

Original Research Article

<https://doi.org/10.20546/ijcmas.2024.1311.001>

Prevalence and Potential Risk Factors of Rift Valley Fever in Sheep in East Nile Locality-Khartoum State, Sudan

Manal Ali El nour¹, Suleiman Adam Ibrahim Hano², M. I. M. Fangama³ and M. A. Abdalla⁴

¹Ministry of Animals Resources and Fishers, Khartoum State, Sudan

²Ministry of Animal Resources and Fishers, West Kordofan, Sudan

³Department of Food Safety, Ministry of Public Health, Qatar

⁴Faculty of Veterinary Medicine, Sudan University of Science and Technology, Sudan

*Corresponding author

ABSTRACT

Keywords

Rift Valley Fever, Sheep, ELISA, Sudan, genus Phlebovirus

Article Info

Received:

15 September 2024

Accepted:

24 October 2024

Available Online:

10 November 2024

Rift Valley Fever is an acute, mosquito-borne viral disease that causes a significant global threat to humans and livestock. This disease holds great importance in terms of its impact on agriculture and public health in African and Middle Eastern countries. RVF primarily affects domestic and wild ruminants such as cattle, sheep, goats, and camels. RVF closely linked to heavy rainfall, floods, and the presence of mosquito species. Recently, the RVF has become endemic in African continent including Sudan. A Cross-section study was conducted during June 2022 to June 2024. A total of 150 of samples were examined using competitive EALISA test to demonstrate the sera-prevalence of RVFIgG immunoglobulins in sheep's serum, in eastern Nile locality - Khartoum State, Sudan. RVFV seropositivity was recorded in 3 out of 150 samples. In addition, the overall prevalence was found 0.02%. Surprisingly, body condition was the only associated risk factor with Rift Valley Fever in the Study area using Univariate analysis (P -value =.009) and (X^2 : 6.921). Other factors Such as animals site, Age, Sex, Breed, Presence of mosquito, grassing system, herd size, mortality in kids, and abortion in sheep were not found significantly associated with Rift Valley Fever. Finally, Rift Valley Fever needs an epidemiological study to understand this disease incidence, prevalence and the effective control methods and the best prevention measures that should be implemented including one health approach.

Introduction

Rift Valley Fever (RVF) is an acute, mosquito-borne viral disease that yields a significant impact to humans and animals as well (Linthicum *et al.*, 2016). This disease holds great importance in terms of its impact on agriculture and public health in African and Middle

Eastern regions. Rift Valley Fever (RVF) virus is a single-stranded segmented RNA virus that falls under the genus Phlebovirus, belonging to the family Phenuiviridae within the order Bunyavirales. Indeed, RVFV primarily affects domestic and wild ruminants such as cattle, sheep, goats, and camels (Nielsen *et al.*, 2020). Clinical manifestations vary from sudden fatalities to mild,

nonspecific symptoms, contingent upon the potency of the virus strain and the species, breed, and age of the afflicted animals (Hise *et al.*, 2015). The disease was initially discovered in sheep in the Rift Valley province of Kenya in 1931 (Daubney *et al.*, 1931). Now days, the disease has become endemic across numerous African countries and the Arabian Peninsula (Nanyingi *et al.*, 2015). In addition, Susceptible animals are primarily infected by vector bites, which are mainly transmitted through various species of vectors. Such, *Aedes* mosquitoes have been identified as primary vectors and potential reservoirs of the rift valley fever disease (Woah, 2019). Moreover, RVF can also be transmitted to humans through mosquito bites, direct contact with infected animals and their bodily fluids (such as blood, discharges, and abortion materials), or by consuming untreated animal products like meat and milk. Its important to know, there is no identified human to human RVF transmission case (Nielsen *et al.*, 2020). Diagnosis of RVF is based on the epidemiological factors including abnormal heavy rains, clinical symptoms and signs, occurrence of storm abortions in small ruminants and serological diagnosis using ELISA. Confirmation of the diagnosis is based on the identification of the organism using RT-PCR techniques. In general, diagnostic techniques such as samples culture, Agar gel immunodiffusion, Histopathology, Virus neutralization, and Haemagglutination inhibition are used to diagnose the RVF disease depends on defined situations and purposes.

RVF outbreaks are associated with the occurrence of floods, increased greenness of vegetation index, and emergence of mosquito vectors that infect susceptible ruminant hosts (Indeje *et al.*, 2006).

