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Seasonal Transmission Dynamics of Rift Valley Fever in Kilimanjaro Region, Tanzania

Kevin M Rwegoshola[a], Richard S Mwakapuja[b], Sixbert I Mkumbaye[c], Pendo M Ibrahim[a], Hadija S Semvua[d], Pius G Homurupembe[e,f], Jaffu O Chilongola[a]

[a]Department of Biochemistry and Molecular Biology, Kilimanjaro Christian Medical University College, Moshi, Tanzania, [b]Tanzania Vaccine Institute, Tanzania Veterinary Laboratory Agency (TVLA), Kibaha, Tanzania, [c]Department of Microbiology and Immunology, Kilimanjaro Christian Medical University College, Moshi, Tanzania, [d]Clinical Trials department, Kilimanjaro Clinical Research Institute, Moshi, Tanzania, [e]Department of Curative Services, Ministry of Health, Dodoma, Tanzania, [f]Department of Public Health and Research, Lugalo Military College of Medical Sciences, Dar es Salaam, Tanzania

Correspondence to Kevin M Rwegoshola (rwegok93@gmail.com)

ABSTRACT

Background: Rift Valley Fever (RVF) is a zoonotic disease that affects both animals and humans. Under reporting, misdiagnosis caused by the broad spectrum of symptoms presented by the disease, and limited access to rapid and accurate laboratory confirmation have led to an undefined burden of RVF. Reports are available that show the circulation of the virus during inter-epidemic periods, implying an endemic circulation of RVFV. This study aimed to determine RVFV transmission across annual seasons and demographic factors that are independently associated with exposure to RVFV.

Methodology: Repeated serosurveys were performed during the long rainy, short rainy, and dry seasons in Lower Moshi area of Moshi district, Kilimanjaro region from January to December 2020. The goal was to determine seroprevalence against RVFV antibodies in humans and factors associated with seropositivity. Detection of RVF antibody was performed by competitive Enzyme-Linked Immunosorbent Assays (cELISA) using serum samples. Stata statistical software version 15 was used for data analysis. Descriptive statistics was carried out, whereby categorical variables were summarised using frequencies and percentages. Numeric variables were summarised using median and interquartile range. Logistic regression was used to assess factors associated with RVF seropositivity.

Results: A total of 446 individuals were involved in the analysis. RVF seroprevalence was highest during rainy season (20.4%) and lowest in the dry season (4%). The overall annual seroprevalence of RVF was 12.8%. Season, participant age, and large number of residents in a given household were found to be significantly associated with RVF seropositivity ($p<.05$).

Conclusion: RVFV demonstrates an endemic circulation in Lower Moshi area of Kilimanjaro region, suggesting the site is a potential RVF hotspot. Based on this study's findings, we recommend close surveillance of RVF in the study area and other areas with similar ecology in Tanzania as a means of preparedness for future unpredicted RVF outbreaks.

BACKGROUND

Rift Valley Fever (RVF) is a Zoonotic disease that is caused by a Rift Valley Fever Virus (RVFV) belonging to family Bunyaviridae of genus Phlebovirus.[1] The disease primarily affects animals but can also infect humans.[2] Human transmission of RVF is through direct contact with infected animals mainly livestock such as; cattle, sheep, goats, buffalo, and camels, and bites from infected mosquitoes especially the Aedes and Culex species. Humans are at risk of being infected by RVFV when they live and engage in activities that bring them into contact with animals, animal products and vector mosquitoes.[3]

Due to under reporting, misdiagnosis caused by a broad spectrum of symptoms the infection can present with, and limited access to rapid and accurate laboratory confirmation, the burden of RVF remains undefined globally.[4] Reports show that RVF has mainly affected the Arabian Peninsula and Africa,[2,5] with over 3000 reported suspected and confirmed cases and approximately 1000 deaths from 2000 to 2007. In Tanzania, recent RVF outbreaks were reported in 2006 and 2007; Overall outbreaks occurred in 39.2% of the districts in Tanzania.[6] Such outbreaks may result in major societal impacts, including significant economic losses due to severe illnesses and abortions in domestic animals, which are significant income sources in different communities. Furthermore, RVF outbreaks have negative consequences on trade resulting from cross-border quarantines of domestic animal movements and animal products.[6]

Despite its rare occurrence and absence of its epidemics in recent years, studies in Tanzania continue to report the existence of RVF during the inter-epidemic period, in different regions of the country for both

animals and humans, hence showing evidence of virus circulation in Tanzania.[7–9] Most of the studies in Tanzania have attempted to determine the point prevalence of RVF with few or no studies that determine the period prevalence of RVF, showing the effect of seasonality on the transmission of RVFV as evidenced by the prevalence of RVF antibodies across seasons. Nonetheless, Regardless, key questions regarding the epidemiology of RVF remain to be; where the virus hides during the inter-epidemic periods, where RVF hotspots are, when will the next outbreak occur and whether seasonality affects RVFV transmission. This study aimed to determine RVFV transmission across annual seasons and demographic factors that are independently associated with exposure to RVFV.

