Detection of Rift Valley Fever Virus Interepidemic Activity in Lower Moshi area of Kilimanjaro Region, North Eastern Tanzania: A Community Survey

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Abstract

Background: Rift Valley fever virus (RVFV) is a zoonotic arbovirus of public health impact infecting livestock, wildlife, and humans mainly in Africa and other parts of the world. Despite its public health importance, mechanisms of RVFV maintenance during inter-epidemic (IEPS) periods and potentially spread to new areas remain unclear. We aimed to comparatively examine exposure to RVFV and RVFV infection among humans, goats and mosquitoes in an agro-pastoral community in Lower Moshi area of Moshi rural district.

Results: Results show that the male gender was related to RVFV seropositivity ($\chi^2 = 5.351$; $p=0.030$). Being 50 years and above was related to seropositivity ($\chi^2=14.430$; $p=0.006$) whereas bed net use, larger numbers of persons living in the same house (>7 persons) and RVFV seropositivity in goats were related to higher seropositivity to RVFV among humans ($\chi^2=6.003$; $p=0.021$, $\chi^2=23.213$; $p=0.000$ and 27.053 ; $p=0.000$), respectively. RVFV antibody concentrations were only marginally higher in humans without statistically significant difference [$t(112) = 0.526$; $p=0.60$]. By the use of RT-qPCR, goats exhibited the highest RVFV infection rate of 4.1%, followed by humans (2.6%), *Aedes spp*(2.3%), and *Culex spp*(1.5%).

Conclusions: In the absence of RVFV infection data in areas nearby the study site, our findings suggest Lower Moshi area as a potential hotspot for RVF, posing the danger of being a source of RVFV spread to other areas. Goats had the highest infection rate, suggesting goats as important hosts in the virus maintenance during IEPs. We recommend the design and implementation of strategies that will warrant effective active surveillance of RVF through the identification of RVF hotspots for targeted control of RVF.

Background

Rift Valley fever virus (RVFV) is a zoonotic arbovirus affecting livestock and humans mainly in Africa and the Arabian Peninsula [1–4] although recent reports indicate the presence of RVF in other parts of the world [5]. According to the World Health Organization (WHO), Rift Valley fever (RVF) is a priority disease due to its considerable public health impact in areas where it occurs and the inadequate interventions to control it [6]. It is also considered an important threat to agriculture in African countries including Tanzania [7–9]. Transmission of RVFV to animals is mainly through bites by infected *Aedes* and *Culex* mosquitoes, whereas human transmission largely through direct contact with tissues of RVFV-infected animals [10].

It has been previously suggested that maintenance of the virus in animals during inter-epidemic (IEPS) periods and potentially spread to new areas through animal movement. Disease pathology and endemic maintenance within mammalian hosts have been reviewed [11, 12]. Although the transmission of RVFV by mosquito vectors to animals and humans has been described, less is known about the role of animals, humans, and vector mosquitoes in maintaining the virus during IEPS. The maintenance mechanisms during IEPS become interesting due to the absence of a clear understanding of where the virus hides during the "silent" periods. Some explanations have been made regarding the possible mechanisms by which the virus is maintained during IEPS. Previous work has documented low-levels of RVFV exposure in northern, central, eastern, and southern Tanzania as a key mechanism of virus maintenance [13, 14]. Most people infected by RVFV remain asymptomatic although a small percentage present with clinical disease. Other reports have

hypothesized critical mechanisms for survival of RVFV during long inter-epizootic periods as vertical transmission through mosquito eggs to mosquito offspring[15–17].

Maintenance of RVFV depend on the presence of competent vectors and hosts but must coincide with multiple factors such as sufficient livestock density, rainfall providing vector breeding sites, and temperatures that support vector development and pathogen replication [18], but differential exposure of RVFV in high-risk agropastoral communities in Northern Tanzania has not been examined. We aimed to comparatively examine exposure to RVFV and RVFV infection among humans, goats and mosquitoes in an agropastoral community in Lower Moshi area of Moshi rural district.