RVF virus activity occur more frequently in old Sudan (FAO, 2003). First report of this disease in Sudan was in 1973, where an outbreak occurred in Kosti city in white Nile state, and some areas of Blue Nile state and Khartoum north (Eisa *et al.*, 1977). In Sudan, Rift Valley fever cases have been reported intermittently across various periods and regions, including Gezira, Sennar, White Nile, Kassala, Khartoum, and River Nile states, with the majority of cases and fatalities reported in Gezira state (Ahmed *et al.*, 2020; Hassan *et al.*, 2011). Various significant factors contribute significantly to the circulation and persistence of Rift Valley Fever Virus in the country especially presence of an appropriate environment for the multiplication and growth of the vector mosquito, the wide distribution of mosquitoes

(Mahmoud *et al.*, 2021). Addition to that keeping the domestic animals in close contact with households, handling freshly slaughtered infected sheep meat, and a lack of public health education, has resulted in massive losses of human lives during RVF outbreaks. Additionally, other factors can increase the risk of human RVF infection, such as age, sex, occupation water, nutrition, socioeconomic status and poor sanitation (Nyakarahuka *et al.*, 2016) these factors may Increase likelihood of RVF outbreak occurrence in Sudan.

The most susceptible hosts to Rift Valley Fever (RVF) infection include sheep (Pepin *et al.*, 2010).

In Sudan, multiple studies have conformed the prevalence of rift valley fever among sheep, ranging from low RVF prevalence of 0.15% among in the central states of Sudan (Mansour *et al.*, 2024) to a high RVF 40.2%, with rates of 51%, 50%, and 14% in Gezira, White Nile, and Sinner and Blue Nile states respectively. Since, the population of sheep in Sudan exceeded 51 million heads according to Wilson (2018). Moreover, sheep are the most raised animals in the area of study and directly linked to export activities particularity to gulf states. As a result, the importance of this current lies in bridging the knowledge gap about RVF disease among sheep in eastern Nile locality, Khartoum State. This study aims to investigate the prevalence of the rift valley fever and to determine potential risk factors associated with occurrence of it, in eastern Nile locality, Khartoum state, Sudan.

Materials and Methods

Study area

The study was cared out in Eastern Nile locality an area of 8000 km square located in the north eastern part of Khartoum state. Khartoum state has semidesert climate, the rainfall is usually 150 to 250mm per year characterized by very hot/dry summer and cold winter, the average temperature ranges from 21 in winter to 47 degree centigrade in the summer. The manual evaporation rate is 7.7mm/day and the average relative humidity range from 21% to 38% (Abdallah *et al.*, 2015).

Study design

Cross-sectional study design was carried out to determine the prevalence of RVF. The study was conducted during period of June 2022 to June 2024.

Study population

The study was primarily focused on sheep herd as reference or target population in locality of eastern Nile. Eastern Nile locality was selected convincingly. Herd and farms which animals were studied were selected randomly.

Sample size Determination

Number of animals sampled were (138) sample according to [Abdallah et al., \(2015\)](#). Sample size was determined according to [Thrusfield \(2005\)](#); $N = (1.922 * PQ / L^2)$.

Where: N= the sample size.

$(1.96)^2 = \text{constant}$.

$P^{\wedge} = \text{expected prevalence of RVF}$.

$Q^{\wedge} = 1 - P^2$

$L^2 = \text{allowable error (5\%)}$.

Sample collection

Blood samples were collected from selected sheep from designated herd and farm in the study area. Serological diagnosis was carried out on collected specimens to demonstrate the presence of IgG immunoglobulines by ELISA for rift valley fever ([FAO, 2003](#)).

RVF prevalence was determined by calculating the ratio of positive cases identified through the test to the total number of sheep sampled in the study area at a specific time point ([Elhassan et al., 2019](#)).

Blood sampling

To determine seroprevalence of rift valley fever in sheep. Investigated animals were physically examined in order to demonstrate breed, age and sex. Moreover, body condition, temperature, respiratory rate and mucous membranes were examined and recorded for individual animals in this study to ensure that selected animals were healthy. Sequentially, blood Samples were collected through vein puncture from each animal in glass tubes without anticoagulant, and serum was separated by centrifugal process and then stored at -20°C until use ([Mahmoud et al., 2021](#)).

Experiment procedure

The experiment was carried out at the virology department within the Rift Valley Fever division at the central veterinary research laboratories. The ID Screen® Rift Valley Fever competition multi-species kits were procured from ID.VETS, an esteemed provider of innovative diagnostics located in Grabels, France. The tests were meticulously conducted following a series of precise steps.