METHODOLOGY

Study Design

The study involved comprehensive seasonal cross-sectional serosurveys to identify the seasonal inter-epidemic transmission dynamics of RVFV and its potential reservoirs across different seasons of the year 2020. The study conducted repeated seasonal cross-sectional serosurveys for a period of 12 months in a distinct ecological area with intimate contact between humans, livestock and vector mosquitoes.

Study Area

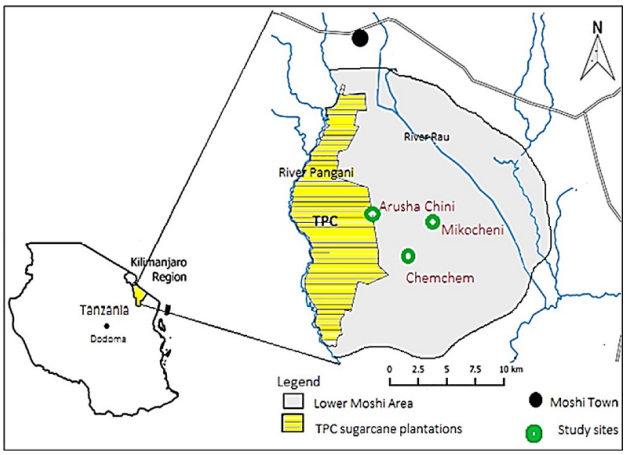
Figure 1 shows the study site as shown elsewhere.[10] The study was conducted in 3 villages, namely; Mikocheni, Chemchem, and Arusha Chini of Lower Moshi in Moshi Rural district of Kilimanjaro region of Tanzania. The site was purposively selected due to the presence of different RVFV hosts (Mosquitoes, humans, and ruminant animals) hence maximising the detection of the virus. Lower Moshi is located on the southern foothills of Mount Kilimanjaro, bordered by Kikuletwa River, Hai District, and Manyara Region on the West, while to the East, it borders Mwanga district. It's elevation ranges between 700 and 800 metres above sea level. Culex spp and Aedes spp are the main RVF vectors in this area.[11,12]

Lower Moshi has numerous water streams forming the irrigation channels for rice and sugar cane, covering an area of about 1100 hectares.[13] The area has 2 rainy seasons, the long rains which run from March to May and the short rainy season from October to December. The average annual rainfall is 900 mm and is highly seasonal with period March to May accounting for 70% of the annual precipitation. The remainder falls during the unpredictable short rains between October and December. Between these 2 rainy seasons is a hot dry season from January to February and a cool dry season from June to September. Aside from paddy production, residents of the area also grow vegetables, maize, peas, and beans. They also keep cattle, goats, sheep, and poultry.

Population and Eligibility Criteria

The study population included all residents of the 3-study village (males and females) aged 10 years and above who were involved in either crop farming or livestock keeping. Individuals who were absent during the time of data collection, who were critically ill, had cognitive impairment and those who had underlying health conditions that interfered with the drawing of blood, were excluded from the study. Consent to participate in the study was obtained directly from adults aged 18 years and above, whereas parents or legal guardians for participants aged below 18 years consented on their children's behalf. Also, before participation, the children assented to participate in the study.

FIGURE 1: Map of Tanzania Showing the Study Site



Source: (Chilongola et al, 2022)

Sample Size and Sampling Technique

The study area was purposively selected since the area has the interaction of different RVFV hosts, mosquitoes, humans and ruminant animals. Stratified random sampling was used to group households based on eligibility, followed by a simple random sample from each stratum. Convenient sampling based on inclusion criteria was used to sample study participants. Sampling was done 3 times in the year, once during each season; the long rainy season (March-May), dry season (June-September) and a short rainy season (October-December). A total of 446 participants in all 3 seasons were sampled through cross-sectional serosurveys such that; 124 participants in the dry season, 172 participants in the long rains season and 150 participants in the short rains season were recruited.

