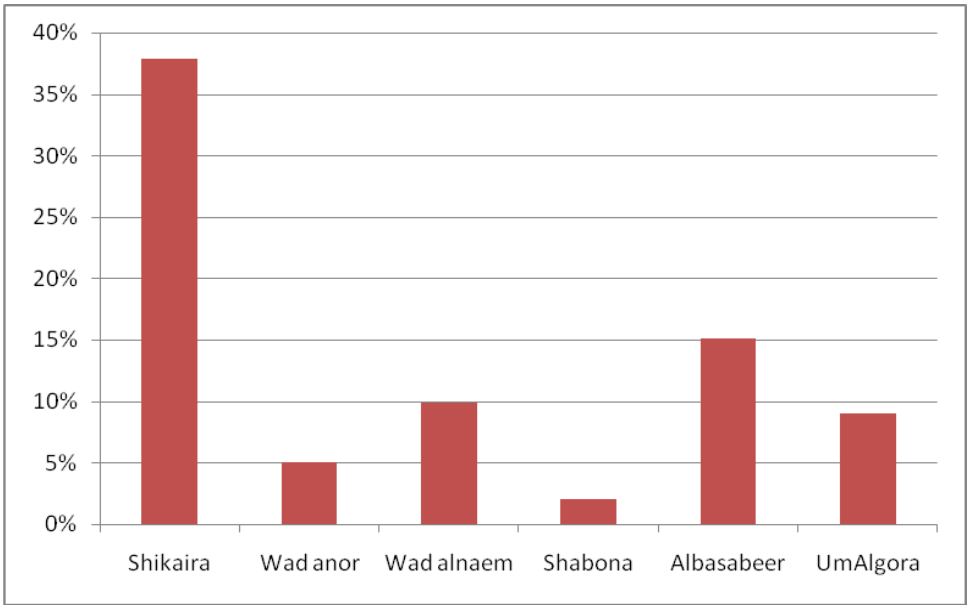

Fig. 2. Prevalence of RVF among sheep in the study area using ELISA

Table 6. Prevalence of RVFV infection by Rt-PCR test according to sex

Area	female (+VE)				% of infection
	Age group				
	<1	1 - 3	3 - 5	>5	
Shikaira	0	1 (3.84%)	3(11. 53%)	1(3.84%)	
Wad anor	0	0	0	0	
Wad alnaem	0	0	0	0	
Shabona	0	7(50%)	2 (14.3%)	0	
UmAlqura	0	0	1 (5.3%)	0	
Overall					22.2%

3.3 Detection of RVFV Using Reverse Transcriptase-Polymerase Chain Reaction

In the current study, due to the fact that none of our reference virological diagnostic laboratories satisfy the minimum requirements for virus isolation (level 3). So we adopted Rt-PCR as standard test to diagnose and confirm RVFV among studied animals. The Rt-PCR provided more sensitive and specific detection of RVFV in serum samples (sensitfity= 95% specificity = 80%). The medium genome of RVFV is preferable and had been tested [26,50,51]. The pair of primers designed for detecting the M segment was produced 342 bp primary Rt-PCR product (Fig. 3). The Rt-PCR amplification products were visualized onto an ethidium bromide stained agarose gel. From Table 6 the Rt-PCR test indicated that positive result was found among animals at Shikaira and Shabona and Umalqura. The Rt-PCR test revealed that the highest prevalence was reported at Shikaira 19%, while in Shabona was 11.10% and Umalqura tested positive for antibodies against RVFV 0.80% (Fig. 4). The lowest prevalence found at Wad anor and Wad alnaeem 0%. The overall prevalence was 15.23% in the study sites. This study did not reveal significant difference between male and female with respect to Rt-PCR (χ2= 2.34, P= 0.1261), similar to IgG result.