Methods

Study Design and Site

A community-based, cross-sectional survey was conducted in three villages of lower Moshi in Moshi district, Kilimanjaro region of Tanzania. Data were collected between March and June 2020 involving 3 villages, namely Mikocheni, Chemchem, and Arusha Chini. Lower Moshi is located on the southern foothills of Mount Kilimanjaro (Figure 1). On the west, Lower Moshi is bordered by the Kikuletwa River, Hai District, and Manyara Region. To the east Lower Moshi borders Mwanga district. Lower Moshi elevation ranges between 700 and 800 m above sea level. The main Rift Valley Fever vectors in this area are *Culex spp*, *Mansonia spp*, *Anopheles spp*, and *Aedes spp*[19]. Numerous water streams cross the area and they form the irrigation channels for rice and sugar cane. The rice irrigation schemes have structured and unstructured canal networks; covering an area of about 1,100 hectares. During the rainy season, temporary pools that serve as malaria vector breeding sites are formed. Their persistence beyond the rains contributes to further malaria transmission. The area has two rainy seasons; the long rains which run from March to May and the short rainy season from November to December. The average annual rainfall is about 900 mm per year[20].

Participants and sample collection

Participants in this study were males and females aged between 10 and 70 years, who were either smallholder crop farmers or livestock keepers and willing to participate in this study. Animal sampling was carried out by animal health experts from the Tanzania Veterinary Laboratory Agency (TVLA). Up to 15 goats were selected from each herd by systematic sampling technique where every 3rd and 5th animal was included depending on the size of the herd. Selected animals were manually restrained and 3 ml of blood collected through jugular venipuncture using a vacutainer needle. Human blood sampling was done by expert phlebotomists from the Kilimanjaro Christian Medical Center (KCMC). Three milliliters of blood were collected from the median cubital vein by venipuncture. Each sample from both animals and humans was divided into two aliquots of 1.5 ml each and placed into plain and EDTA vacutainer tubes, respectively. To each sample in an EDTA tube, 4.5ml of Tri Reagent (Zymo Research, Irvine, CA, U.S.A.) were added. The mixture was gently mixed by shaking for 1 minute and immediately shipped to the KCRI biotechnology laboratory at 4°C, for analyses. Demographic data from participants were collected using electronic forms designed using Open Data Kit (ODK)tools (<https://opendatakit.org/>) deployed in Android tablets.

Mosquito trapping

BG Sentinel trap (BGS) (Biogents AG, Regensburg, Germany) to target outdoor host-seeking adult mosquitoes particularly *Aedes spp*, *Ochlerotatus spp*, *Culex spp*, *Mansonia spp*, and *Anopheles spp*[21]. BGS traps were used in combination with the BGS-Lure, a dispenser that releases emanations such as those found on human skin (lactic acid, ammonia, and caproic acid)[22]. The BGS-Trap, developed by BioGents GmbH (Regensburg, Germany), consists of an easy to transport, collapsible white bucket with white gauze covering its opening. In the middle of the gauze cover, there is a black tube through which a downflow is created by 12V DC fan that causes any mosquito in the vicinity of the opening tube sucked into a catch bag[22]. Mosquitoes were immediately morphologically identified in the field and consequently sorted according to species, sex, and whether fed or unfed.

Laboratory procedures

RVFV competitive ELISA (cELISA)

Serum was extracted from the plain vacutainer tubes at the end of each day by centrifugation of clotted blood at 3000 rpm for 5 min. An extracted serum sample was then transferred into 2 ml sterile cryovials using a sterile Pasteur pipette. All serum samples were tested for the presence of antibodies against RVFV using a competitive ELISA (cELISA) using the ID Screen RVF Competition Multi-Species kit (ID-vet, Grabels, France), which detects both IgG and IgM antibodies directed against the RVFV nucleoprotein (NP). Validation tests for the test kit have shown a sensitivity of between 91 and 100% and a specificity of 100%. The cELISA was performed according to the instructions of the manufacturer and as described previously [23, 24]. To control the validity of each plate, the mean value of the two negative controls (OD_{NC}) was computed whereby a plate was considered valid if the OD_{NC} was >0.7 . For a valid plate, the mean value of the two positive controls divided by OD_{NC} had to be <0.3 . For each sample, the competition percentage was calculated by dividing $OD_{sample}/OD_{NC} \times 100$. If the value was ≤ 0.4 , the sample was considered positive while a value > 0.5 was considered negative. Only samples that tested positive for cELISA were subjected to RT-qPCR for RVFV detection.