Data Collection Methods

Data collection involved the use of electronic forms designed using Open Data Kit (ODK) tools (https://opendatakit.org/) deployed in Android tablets for participants' demographic data collection, then followed by blood sample collection.

Blood Sample Collection

The blood sample collection was performed by a team of expert phlebotomists from Kilimanjaro Christian Medical Centre (KCMC). Venipuncture was used to draw 3 millilitres of blood from the median cubital vein. Each sample was split into two 1.5 ml aliquots, which were then put into plain and EDTA vacutainer tubes,

respectively. Each sample was placed in an EDTA tube with 4.5 ml of Tri Reagent (Zymo Research, Irvine, CA, USA), gently mixed by shaking for 1 minute, and then sent directly to the Kilimanjaro Clinical Research Institute (KCRI) biotechnology lab at 4°C for Ribonucleic acid (RNA) extraction and Polymerase Chain Reaction (PCR) analysis. After allowing the samples to clot for not more than 20 minutes at room temperature, they were spun at 2000g for 10 minutes in a refrigerator-based centrifuge to produce clear serum, which was then transferred to sterile, clean serum tubes. Immunoglobulin G (IgG) and Immunoglobulin M (IgM) to RVFV were screened for in serum samples.

RVFV Competitive ELISA

The ID Screen RVF Competition Multi-Species kit (ID-vet, Gables, France) was used to conduct a Competitive Enzyme-Linked Immunosorbent Assay (cELISA) to check for antibodies against RVFV in all blood samples. This kit can identify both IgG and IgM antibodies against the RVFV nucleoprotein (NP). The kit's sensitivity range from 91% to 100%, and it's specificity is 100%.[14] According to the manufacturer's directions and as previously mentioned, the cELISA technique was carried out.[8] The mean value of the 2 negative controls (ODNC) was calculated to control each plate's validity, and a plate was considered as being valid if the OD_{NC} was greater than 0.7. The mean value of the 2 positive controls divided by OD_{NC} needed to be 0.3 for a plate to be considered valid. The competition percentage for each sample was obtained by multiplying (OD_{sample}/OD_{NC}) by 100. A sample was considered positive if the result was less than 0.4 and negative if it was greater than 0.5.

Data Management

Each season had its dataset which was recorded in Microsoft Excel. The principal investigator ensured the confidentiality of the data throughout the study and data collected was only used for the study.

Statistical Analysis

The 3 datasets were initially integrated into a single Microsoft Excel file, with seasonal data indicated, for the examination of seasonal seroprevalence of RVF. Following that, the datasets were imported for analysis into STATA software 15. (Stata Corp LLC, College Station, Texas, USA). Data cleaning was performed to ensure consistence of the variables in the 3 datasets. Encoding, labelling, defining, recording, and variable generation were carried out to produce clean datasets. The variables in the dataset that had complete data were used in the analysis. A single, consolidated dataset was then created by combining the 3 original datasets.

A univariate logistic regression model was first fitted to obtain the crude odds ratios (cORs). Variables with a p-value <.05 were considered to be statistically significantly associated with the outcome variable. Akaike information criterion was used to assess the best form to fit the variable age and number of people living in the same household, either as a categorical or a numeric variable. A model with a lesser Akaike value was selected as a good fit model. A likelihood ratio test was conducted to select variables to be used in a multivariable logistic regression model. All the exposure variables were fitted one at a time with the exposure variable of interest (Seasonal distribution). When the test produced a p-value <.05, then the corresponding variable was entered into a multivariable logistic regression model. A multivariable logistic regression model was then performed on the selected variables to obtain the Adjusted OR (aOR) and variables with a p-value <.05 were considered statistically significantly associated with the outcome variable.

Ethical Considerations

Ethical clearance certificate PG 42/2021 was granted by Kilimanjaro Christian Medical University's College Research and Ethics Review Committee (CRERC). Relevant permissions to collect specimens from the field were obtained from the district and regional medical officers and respective district and regional administrative secretaries for Moshi CBD and Kilimanjaro region. In all cases, confidentiality was maintained.

RESULTS

Social Demographic Characteristics of the Study Participants

A total of 446 individuals who met the inclusion criteria were included for analysis in this study. The overall median age of the participants was 40 (IQR=26-54) whereby, 237 (53.6%) of the participants were aged between 21 and 50 years. Of all the participants, 286 (54.2%) were females (Table 1).