In the ELISA micro-plate, 50µL of Dilution Buffer 19 were added to each well. Subsequently, 50µL of the positive control was dispensed into wells A1 and B1, while 50µL of the negative control was placed in wells C1 and D1. Following this, 50µL of each sample to be tested was carefully introduced into the remaining wells.

The plates were securely covered and incubated for a duration of 60 minutes with a tolerance of ± 6 minutes at a temperature of 37°C with a variance of $\pm 2^{\circ}\text{C}$.

After incubation, the wells were carefully emptied and washed three times using 300µL of the designated wash solution.

The Anti-RVF-NP Conjugate 1x was prepared by diluting the Anti-RVF-NP-PO Conjugate 10X to a 1:10 ratio in dilution buffer 19. Subsequently, 100µL of the Conjugate 1x was dispensed into each well. The plates were then covered and incubated for 30 minutes with a variance of ± 3 minutes at 21°C ($\pm 5^{\circ}\text{C}$). Following this, the wells were emptied and underwent three washes with 300µL of the wash solution.

Next, 100µL of the Substrate was added to each well, followed by covering the plates and incubating for 15 minutes with a variance of ± 2 minutes at 21°C ($\pm 5^{\circ}\text{C}$) in darkness. To halt the reaction, 100 µL of stop solution was introduced into each well. The outcomes were subsequently measured and recorded at O.D. 450 nm.

Investigation of potential risk factors

All animals included in this study were subjected to questionnaire, which was filled out by the animal owners, the questionnaire was included individual risk factor attributes including age (Younger, older animals), sex (male, female), body condition (emaciate, fat) and management risk factor attributes including herd size (small, medium, large), grazing system (nomadic, semi-

nomadic, stationary), rainfall (high, low), mosquito control (practiced or not), vegetation (low, high), water bonds (presence or absence).

Statistical data analysis

The collected data was organized and managed using excel spread-sheet (WPS Office). as well as, the data were analyzed using statistical software package for social sciences (SPSS version 21.1). Univariate analysis using the Chi Square test was used to investigate risk factors associated with rift valley fever (Kim, 2017).

Results and Discussion

The overall prevalence of rift valley fever

The study was carried out during period of June 2022 to June 2024. 150 serum samples were collected from healthy sheep were investigated to determine the prevalence of rift valley fever, to investigate the potential risk factors which may associated with it. Moreover, the study aimed to measure the level of animals owners in regard of rift valley fever. As a result, our finding shows that, Out of 150 serum samples examined, 3 samples were tested positive to rift valley Fever in sheep, with an overall prevalence of 0.02%. (Table 3.1). Among all factors which included in this study, body condition was the only one Statistically associated with rift valley fever at level of (P-value = 0.009). Other factors were not found Significantly associated with rift valley fever in our study (table 3.2.1).

Prevalence of Rift Valley fever in relationship to the potential risk factors

Risk factors that had a significant association with Rift Valley Fever

Among all risk factors which included in our study; body condition score showed significant association with the infection with rift valley fever based on chi-squared analysis at (P-value = 0.009).

(Table. 2) showed that, 104 samples out of 150 sample found in good body condition in the other hand, 43 animals out of 150 were found as bad condition. In addition, all positive sample which 100% of positive sample were found among the animals with bad condition.

Risk factors that had no significant association with rift valley fever

No significant association ($P\text{-value} \leq 0.05$) had been found between the prevalence of rift valley fever and animals side, sex, breed, herd size, grazing system, presence of mosquito, vaccination, abortion, mortality in kids, and water sources. Furthermore, factors related to owners were found not significantly associated with RVF disease in our study.

Rift Valley Fever (RVF) is an acute, mosquito-borne viral disease that causes a significant global threat to humans and livestock. This disease holds paramount importance in terms of its impact on agriculture and public health in African and Middle Eastern regions. RVF primarily affects domestic and wild ruminants such as cattle, sheep, goats, and camels.

The RVF virus spread after heavy rainfall, which is reported to be significantly associated with weather abnormalities in the Greater Horn of Africa, leading to RVF outbreak especially in East African countries (Davies *et al.*, 1985; Nanyingi *et al.*, 2015). The Disease made its debut outside of Africa in the early 2000s when an RVF outbreak surfaced in Saudi Arabia and Yemen.

150 serum samples of sheep were analyzed using competitive ELISA test in the eastern Nile locality, Khartoum State, Sudan. The research has revealed that the overall prevalence was 0.02%.