Ribonucleic acid (RNA) isolation, purification, and real-time PCR amplification.

For detection of RVFV RNA in humans and goats, RNA was extracted from Trizol archived blood in EDTA tubes using DirectZol miniprep kit (Zymo Research, Irvine, CA, U.S.A.) by using the Boom method. To isolate RVFV RNA from mosquitoes, pools of 10-50 unfed monospecific female mosquitoes were placed in cryovials and transferred into Lysing Matrix, impact-resistant tubes containing 1.4 mm ceramic beads (MP Biomedicals, CA, USA). Samples were disrupted by bead beating at 10,000 x g for 1 min, spun at 1000 g for 10 min at 4°C. The supernatant was transferred into labeled RNase-free tubes. Purification procedures were done using Direct-zol™ RNA miniprep kit (Irvine, CA, U.S.A) following the manufacturer's instructions.

For both human/goat and mosquito samples, RNA concentration and quality check were performed using NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, NY, USA) before storage at -80°C. RVFV RNA was

detected using TaqMan probe-based one-step RT-qPCR targeting the RVFV Gn gene as described by Gudo and colleagues [2] using Applied Biosystems ViiA7 PCR platform, Thermo Scientific, NY, USA).

Nature of data and data Analysis

Data analysis was performed using IBM SPSS v.26 (IBM® Corp., Armonk, NY, USA). Descriptive data were presented as frequencies and percentages, means, and medians. Categorical data were reported as a tabulation of proportions and compared between humans and goats. Chi-squared statistic (χ^2) was used to examine associations between seropositivity to RVFV and RVFV infection in both humans and goats. Mean IgM and IgG concentrations were compared between humans and goats by paired t-test. Percent positivity to RVFV infection in goats, humans, and mosquitoes were reported as histograms.

Ethical issues

This study obtained approval by the Kilimanjaro Christian Medical University College (KCMUCo) Research and Ethics Committee (CRERC) with approval certificate #2419. This study was also approved by the Kilimanjaro Regional and District Administrative Secretaries, District Medical and Veterinary Officers, and local village and ward executive officers of respective villages. Before commencement of sample collection, written informed consent was obtained from all study participants aged 18 years and above by signing consent forms whereas parents and/or legal guardians of participants under 18 years and participants who could not read or write consented on behalf. All authors hereby confirm that all procedures in this study were approved by CRERC and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. Authors also confirm that all procedures that involved animals in this study were conducted in compliance with the ARRIVE guidelines.

Results

Demographic Characteristics of Human Participants

A total of 266 human participants were enrolled in the study. Of the participants, more than half (56.4%) were females. The median age (interquartile range) of participants was 45 (30–55). The majority of participants (74.4%) came from households with more than 4 persons in the same house. With regards to the participant's education, 63.2% of participants had attained primary school education. Most participants (72.9%) kept livestock (cattle, sheep, goats, and/or chicken). Three quarters (75.2%) of the participants reported having used an insect side treated bed-net the night before the interview. (Table 1)

Table 1
Demographic Characteristics of Participants

Characteristics	n	%
Age group (years)		
≤ 20	28	10.5
21–50	140	52.7
> 50	98	36.8
(Median, IQR) years	45(30–55)	
Sex		
Male	116	43.6
Female	150	56.4
Individuals living in a household		
< 4	68	25.6
≥ 4	198	74.4
Highest education		
No formal	51	19.2
Primary	168	63.2
Tertiary	47	17.7
Type of animals kept by the participant		
Animal Keeping	194	72.9
None	72	27.1
Bed-net use*		
Yes	66	24.8
No	200	75.2
IQR, Interquartile Range; 72 missing entries*		