Seasonal Seroprevalence of RVF

As shown in Figure 2, the overall seroprevalence of RVF in the year 2020 in the Lower Moshi area of the Moshi district of the Kilimanjaro region was 12.8%. The long rainy season had the highest RVF seroprevalence of 20.4% whereas the dry season had the lowest (4%).

FIGURE 2: Seasonal Seroprevalence of RVF in Lower Moshi district (N=446)

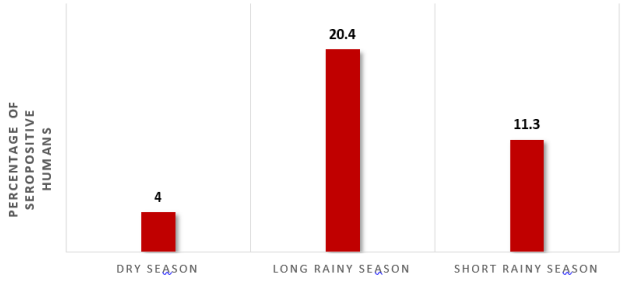


Figure 2 shows the overall RVF seroprevalence across each season. The y-axis shows the percentage for RVF seroprevalence whereas the x-axis presents seasons of the year. Participants were most seropositive (exposed) to RVFV in the long rainy season, but least exposed in the dry season.

Proportional difference of Participants' Demographic Characteristics with RVF Seropositivity

RVF seropositivity significantly differed across the seasons of the year, age of participant and education level. There were marginal associations between the number of

individuals living in the same house and travelling outside the residence area. A high proportion of participants who were RVF seropositive were in the long rainy season, (χ^2 =17.634, *p<.01*) and participants who were aged above 50 years were more seropositive (χ^2 =17.058, *p<.01*). Also, participants with primary education were more seropositive compare to other education groups (χ^2 =7.750, p<.01). Results are presented in Table 2.

Factors Associated with RVF Seropositivity

Results on the participants' factors that are associated with RVF seropositivity are summarised in Table 3. During the long rainy season of the year, participants had 6.08 times higher odds of being seropositive with RVF compared to the dry season. in the general population [OR:6.08, (95%CI, 2.31-16.0)], while participants in the short rainy season had 3.04 times higher odds of RVF seropositivity compared to the dry season [OR:3.04, (95% CI, 1.08-8.50)]. Age in numerical form was chosen by the Akaike information criterion as the optimum form, therefore, for every additional year of age, the odds of being seropositive for RVF in the general population rise by 1.03 times higher [(OR:1.03, (95% CI, 1.01-1.04)].

After performing the likelihood ratio test, the variables chosen for the adjusted logistic regression analysis were; season, sex, participant's age, and the number of persons residing in the same house. Except for sex, all other chosen factors in the final model had a statistically significant association with RVF seropositivity at a p-value <.05. We also found the variable 'season' to confound the variable "number of people living in the same house" by 29.2%. After adjusting for sex, age of the participant, and the number of people residing in the same household in the general population, participants in the long rainy season had 7.30 times higher odds of RVF seropositivity compared to the dry season [(OR:7.3, (95% CI, 2.46-21.67)], whereas, they had 3.35 times higher odds of being RVF seropositive during the short rainy season than they did during the dry season [(OR:3.35, (95% CI, 1.06-10.56)].

Additionally, after adjusting for a season, sex and the number of persons living in the same house, it was observed that, for every one-year increase in age, there was a significant increase of seropositivity to RVFV by 1.03 times higher odds [(OR:1.03, (95%CI 1.01-1.04)]. Individuals living in the same house at a number greater than 5 had 2.77 times higher odds of being RVF seropositive compared to those who shared a home with less than or equal to 5 persons [OR:2.77, (95% CI, 1.27–6.03)].

TABLE 1: Participant's Social Demographics Characteristics (N=446)