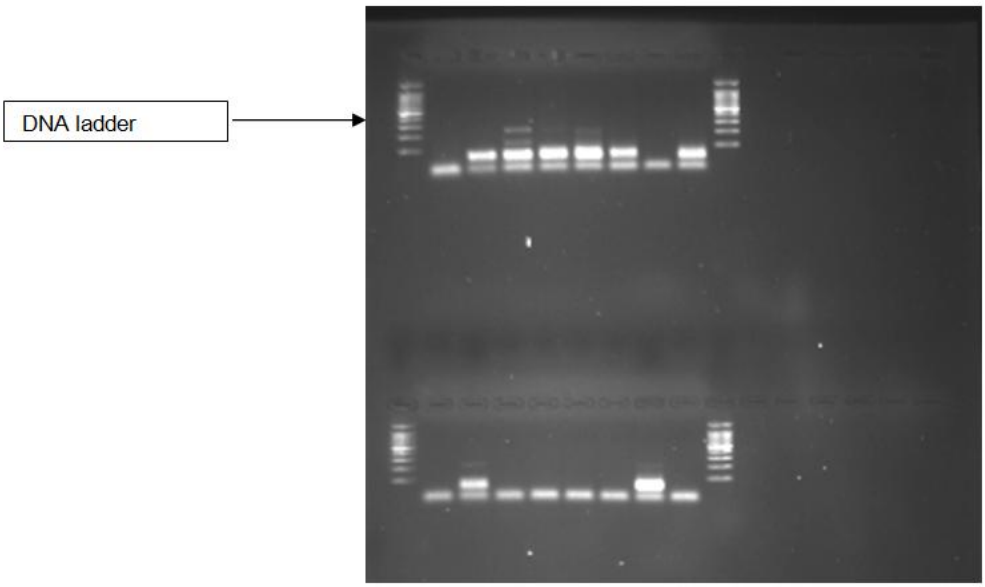

Fig. 3. The 1.5% ethidium bromide stained agarose gel for detecting 342 bp : Lane 1DNAladder, Lane 2 negative control, Lane 4 positive sample 342bp

Table 7. Prevalence of RVFV infection by Rt-PCR test according to age

Area	Male (+ve)				% of infection
	Age group				
	>1	<1 - 3	3 - 5	<5	
Shikaira	1(10%)	1(10%)	0	0	
Wad anor	0	0	0	0	
Wad alnaem	0	0	0	0	
Shabona	1(4.8%)	1(4.8%)	0	0	
UmAlqura	0	0	0	0	
Total					7.7%