Furthermore, our findings indicate that among all the risk factors considered in the study, only the body condition score showed a significant association with Rift Valley fever in the eastern Nile locality. Our findings align with a previous study conducted by Mansour *et al.*, (2020), indicating a prevalence rate of 0.15% among sheep in the central states of Sudan. This result is in agreement with other study conducted by Mansour *et al.*, (2021) his finding was the overall seroprevalence for RVF was 0.01% in Khartoum and Sennar states.

On the contrary, Our findings sharply contradict the conclusions drawn by previous studies regarding the prevalence of Rift Valley Fever in sheep in Sudan; the prevalence has been determined to be lower than the results obtained by Eisa (1984), which reported a prevalence of 32.2%.

Table.1 The overall prevalence of Rift Valley Fever in sheep. in eastern Nile locality, Khartoum state – Sudan

Number of Animals tested	Number of negative	Number of positive	%
150	147	3	0.02%

Table.2 Risk factor Significantly associated with rift valley fever in study area.

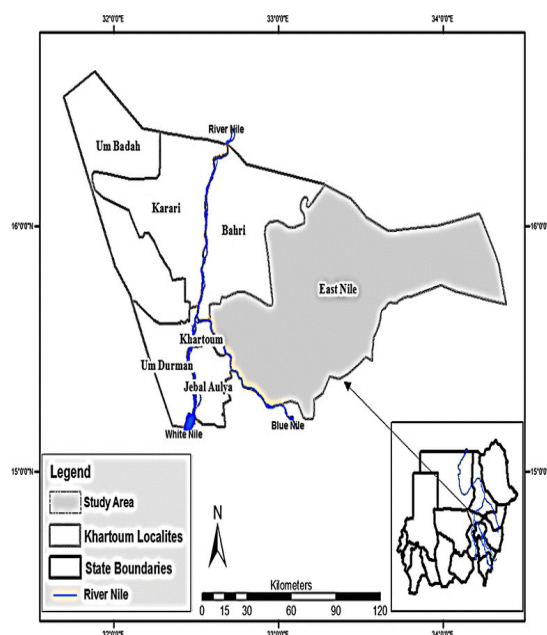
Body Condition	Number of negative	Number of positive	Percentage	Value	df	value
Good	0 (0.0%)	104 (1005)	0.0%	6.921	1	.009
Bad	46	3 (6.52%)	43 (93.48%)			

Table.3 Percentage, *P*-value of not Statistically significant risk factors

Risk Factors	No. of positive	No. of negative	Total	df	<i>P</i> - value
Vallege: El Hwata	2 (7.4%)	25 (92.5)	27	8	.531
Abosial	0 (0.0%)	10 (100%)	10		
Wd Hasuna	0(0.0%)	20 (100%)	20		
Um Rkham	0(0.0%)	23 (100%)	23		
EL Nala	0 (0.0%)	15 (100%)	15		
Al Kekel	1 (5%)	19 (95%)	20		
Farag alla	0 (0.0%)	12 (100%)	12		
Bastat	0 (0.0%)	8 (100%)	8		
Eid Habob	0 (0.0%)	15 (100%)	15		
Age: < 1 year	2 (5%)	38 (95%)	40	2	.242
1-3 year	0 (0.0%)	46 (100%)	46		
> 3year	1 (1.56%)	63 (98.4%)	64		
Sex: Male	1 (2%)	49 (98%)	50	1	1.000
Female	2 (2%)	98 (98%)	100		
Breed: local breed	3 (2%)	147 (98%)	150	1	-
Foreign breed	0 0.0%	0 0.0%	0		
Mosquito:presence	2 (1.8%)	108 (98.1)	110	1	.792
Not presence					
	1 (2.5%)	39 (97.5%)	40		
Grazing system :nomadic	3 (2.0%)	142 (98%)	145	1	.745
Semi nomadic	0 (0.0%)	5 (100%)	5		
Herd size: small	1 (1.61%)	61(98%)	62	2	.757
Medium	0 (0.0%)	15 (100%)	15		
Large	2 (2.73%)	71 (97.2)	73		
Food additive: added	2 (2.05)	95 (97.9%)	97	1	.759
Not added	1 (1.58%)	62 (98.2%)	63		
Vet services :Found	2 (1.6%)	130 (98.4%)	132	1	.251
Not found	1 (5.5%)	17 (94.5%)	18		
Lambs mortality: yes	0 (0.0%)	48 (100%)	48	1	.230
No	3 (3%)	99 (97%)	102		
Abortion: Aborted	0 (0.0%)	45 (100%)	45	1	.252
Not aborted	3 (2.8)	102 (97.2%)	105		

*df: degree of freedom.