Factors Associated With Rvfv Seropositivity In Humans And Goats

Human RVFV seropositivity was analyzed for any associations with participant age, bed net use within the last 24 hours, positivity for RVFV infection, number of persons living under the same roof, recent travel outside the study site, highest education of the participant, and RVFV infection and seropositivity in goats,

results are presented in Table 2. Results show that the male gender was significantly more related to RVFV seropositivity ($\chi^2 = 5.351$; $p = 0.030$). Likewise, participants aged 50 years and above were more seropositive as compared to their younger counterparts ($\chi^2 = 14.430$; $p = 0.006$). Bed net use, larger numbers of persons living in the same house (> 7 persons), and RVFV seropositivity in goats were related to higher seropositivity to RVFV ($\chi^2 = 6.003$; $p = 0.021$, $\chi^2 = 23.213$; $p = 0.000$ and 27.053 ; $p = 0.000$), respectively (Table 2). Among the selected factors analyzed for possible association with IgM/IgG RVFV seropositivity in goats, only IgM/IgG RVFV seropositivity in humans had a significant relationship ($\chi^2 = 27.053$; $p = 0.000$) (Table 3).

3.3 Comparison of mean IgM/IgG concentrations in humans and goats

When mean concentrations of antibodies to the RVF virus were compared between goats and humans, it was observed that RVFV antibody concentrations were only marginally higher in humans without statistically significant difference [$t(112) = 0.526$; $p = 0.60$] (Table 4). Percentages of RVFV seropositive humans and goats as well as PCR results for viral infections were determined (Fig. 2). Compared to humans, goats were more seropositive to RVFV (23.3% seropositive goats against 13.2% seropositive humans).

Table 2
Factors associated with RVFV seropositivity in humans

Variable	Level	Negative, n(%)	Positive, n(%)	$\chi^2(p)$
Sex	Male	47(71.2)	19 (28.8)	5.351 (0.030)
	Female	90 (85.7)	15 (14.3)	
Age Group	11–20	18 (94.7)	1 (5.3)	14.430 (0.006)
	21–30	26 (92.9)	2 (7.1)	
	31–40	23 (88.5)	3 (11.5)	
	41–50	19 (82.6)	4 (17.4)	
	> 50	51 (67.1)	25 (32.9)	
Human RVFV PCR	Positive	0 (0)	7 (100)	0.248 (1.000)
	Negative	1 (3.4)	28 (96.6)	
Bed-Net Use	Yes	24 (64.9)	13 (35.1)	6.003 (0.021)
	No	110 (83.3)	22 (16.7)	
Number of persons in a HH	1–3	45 (90)	5 (10)	23.213 (0.000)
	4–6	74 (85.1)	13 (14.9)	
	7+	17 (50)	17 (50)	
Travel outside site	Yes	48 (82.8)	10 (17.2)	0.521 (0.551)
	No	89 (78.1)	25 (21.9)	
Destination	Urban	22 (88)	3 (12)	2.545 (0.346)
	Peri-urban	10 (90.9)	1 (9.1)	
	Rural	16 (72.7)	6 (27.3)	
Highest Education	No Formal Education	34 (81)	8 (19.8)	0.465 (0.830)
	Primary	91 (78.4)	25 (21.6)	
	tertiary	12 (85.7)	2 (14.3)	
RVFV Infection in goats	Yes	1 (50)	8 (80)	0.800 (1.000)
	No	1 (50)	2 (20)	
RVFV seropositivity in goats	Yes	11 (9.4)	24 (43.6)	27.053 (0.000)
	No	106 (90.6)	31 (56.4)	

Table 3
Factors associated with RVFV seropositivity in goats

Variable	Level	Negative, n(%)	Positive, n(%)	$\chi^2(p)$
RVFV infection in goats	Positive	3 (75.0)	8 (89.9)	0.410 (1.000)
	Negative	1 (25.0)	1 (11.1)	
Herd Size	< 20	19 (39.6)	11 (39.3)	0.284 (0.913)
	20–50	16 (33.3)	8 (28.6)	
	> 50	13 (27.1)	9 (32.1)	
IgM/IgG seropositivity in Humans	Positive	11 (9.4)	24 (43.6)	27.053 (0.000)
	Negative	106 (90.6)	31 (56.4)	
RVFRV infection in Humans	Positive	2 (16.7)	5 (20.8)	0.089 (1.000)
	Negative	10 (83.3)	19 (79.2)	