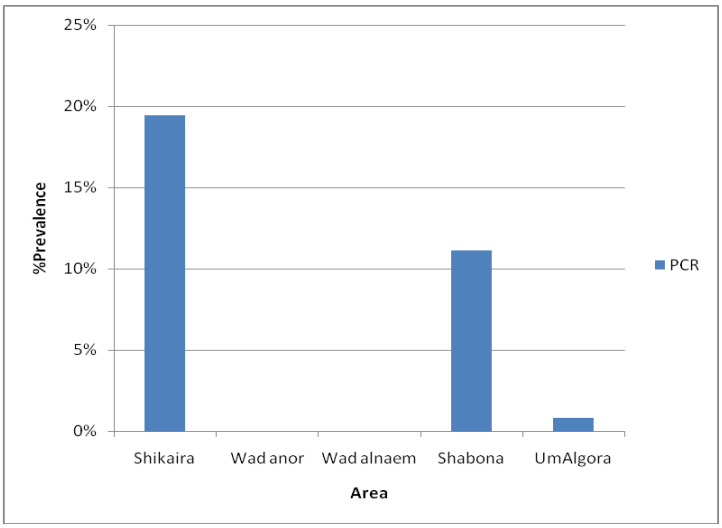

Fig. 4. Prevalence of RVF in the study area by using Rt- PCR

Table 8. Prevalence of RVFVaccording to sheep gender using Rt-PCR

	+ve	-ve	$\chi 2$	*P. value*
Male	4	52	2.34	0.1261
Female	15	81		

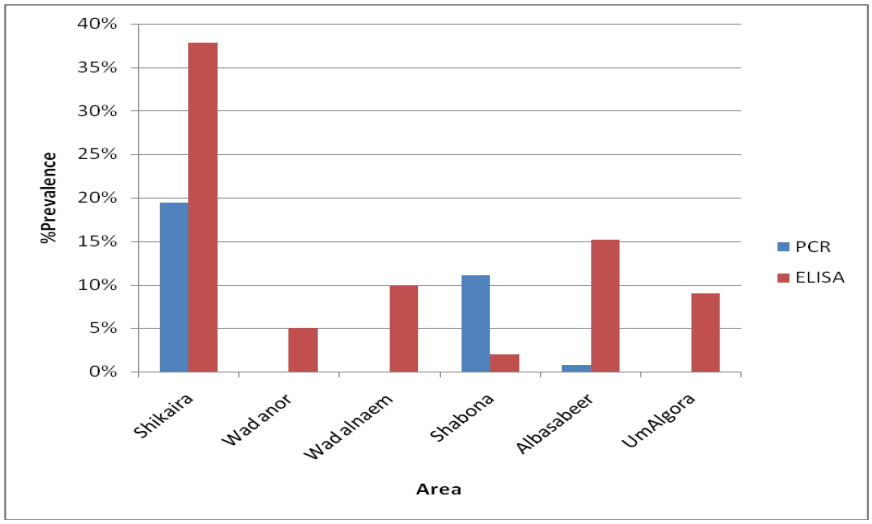

Fig. 5. Prevalence of RVF in the study area by using Rt- PCR and ELISA

3.4 Validation of Rt-PCR and ELISA in detecting RVFV

Fig. 4 showed that the percentages of RVFV infections were high when using the ELISA for the five areas expect Shabona site. It was stated that the Rt-PCR is suitable for detection of recent infection of the virus within first three days, while the ELISA may detect the antibodies after infection during period ranging from one week to months by identifying the Immunoglobulin M (IgM) and Immunoglobulin G (IgG) [56]. There is high percentage of infection in Shabona site, when using Rt-PCR, a result likely due to circulating of the virus during data collection. These findings taken together highlight the complementary nature of molecular detection assays and serologic tests and the importance of using a combination of assays for reliable diagnosis of virus infection.

Analysis of variance was performed using the Statistix 8 software. The analysis was carried out for the pooled data from the study areas and for each area separately for both ELISA and Rt-PCR tests. As shown in Table 4 and Table 5 the analysis of variance showed that there is no significant difference between the age groups and sex (male and female) with respect to both tests. This result indicates that the infection with this virus doesn't depend on age or sex of the sheep. All age groups and both sexes are vulnerable to virus infection.

3.5 Sensitivity and Specificity of ELISA and Rt-PCR

The sensitivity test is defined as the ability of a screening test to detect a true positive, being based on the true positive rate, reflecting a test ability to correctly identify all infected animals. Table 6 showed that there is a high-sensitivity and low specificity test. In other words, they are good for catching actual cases of the virus. It was reported [57] that the rapidity, sensitivity and specificity of the RT- PCR assay would greatly facilitate detection of RVFV infection during an outbreak of the disease among humans and susceptible animals. The RT-PCR is often used as a supportive diagnostic assay to the time consuming and cumbersome conventional virus isolation laboratory procedure.

3.6 Receiver Operating Characteristic (ROC) Curve Analysis

ROC analysis was used to create cumulative values for failure and success then the values of the False Positive Rate (FPR) and the True Positive Rate (TPR) for each row was calculated. An excellent model of ROC analysis has AUC near to the 1 which means model has good

measure of selection based on the performance. To distinguish between the ELISA (actual class) and the Rt-PCR (predicted class) we used the class predictions produced by the model. Table 10 and Fig. 6 illustrated that the AUC is 0.79 which indicate that the Rt- PCR is gold standard test.

Table 9 (a,b,c,d,e,f). Sensitivity and specificity values for the ELISA and RT-PCR assays for RVFV for study sites

(a)

Area	Test	+VE	-VE		Likelihood Ratio
Um alqura	Rt-PCR ELISA	1 9 Sensitivity= 90%	15 89 Specificity= 52%	Positive predictive value 0.9% (95% CI) Negative predictive value 99% (95	**1.88**

(b)

Area	Test	+VE	-VE		Likelihood Ratio
Wad alneem	Rt-PCR ELISA	0 10 Sensitivity= 100%	24 53 Specificity= 52.63%	Positive predictive value 0.1000% (95% CI) Negative predictive value 1.000 (95% CI)	**2.11**

(c)