Variable	The dry season (n=124) n (%)	The long rainy season (n=172) n (%)	The short rainy season (n=150) n (%)	Total (N=446) n (%)
Sex				
Female @	51 (41.1)	105 (61.4)	85 (56.7)	241 (54.2)
Male	73 (58.9)	66 (38.6)	65 (43.3)	204 (45.8)
Age (years) #				
< 20	1 (0.8)	19 (11.1)	46 (30.9)	66 (14.9)
21-50	94 (77.7)	77 (44.8)	66 (44.3)	237 (53.6)
>50	26 (21.5)	76 (44.1)	37 (24.8)	139 (31.5)
Median (IQR)	41 (32-49)	48 (29-60.5)	35 (16-50)	40 (26-54)
Number of people in the same household δ				
≤ 5	58 (46.8)	83 (48.5)	9 (6.0)	150 (33.7)
> 5	66 (53.2)	88 (51.5)	141 (94.0)	295 (66.3)
Median (IQR)	5 (3-6)	5 (3-6)	8 (7-11)	6 (4-8)
Travelled outside domicile				
No	76 (61.3)	114 (66.3)	126 (84.0)	316 (70.9)
Yes	48 (38.7)	58 (33.7)	24 (16.0)	130 (29.1)
Area of destination (n=130)				
Rural	15 (31.2)	22 (37.9)	8 (33.3)	45 (34.6)
Peri/Urban destination	33 (68.8)	36 (62.1)	16 (66.7)	85 (65.4)
Education level				
No formal education	44 (35.5)	42 (24.4)	26 (17.3)	112 (25.1)
Primary education	12 (9.7)	116 (67.4)	106 (70.7)	234 (52.5)
Tertiary education	68 (54.8)	14 (8.2)	18 (12.0)	100 (22.4)

The symbols @, # and δ represent the following: @= One missing value on sex on the long rainy season; #= Four missing values on age (3 during the dry season and 1 during

TABLE 2: Proportional of RVF Seropositivity Across the Study Participants' Characteristics (N=446)

Variable	Total	Rift Valley Fever n (%)	Seropositivity 95% CI	$\chi 2$	P-value
Season					
Dry Season	124 (27.8)	5 (4.0)	1.6-9.3	17.634	<.001
Long rainy season	172 (38.6)	35 (20.4)	15.0-27.0		
Short rainy season	150 (33.6)	17 (11.3)	7.1-17.5		
Sex*					
Female	241 (54.2)	25 (10.4)	7-14.9	2.336	.126
Male	204 (45.8)	31 (15.2)	10.9-20.8		
Age of Participants (Years)#					
<20	66 (13.9)	6 (9.1)	4.1-18.9	17.058	<.001
21-50	237 (55.6)	19 (8.0)	5.2-12.3		
>50	139 (29.8)	31 (22.3)	16.1-30.0		
Number of people living in the same household"					
≤ 5	150 (33.7)	13 (8.7)	5.1-14.4	3.476	.062
> 5	295 (66.3)	44 (15.0)	11.3-19.5		
Travelled outside domicile					
No	316 (70.9)	45 (14.2)	10.8-18.6	2.074	.150
Yes	130 (29.1)	12 (9.2)	5.2-15.6		
Area of travel (n=130)					
Rural destination	45 (11.7)	7 (15.6)	7.5-29.6	3.286	.070
Peri/Urban destination	85 (20.4)	5 (5.9)	2.4-13.5		
Highest education level					
No formal education	112 (22.4)	14 (12.5)	7.5-20.1	7.950	.019
Primary education	234 (53.0)	38 (16.2)	12.0-21.6		
Tertiary education	100 (24.6)	5 (5)	2.1-11.5		

Table 2 presents associations between participant characteristics and RVF seropositivity. Rows displays the total number of participants in each variable level, the frequency and proportion of RVF-positive individuals in each category with their 95% CI, the chi-square test (2) value, and their corresponding P-values for statistical significance. Column show independent categorical variables assessed. The symbols *, #, and " each show one missing value for the sex column, four missing values for the age column, and one missing value for the number of individuals residing in the same household

TABLE 3: Factors Associated with RVF Seropositivity

Variable	cOR (95%CI)	P-value	aOR (95%CI)	P-value
Season				
Dry Season	Ref		Ref	
Long rainy season	6.08 (2.31-16.01)	<.001	7.30 (2.46-21.67)	<.001
Short rainy season	3.04 (1.09-8.50)	.034	3.35(1.06-10.56)	.039
Sex				
Female	Ref		Ref	
Male	1.55 (0.88-2.72)	.128	1.42 (0.77-2.63)	.261
Age of Participant	1.03 (1.01-1.04)	<.001	1.03 (1.01-1.04)	.001
Number of people living in the same				
≤ 5	Ref		Ref	
> 5	1.85 (0.96-3.54)	.065	2.77 (1.27-6.03)	.01

Continue

TABLE 3: Continued

Variable	cOR (95%CI)	P-value	aOR (95%CI)	P-value
Travelled outside domicile				
No	Ref			
Yes	0.61 (0.31-1.20)	.153	-	-
Area of travel				
Rural destination	2.95 (0.88-9.89)	.08	-	-
Peri/Urban destination	Ref			
Highest education level				
No formal education	Ref			
Primary education	1.36 (0.70-2.62)	.364	-	-
Tertiary education	0.37 (0.13-1.06)	.065	-	-

Table 3 summarises the analysis of factors associated with RVF seropositivity. The column shows the factors that are being assessed while the row shows the crude odds ratio (cOR) and adjusted odds ratio (aOR) with their 95%CI and P-values to confirm any statistically significant associations of the factors with RVF seropositivity.