RvfV Rna Detection In Human, Goat, And Mosquito Samples

Aedes spp and *Culex spp* were the dominant species among collected mosquitoes. However, *Mansonia spp* and *Anopheles spp* mosquitoes were also collected in smaller numbers. Our analyses were focused on *Aedes spp*, *Culex spp* as the main documented vectors for RVFV. When virus detection was performed using polymerase chain reaction (RT-qPCR), goats exhibited the highest infection rate of 4.1%, followed by humans (2.6%). *Aedes spp* had a higher infection rate of 2.3% compared to that of *Culex spp* which was recorded to be 1.5%.

Table 4
Comparison of mean IgM/IgG anti-RVFV antibody concentrations between goats and humans

RVF HUMAN - RVF ANIMAL	Paired Differences					t	df	P- value
	Mean (difference)	SD	SE of Mean	95% CI of the Difference				
				Lower	Upper			
	0.0363451	0.7346983	0.0691146	-0.1005966	0.173287	0.526	112	0.600

Discussion

The main aim of this study was to examine the degree of exposure to RVFV in goats and humans. This study also sought to isolate RVFV in humans, goats, and key RVFV vector mosquitoes; *Aedes spp* and *Culex spp*. Results from this study show that, although there has been no RVF outbreak reported in Tanzania since 2006–2007, antibodies to RVFV and the virus has been detected in humans and goats in Lower Moshi area. Findings from this study indicate that 13.2% and 23.3% of tested humans and goats had circulating

antibodies to RVFV, respectively. Our findings emphasize an active exposure to RVFV during IEPs as previously reported by some studies across geo-ecological zones of Tanzania [19, 25–28].

In this study, goats had higher exposure rates to RVFV compared to humans. *Aedes spp*, the major vector for RVFV, is known to have bimodal daily feeding behavior with both exophagic and exophilic behaviors[29], feeding on a wide range of mammalian hosts. Consequently, this behavior can be implicated as a key behavior in its role as a vector for many zoonotic infections. Despite its preference for human hosts [29], we report higher seropositivity in goats. The transmission of RVFV is not absolutely dependent on the presence of vector mosquitoes. Direct human contact with infected animal tissues has been reported as a significant factor for its transmission from animals to humans[30–32]. Not all of the human participants in this study were directly involved in activities that bring them into direct contact with infected tissues such as infected aborted fetuses and those working in slaughterhouses, which could partly explain the lower seropositivity to RVFV in humans compared to goats.

In the current study, RVFV RNA was detected in humans, goats, and mosquitoes. Goats exhibited the highest infection rate of 4.1%, followed by humans (2.6%). Viral RNA was also detected in 2.3% and 1.5% of tested *Aedes spp* and *Culex spp* mosquito pools. This study was conducted to shed light on the maintenance mechanisms of RVFV by investigating both exposure and infection rates in mammalian and arthropod vectors. To our knowledge, this is the first study conducted in Tanzania to concomitantly report on RVFV diagnosis in humans, animals, and mosquitoes. Many of the previous studies that sought to understand the epidemiology of RVFV in Tanzania, either focused on sero-epidemiology or could not detect RVFV RNA in mammalian and arthropod vectors. Although the interactions of arboviruses and their vectors are complex and their epidemiology is poorly understood, our findings support the hypothesis that during IEPs, RVFV is likely maintained by localized low-level transmission between mosquito vectors and mammalian hosts without any noticeable clinical symptoms[15, 32–34]. Evidence for RVFV transmission during IEPs has previously been reported among humans, livestock, and wild animals in Tanzania and elsewhere [1–4, 6, 19, 24–28, 33, 35–38]. Although goats were more seropositive to RVFV compared to humans, paired comparison of mean anti-RVFV IgG/IgM concentrations revealed no difference that existed between humans and goats.