Area	Test	+VE	-VE		Likelihood Ratio
Shabona	Rt-PCR ELISA	11 1 Sensitivity = 0%	24 91 Specificity=47%	Positive predictive value 0% (95% CI) Negative predictive value 89% (95% CI)	**0.00**

(d)

Area	Test	+VE	-VE		Likelihood Ratio
Albasabeir	Rt-PCR ELISA	0 17 Sensitivity = 100%	118 88 Specificity= 53.88%	Positive predictive value 14.4% (95% CI) Negative predictive value 100% (95% CI)	**2.17**

(e)

Area	Test	+VE	-VE		Likelihood Ratio
Wad anor	Rt-PCR ELISA	9 5 Sensitivity= 100%	20 72 Specificity= .51.3%	Positive Predictive Value 0.0500 (95% CI) value 99% (95% CI) Negative predictive value 1.00 (95% CI)	**2.05**

(f)

Area	Test	+VE	-VE		Likelihood Ratio
Shikaira	Rt-PCR ELISA	7 11 Sensitivity = 90%	28 24 Specificity= 52%	Positive predictive value 0.9% (95% CI) Negative predictive value 99% (95% CI)	**1.88**

Table 10. Receiver operating characteristic curve for ELISA and PCR

ROC for +Ve ELISA and+ Ve Rt-PCR							
			Cumalitive				
Age	**+ve ELISA**	**+ve Rt-PCR**	**+ve ELISA**	**+ve Rt-PCR**	**FPR**	**TPR**	**AUC**
			0	0	1	1	0.21
<1	12	2	12	2	0.79	0.89	0.52
>1-3	34	10	46	12	0.21	0.33	0.057
3-5	10	5	56	17	0.034	0.06	0.0019
>5	2	1	58	18	0	0	0
	58	18					0.79

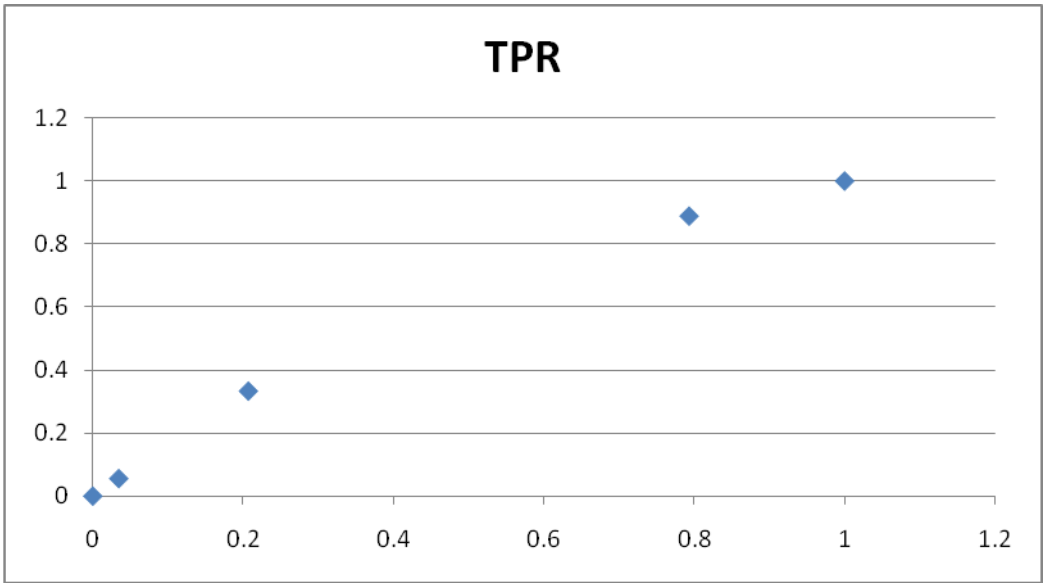

Fig. 6. Receiver Operating Characteristic curve

4. CONCLUSIONS

The results from this study showed the presence of anti-RVFV antibodies in all study sites, suggesting that RVFV is actively circulating among sheep in Gezira state.

The high seroprevalence of RVFV infection in Gezira state indicates its endemicity.

The present study highlights the importance of tracking the spread of the virus and its implication for analyzing changes in RVFV endemicity in specified areas over time.

Our results revealed that the seroprevalence for RVF virus was (14.2%) in Gezira state.