DISCUSSION

The overarching aim of this study was to determine the seasonal variations of RVF seroprevalence and identify demographic factors that are likely to influence RVF seropositivity in humans. The study reports an overall annual RVF seroprevalence in the Lower Moshi area of the Kilimanjaro region in Tanzania to be 12.8%, with the rainy season having the highest seroprevalence at 20.4% against 4% in the dry season. This prevalence suggests that despite the apparent seasonal fluctuations in RVF seroprevalence, individuals are continuously exposed to RVFV throughout the year, even in the absence of an outbreak. Several previous studies have reported endemic RVF transmission during inter-epidemic/epizootic periods.[5,8,9,11,12,15–27] The mechanisms for RVFV maintenance have been described in previous studies.[2,12,28–31]

Climate is known to affect the geographic, temporal distribution, life cycles of arthropod vectors, and the spread and evolution of the viruses they transmit. It also defines the efficiency with which arboviruses are transmitted from arthropods to vertebrate hosts.[32] Climatic variables indirectly affect vector abundance and distribution, and their ability to vector arboviral diseases.[33] Lower Moshi sugarcane and paddy irrigation area have no previous reports on RVF outbreaks. However, our results suggest an endemic prevalence of the disease in the area, which labels it as a potential hotspot for RVF transmission in north-eastern Tanzania.

Lower Moshi is characterised by having numerous water streams with abundant populations of Culex spp and Aedes spp mosquitoes,[18] and these are the main RVF vectors.[11] The epidemiology of RVF in East Africa is closely associated with the ecological factors prevalent in the Great Rift Valley, which spans Ethiopia, Kenya, and northern Tanzania. Usually, the wet and marshy environments within East Africa cause the transovarially infected dormant eggs of Aedes mosquitoes in the soil to hatch, making Aedes mosquitoes the principal vector responsible for RVFV maintenance.[34] Hatched infectious Aedes mosquitoes transmit the virus to nearby livestock and wildlife vertebrate hosts, which serve as amplifiers of the virus, infecting more mosquitoes, and thereafter secondary vectors of the virus (Culex, Anopheles and Mansonia mosquitoes) amplify the transmission of the virus to non-infected domestic animals and humans.[35] Accordingly, the presence of irrigation schemes, abundant vector mosquitoes, and close interaction between ruminants and persons in our study site makes the site an ideal environment for RVFV maintenance.

Consistent with previous findings, old age and large number of household members were associated with higher seropositivity to RVF.[36,37] As mentioned in studies conducted elsewhere, older male persons are more likely to have been previously exposed to RVFV as a result of their long-term involvement in milking, animal herding and contact with infected vector mosquitoes. Old age predisposition suggests an endemic circulation of RVFV in the study area, rather than a single outbreak event, as a reason for the detected seroprevalence.[8,9] With this information, lower Moshi can be considered as a potential area for future outbreaks of RVF. Therefore, concerns in controlling the spread of the disease should be taken into consideration. The association between seasonality and RVF will raise awareness on where to concentrate and aid in resource allocation towards the prevention and control of the disease in this area. Furthermore, the study has helped to shade light on the possibility that the study areas continue being infected with RVF during the inter-epidemic period despite the absence of previous reports on outbreaks of the disease.

CONCLUSIONS

We observed the highest RVF seroprevalence during the long rainy season compared to other seasons. Moreover, seasonal distribution was found to be significantly associated with RVF seropositivity. An increase in age and the number of people living in the same household also

increased the chances of RVF seropositivity in human population residing in Lower Moshi district. Furthermore, the study has raised awareness of the possibility of RVF circulation during the inter-epidemic period, even in areas that have not been previously reported with RVF outbreaks.

Study limitations

The study used cross-sectional data and thus we could not establish the temporality or causality of associations between seasonality or any other variable with RVF.

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