Some factors were significantly associated with seropositivity to RVFV in humans including male gender, living more than 4 persons in a household, being older than 50 years, not using an insecticide-treated bed-net, and higher RVFV seropositivity in goats. RVFV seropositivity in Humans was consequently associated with seropositivity in goats. Males, especially in agropastoral communities seem to be more active outdoors for various subsistence activities including farming and grazing which bring them into frequent contact with RVFV susceptible or infected animals. This finding stresses the need for continued distribution, access, and usage of LLINs, especially among rural and agro-pastoral communities that are more prone to zoonotic diseases.

The study site is characterized by features that are supportive of vector mosquito breeding and intimate human-animal interaction. In the absence of reports on RVFV infection in areas nearby the study area, [19] the detection of antibodies to RVFV in humans and goats and detection of RVFV in humans, goats, and

mosquitoes in the study area suggests the site to be a potential RVF hotspot. The dominant pastoral grazing system in the study area and surrounding areas is manifested as unlimited movements of livestock as a result of environmental degradation of the wetland due to overstocking and overgrazing increases chances for introducing the disease into new areas. The absence of clinical manifestations among livestock and humans in the study area, which could be a consequence of herd immunity, seems to have escaped the knowledge of the veterinary and public health authorities, raising concerns about the available local and national capacity for preparedness and response machinery against zoonotic infectious with potential to cause fatal epidemics. Thus, there exists a critical need for improved surveillance of RVF transmission through detection of RVFV activity among humans, livestock, and vector mosquitoes.

Since passive surveillance of RVF is challenging in the absence of clinical features among humans and livestock, active surveillance is recommended and, where resources may be limited, targeted surveillance in high-risk areas (hot spots) will help prevent future RVF outbreaks. It is critically important to relook the national contingency plans used in RVF surveillance and response to RVF outbreaks, bearing in mind that observed active transmission of the virus occurs in the absence of expected clinical manifestations that have been the traditional RVF pointers for a long time such as massive abortions in livestock.

Conclusion

Here, we present data that reveals the detection of anti-RVFV antibodies in humans and goats and isolation of RVFV from humans, goats, and mosquitoes in an area with the necessary features for mosquito breeding. Collected during a dry season of IEP, our data suggests the Lower Moshi area as a potential hotspot for RVF, posing the danger of being a source of RVFV to other areas. Strategies for effective active surveillance of RVF that involve the identification of RVF hotspots for targeted control are recommended.

List Of Abbreviations

RVF: Rift Valley Fever; RVFV: Rift Valley Fever Virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IEP: Inter epidemic Period; cELISA: competitive ELISA; RT-qPCR: Real Time quantitative Polymerase Chain Reaction; RNA: Ribonucleic acid.

Declarations

Ethics approval and consent to participate

This study was conducted after the approval of the Kilimanjaro Christian Medical University College (KCMUCo) Research and Ethics Committee (Certificate #2419). Permission to conduct the study was also obtained from Kilimanjaro Regional and District Administrative Secretaries, District Medical and Veterinary Officers, and local village and ward executive officers of respective villages. After an explanation of the study aims and procedures to the study communities through community sensitization meetings, individuals were asked to voluntarily agree to participate in the study. Written informed consent was obtained from all study participants aged 18 years and above by signing consent forms whereas parents and/or legal guardians of participants under 18 years and participants who could not read or write consented on behalf. All authors

hereby declare that all procedures in this study were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Consent for publication

Not applicable

Availability of Data and Materials

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

Competing interests

The authors declare that they have no competing interests

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Authors' Contributions

JOC, RSM, BTM conceived the project, overall study implementation and wrote the manuscript.

MSK, RMB, PGH, SIM participated in data collection and made significant inputs in writing the manuscript. MSK, JOC and JMV analyzed and interpreted data

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Figures

Figure 1

Map of Tanzania developed from Google earth showing study site. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Comparative IgM/IgG seropositivity and PCR positivity between goats, mosquitoes and humans