The Rt-PCR used in the present study is reliable to detect precisely the M segment of RVFV. It is valuable as a confirmatory test and should be considered by reference laboratories in the region.

ETHICAL APPROVAL

The field work and laboratories investigations has been conducted at Gezira State and Blue Nile National Institute for Communicable

Diseases. the author received an ethical permission from the general directorate of animal health, federal ministry of agriculture and animal resources;the author also received an ethical clearance from veterinary ethics committee (vec). animals sampled were housed and cared in accordance with national international legislation and local animal regulation requirements. the blood collection procedure from sampled sheep was performed by qualified veterinarian following proper physical restraint of animal to ensure both personnel and animal safety.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Food and Agriculture Organization of the United Nations (FAO) Statistic Year Book 2019.
2. Food and Agriculture Organization of the United Nations (FAO) Statistic Year Book 2016.
3. Peters C.J, Meegan J. Rift Valley fever p. 403-420. In:Beran G W (ed.), CRC handbook series in zoonoses, section B:viral zoonoses, vol. 1. CRC Press, Inc, Boca Raton, Fla;1982.
4. Daubney R, Hudson J.R, Garnham PC. Enzootic hepatitis or rift valley fever an undescribed virus disease of sheep, cattle and man from east Africa. J. Pathol. Bacteriol. 1931;34:545-579.
5. Anymaba A, Linthicum KJ, Tucker C. Climate-disease connections:Rift Valley fever in Kenya. Cad Saude Publica, Rio de Janeiro. 2001;17(Suppl):133-140.
6. Eisa M, Kheirelseed ED, Shommein AM, Meegan JM. An outbreak of rift valley fever in sudan-1976. Trans. R. Soc Trop, Med.Hygein. 1980;74:417-419.
7. Meegan JM, Bailey CL. Rift Valley Fever. In: Monath, TP. (ed.). the arboviruses:Epidemiology and Ecology. CRC Press, Boca Raton, Florida. 1989;4:51-76.
8. Diallo B, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of chikungunya virus in Sen-egal:current data and transmission cycles. Am. J. Trop. Med. Hyg. 1999;60:281–286.
9. Diallo M, Lochouarn L, Ba K, Sall A.A, Mondo M, Girault L, Mathiot C. First isolation of the Rift Valley fever virus from Culex poicilipes (Diptera:Culicidae) in nature. Am. J. Trop. Med. Hyg. 2000;62:702–704.
10. Wilson ML. Rift Valley fever virus ecology and the epidemiology of disease emergence. Ann N Y Acad Sci. 1994;740:169–180.
11. Njenga, MK, Wanjala, R, Rao CY, Weiner M, Omballa V, et al. Using a field Quantitative Real- time PCR Test to Rapidly Identify Highly Viremic Rift Valley Fever Cases. Journal of Clinical Microbiology. 2009;47(4):1166-1171.
12. Sossah C. RE-emergence of RVF in southern Africa:How to better predict and respond?, . OIE regional seminar on South Africa;2009.
13. Jeffrey Mariner. Rift Valley Fever Surveillance. FAO Animal Production and Health Manual No. 21. Rome. Food and Agriculture Organization of the United Nations (FAO). 2018;80.
14. World Health Organization, Regional Office for the Eastern Mediterranean. Technical guide of the diagnosis, prevention and control of Rift Valley Fever in man and animal;1983. Accessed 11/11/2009. Available http:// www.who.org.
15. Flick, RandBolouy M. Rift valley fever virus. Current Molecular Medicine. 2005;5:827-834.
16. Elliott RM. Emerging viruses:the Bunyaviridae. Mol Med. 1997;3:572-577.
17. Hoogstraal H, Meegan JM, Khalil GM. The Rift Valley fever epizootic in Egypt 1977–78, II.Ecological and entomological studies. Trans. R. Soc.Trop. Med. Hyg.. 1979;73:624–629.
18. Erasmus BJ, Coetzer JA.. the symptomatology and pathology of Rift Valley fever in domestic animals pp. 77–82 in:Swartz TA, Klinberg M.A, Goldblum N, Papier C.M. (Eds.). Contributions to epidemiology and biostatistics:Rift Valley fever. S. Karger AG, Basel. 1981
19. Morvan, G;Rollin PE, Laventure, S, Rakotoarivony I, Roux J. Rift Valley Fever epizootic in central highlands of Madagascar. Research in Virology. 1992;14:407-415.
20. Youssef BZ. The potential role of pigs in the enzootic cycle of rift valley Fever at Alexandria governorate. Egypt. J Egypt Public Health Assoc. 2009;84:331–334.
21. Seufi AM. Galal FH. Role of Culex and Anopheles mosquito species as potential vector of Rift valley fever virus in Sudan