Map.1 The study area eastern Nile locality (area highlighted in Grey) (Mohammed *et al.*, 2016)



Moreover, other research indicates that the occurrence of Rift Valley Fever (RVF) in sheep is 17%, in cattle is 33%, and in goats is 50% (Mohammed *et al.*, 2016). Additionally, our findings contrast with a study conducted by Abdallah *et al.*, (2015), revealing an overall prevalence rate of 9.6% of RVFV antibodies among camels in Khartoum State of Sudan. In addition, Elhaj *et al.*, (2021) the prevalence of RVF among sheep was 14.2% its prevalence was much higher than which we found.

Additionally, a study conducted by Mansour *et al.*, (2024) revealed varying findings, with RVF prevalence rates of 37% in Gezira, 6.4% in Sennar, 23% in White Nile, 6.7% in Blue Nile, 2.8% in Khartoum, and 9.8% in River Nile.

Moreover, our findings were in contrast with another study conducted by Elhassan *et al.*, (2014) showing an overall prevalence of 40.2%, with rates of 51%, 50%, and 14% in Gezira, White Nile, and Sinner and Blue Nile states respectively. This diversity in prevalence rates may be attributed to ecological variations of the disease and the presence of control measures. The reasons why the prevalence in our study is lower than in other studies conducted in the area is due to the fact that the data were obtained from animals during a period in which the virus was suspected to be circulating in the area. In other words, serum samples were collected during an outbreak

of RVF. Unlike other areas in central Sudan, the ecological conditions in the area where our study was conducted were significantly different. The majority of the population lived in a semi-arid to desert climate zone, which is not conducive to the proliferation of RVF vectors. Another contributing factor could be the absence of animal movement into or out of our study area. Likewise, our finding is also in disagreed with a study that reported a prevalence of 18.3% for RVF in abattoir workers in Kenya (Nyamota *et al.*, 2023).

Also, our research indicates a statistically significant association between body condition score and RVF in the eastern Nile region. Furthermore, other factors included in the study were not found Statistically Significant with Rift valley fever in our study.

Factors such age, sex, breed were not found statistically associated with rift valley fever. This may due to absence of RVFV in the study area. Additionally, the presence of mosquito were not found statistically associated with RVF in sheep in eastern Nile locality this result was in agreement with (Abdallah *et al.*, 2015).

This in discordance with fact that the disease transmitted by mosquito species Eisa (1984) he suggests that RVF outbreaks are associated with insect vectors. Factors like local, and herd size were not found statistically associated with RVF in sheep this finding is in agreeable

with Mansour *et al.*, (2020), which was Khartoum state, locality, species and animal population were not statistically associated with RVF seroprevalence (Abdallah *et al.*, 2015).

Even that, (Abdallah *et al.*, 2015) survey was conducted at camels it give us good opportunity to understand the epidemiology of disease from other aspect among animals species in Khartoum state.

Our study provides evidence of circulation of RVFV among sheep in eastern Nile locality - Khartoum State, Sudan. as determined by the presence of RVFV antibodies using competitive ELISA technique.

Study recommends that, further studies should be done in sheep in Khartoum state during different seasons. As well as, more epidemiological tools were needed to investigate the determinants of the disease in sheep in the Sudan.

Surveillance for RVF-vectors should implement to provide public health authorities an opportunity to anticipate and prepare for a possible RVF outbreak in Khartoum State, Sudan. Furthermore, our study recommends that One Health Approach should be implemented to address the Rift Valley Fever among animals and humans as well.

Acknowledgements

Authors expressed their deep thanks Department of Rift Valley Fever at the Central Veterinary Research Laboratories. Soba, Khartoum, Sudan for providing assistance in laboratory diagnosis of Study samples.

Author Contributions

Manal Ali El nour: Investigation, formal analysis, writing—original draft. Suleiman Adam Ibrahim Hano: Validation, methodology, writing—reviewing. M. I. M. Fangama:—Formal analysis, writing—review and editing. M. A. Abdalla: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Manal Ali El Nour, Suleiman Adam Ibrahim Hano, M. I. M. Fangama and Abdalla, M. A. 2024. Prevalence and Potential Risk Factors of Rift Valley Fever in Sheep in East Nile Locality-Khartoum State, Sudan. *Int.J.Curr.Microbiol.App.Sci*. 13(11): 1-8. doi: <https://doi.org/10.20546/ijemas.2024.1311.001>