outbreak, 2007. BMC Infectious Diseases. 2010;10 (65).
22. Anderson GW, Saluzzo Jr, Ksiazek JF, Smit TG, hJF, Ennis W, Thureen D et al. Comparison of in vitro and in vivo systems for propagation of Rift Valley fever virus from clinical specimens. Res. Virol. 1989;140:129–138.
23. Fukushi S, Nakauchi M, Mizutani T, Saijo M, Kurane I, Morikawa S. Antigen-capture ELISA for the detection of Rift Valley fever virus nucleoprotein using new monoclonal antibodies. Journal of Virological Methods. 2012;180 (1) :68-74.
24. van der Wal FJ, Achterberg RP, de Boer SM, Boshra H, Brun A, Maassen CB, et al. Bead-based suspension array for simultaneous detection of antibodies against the Rift Valley fever virus nucleocapsid and Gn glycoprotein. J Virol Methods. 2012;183:99–105.
25. Kortekaas J, Kant J, Vloet R, Cêtre-Sossah C, Marianneau P, Lacote S, et al. European ring trial to evaluate ELISAs for the diagnosis of infection with Rift Valley fever virus. J Virol Methods. 2013;187:177–181.
26. Sall A.A, Macondo EA, Se ne OK, Diagne M, Sylla R, Mondo M, et al. Use of reverse transcriptase PCR in early diagnosis of Rift Valley fever. Clin. Diagn. Lab. Immunol. 2002;9:713–715.
27. Wilson WC, Weingartl HM, Drolet BS, Davé K, Harpster MH, Johnson PA, Faburay B, Ruder MG, Richt JA, McVey DS. Diagnostic approaches for Rift Valley fever. Dev Biol (Basel). 2013;135:73-80.
28. Anderson GW Jr, Saluzzo, JV, Ksiazek TJ, Smith JF, Ennis W, Thureen D et al. Comparison of in vitro and in vivo systems for propagation of Rift Valley fever. Res Virol. 1989;140 (2):129-138.
29. Coetzer, J. The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted foetuses. Onderstepoort J vet Res.1982;49(1): 11-17.
30. Williams R, Ellis C, Smith S, Potgieter C, Wallace D, Mareledwane, V, Majiwa P. Validation of an IgM antibody capture ELISA based on a recombinant nucleoprotein for identification of domestic ruminants infected with Rift Valley fever virus. Journal of Virological Methods. 2011;177(2):140-146.
31. Fukushi S, Nakauchi M, Mizutani T, Saijo M, Kurane I, Morikawa S. Antigen-capture ELISA for the detection of Rift Valley fever virus nucleoprotein using new monoclonal antibodies. Journal of Virological Methods. 2012;180(1):68-74.
32. Gerdes G. Rift Valley Fever. Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees) 5th ed. Office International des Épizooties, Paris. 2004;185-194.
33. Sellers RF, Pedgley DE. Rift Valley fever in Egypt 1997. Disease spread by wind-borne vector. Veterinary Record. 1982;110:73 - 77.
34. Abdel Wahab KSE, EL Baz LM, Eltayeb EM, Omar H, Ossman Mam. Yassin W. Rift Valley fever virus infections in Egypt:Pathological and virological findings in man. Tran8actions of the Royal Society of Tropical Medicine and Hygiene. 1978;72:392-396.
35. Meegan ML, Niklasson B, Bengtsson E. Spread of Rift Valley fever virus from continental Africa. Lancet. 1979;2(8153): 1184 -1185.
36. Laughlin LW, Meegan JM, Strausbaugh LJ. Epidemic Rift Valley fever in Egypt:Observations of the spectrum of human illness. Trans. R. Soc. Trop. Med. Hyg. 1979;73:630–633.
37. Hassan OA, Ahlm C, Sang R, Evander M. the 2007 Rift Valley fever outbreak in Sudan. PLoS Negl. Trop Dis. 2011;5(9):e 12229
38. Saleh A, Mohammed KA, Hassan MM, Thomas J. Antibodies to Rift Valley fever virus in human population of Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1980;75 (1):129-130.
39. Tamador MAE. Epidemiology of Rift Valley fever in Sudan PhD thesis. Faculty of Veterinary Science, University of Khartoum. Sudan; 2007.
40. House JA, Kocan KM, Gibbs EPJ. Editors. Tropical veterinary medicine diseases:control and prevention in the context of the new world order. New York:Annals of the New York Academy of Science;. 2000.
41. The Integrated Framework Program. Revitalizing Sudan's non-oil exports: a diagnostic trade integration study. 2008;41–115.
42. El Imam M. El Sabiq M, Omran M, Abdelkareem A, El Gaili Mohamed MA, et al. Acute renal failure associated with the Rift Valley Fever:a single center study.

Saudi J Kidney Dis Transpl. 2009;20:1047–1052.
43. Eisa M, Obeid HMA, El Sawi ASA. Rift Valley fever in the Sudan. Results on field investigations of the first epizootic in Kosti District 1973. Bull Anim Health Prod Afr. 1977;25:343–347.
44. Ahmed Kamal S. Observations on Rift Valley fever virus and vaccines in Egypt. Virol J. 2011;8(1):532.
45. Food and Agriculture Organization of the United Nations (FAO) Stat Data Rome. 2018).
46. Behnke R. The economics of pastoral livestock production in Sudan. Somerville MA:Feinstein International Center, Tufts University; 2012.
47. Ministry of Animal Resources and Fisheries (MARF). Statistical Bulletin for Animal Resources No 19. 2009. Khartoum (Sudan).
48. Blow JA, Dohm DJ, Negley DL, Mores CN. Virus inactivation by nucleic acid extraction reagents. J. Virol. Methods. 2004;119:195 –198.
49. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots:a fundamental evaluation tool in clinical medicine. Clin Chem. 1993;39:561–577.
50. Jose Fafetine, Luis Neves, Peter N, Thompson, Paweska, Victor P, Rutten MG Coetzer J.AW. Serological Evidence of Rift Valley Fever Virus Circulation in Sheep and Goats in Zambe´ zia Province, Mozambique. Neglecte Tropical Diseases. 2013;7(2):e2085.
51. Byomi AM, Samaha HA, Zidan SA, Hadad, GA. Some Associated risk factors with the occurrence of rift valley fever in some animals and man in certain localities of nile delta, Egypt. Assist Vet, Med. J. 2015;61:144 :10-17.
52. Pepin M, Bouloy M, Bird BH, Kemp A, Paweska J. Rift Valley fever virus (Bunyaviridae:Phlebovirus):an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. Vet. Res. 2010;41(6):61.
53. Elsaddig. personal communication in Certain localities of Nile Delta, Egypt. Assiut Vet. Med. J. 2017;63(154).
54. Maiy MMA, Ibrahim AA, Tamadur MA, Sanaa AA, Mohamed EA. Imadeldin EA.. A survey of rift valley fever and associated risk factors among the one-humped camel (Camelus dromedaries) in Sudan. Irish Veterinary Journal. 2016;69:6.
55. Tshilenge MG, Masumu J, Mbao V, Kayembe JM, Rwweyemamu M ark, Mulumba Kl. Seroprevalence and Virus Activity of Rift Valley Fever in Cattle in Eastern Region of Democratic Republic of the Congo. Journal of Veterinary Medicine. 2018;Volume 2018, Article ID 4956378, 8 pages.
56. Bird BH, Albarino CG, Nichol ST. Rift valley fever virus lacking nsm proteins retains high virulence in vivo and may provide a model of human delayed onset neurologic disease. Virology. 2007;362: 10–15.
57. Mohamed AE, Imadeldin EA. A simple and rapid method for detection of Rift Valley Fever Virus in cell culture using RT-PCR. International Journal of Tropical Medicine. 2006;1(1):44-47.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/75606