Tanzania Journal of Health Research
Volume 17, Number 4, October 2015
Doi: http://dx.doi.org/10.4314/thrb.v17i4.3

Rift Valley fever potential mosquito vectors and their infection status in Ngorongoro District in northern Tanzania

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Abstract

Background: Rift Valley fever (RVF) is a mosquito-borne viral zoonotic disease. Rift Valley fever virus (RVFV) has been isolated from more than 40 species of mosquitoes from eight genera. This study was conducted to determine the abundance of potential mosquito vectors and their RVFV infection status in Ngorongoro District of northern Tanzania.

Methods: Adult mosquitoes were collected outdoors using the CDC light traps baited with carbon dioxide in five randomly selected villages namely, Meshili, Malambo, Osinoni, Endulen and Nainokanoka. The study was carried out towards the end of rainy season in May 2013. The traps were set in proximity to potential breeding sites and cattle kraals. The collected mosquitoes were identified to genus and species using morphological keys. They were tested for RVFV RNA using real time reverse transcription-polymerase chain reaction (rRT-PCR).

Results: A total of 2,094 adult mosquitoes belonging to three genera and nine species were collected. Most of them (87.5%) were collected in Meshili, followed by Malambo (8.2%) and Osinoni (4%) villages. No single mosquito was collected in Nainokanoka or Endulen. The nine species collected were *Culex pipiens* complex, *Cx. antennatus*, *Cx. tigripes*, *Cx. annulioris*, *Cx. cinereus*, *Anopheles arabiensis*, *An. squamosus*, *An. pharoensis* and *Mansonia uniformis*. No RVFV RNA was detected in the mosquito specimens.

Conclusion: Various RVFV potential mosquito species were collected from the study villages. These mosquito vectors were heterogeneously distributed in the district suggesting a variation in RVF transmission risk in the study area.

Keywords: Rift Valley fever, virus, mosquito infection, transmission, Tanzania

Introduction

Rift Valley fever (RVF) is an acute febrile arthropod-borne viral zoonotic disease of mainly human and ruminants caused by a member of *Phlebovirus* genus of the family *Bunyaviridae*. Rift Valley fever outbreaks have been reported in Kenya, Tanzania, South Africa (WHO, 2007; WHO, 2010; Archer *et al*., 2011), Mauritania (Ahmed *et al*., 2011), Senegal (Thonnon *et al*., 1999), Sudan (Adam *et al*., 2010) and Madagascar (Jeanmaire *et al*., 2011). In 2001-2002 RVF outbreaks were reported beyond Africa in Saudi Arabia and Yemen (Shoemaker *et al*., 2002). The disease affects cattle, sheep, goats and camels with mortality rate reaching 30% and 100% in adult and young animals, respectively (Madani *et al*., 2003). RVF is characterised by abortions in cattle, sheep and goats (Nichol *et al*., 2001) and by mild, acute febrile illness with spontaneous recovery in humans. In small proportion of cases the disease in human it can be associated with severe jaundice, rhinitis, encephalitis and haemorrhagic manifestations and death (Davies, 1980).

Rift Valley fever virus is transmitted between animals and humans by mosquitoes, particularly those belonging to the *Aedes, Culex* and *Anopheles* genera (Easterday *et al*., 1962; Laughlin *et al.*, 1979). Humans also acquire infection through direct contact with blood or aborted materials from infected animals (Swanepoel & Coetzer, 2004). It is believed that during the inter-epidemic period (IEP) the RVFV is maintained in the eggs of Aedes mosquitoes (primary vector)

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Doi: http://dx.doi.org/10.4314/thrb.v17i4.3

which breed in isolated depressions called *dambos* during periods of extensive rainfalls and floods (Linthicum *et al*., 1985; Chevalier *et al*., 2005; Mohamed *et al*., 2010). When Aedes mosquito infect animals with RVFV, virus amplification occurs in these vertebrate hosts, and then Culex and Anopheles species (secondary vectors) transmit the virus further to a wider area beyond the area of the original outbreak (McIntosh, 1972).

Rift Valley fever virus has been isolated from more than 40 species of mosquitoes from eight genera (Ratovonjato *et al*., 2011). Laboratory studies indicate that numerous species of mosquitoes and sand flies are susceptible to oral infection, some of which are able to transmit RVFV by bite (Sang *et al*., 2010). In Tanzania, RVF outbreaks have been reported in Arusha, Dodoma, Iringa, Kilimanjaro, Manyara, Mara, Morogoro, Mwanza, Pwani , Shinyanga, Singida, Tabora and Tanga regions (Sindato *et al*., 2014). Besides the long persistence of RVF in Ngorongoro district in Arusha Region (Sindato *et al*., 2014, 2015), little is known about the distribution of potential mosquito vectors and their RVFV infection status. Limited number of studies has reported presence of potential mosquito vectors of RVF in Ngorongoro district (Mweya *et al*., 2013; 2015). The objective of this study was therefore to determine the abundance and RVFV infection status of potential mosquito vectors during the IEP in Ngorongoro District of northern Tanzania.

Materials and Methods

Study site

This study was carried out in Ngorongoro Conservation Area (NCA) in Ngorongoro district in northern Tanzania. NCA is one of the three divisions of Ngorongoro District and covers an area of 8,292m^2. The main features of the Division include the Ngorongoro Crater, The Serengeti Plains and the catchment forest (http://www.ngorongorocrater.org/welcome.html). This area is largely of hilly terrain interspersed with broad U-shaped valley. The vegetation consists mainly of various shrubs and *Acacia* bushes.

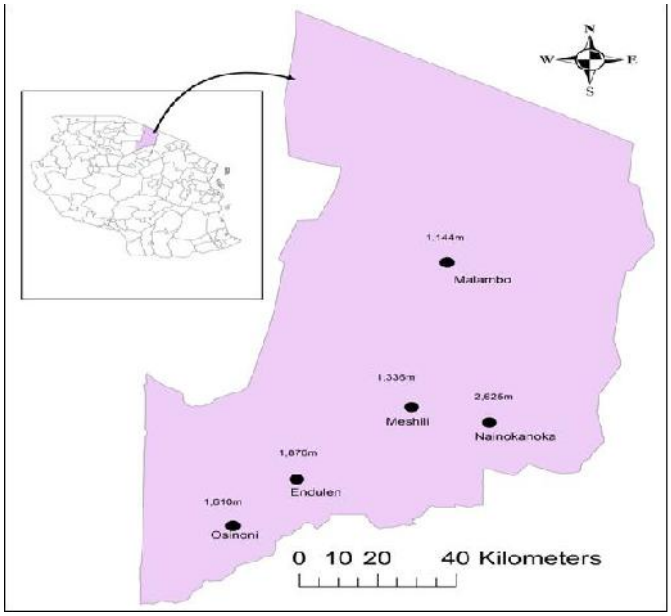

Figure 1: Map of Ngorongoro district showing location of the study villages

The area experiences two rainy seasons: a short rainy season between October and December, and a long rainy season between February and May. Typically, the annual precipitation averages between 500 and 1,000 mm. The area is occupied predominantly by Maasai pastoralists. Other minority ethnic groups living in this area are Hadzabe, Ndorobo and Sonjo. The livestock species

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kept in the area are primarily cattle, goats, sheep and donkeys. The study villages were Malambo (1,144m), Meshili (1,336m), Osinoni (1,610m), Endulen (1,876m) and Nainokanoka (2,545m). These study villages were randomly selected from a sampling frame of 17 villages of Ngorongoro Conservation Area.

Mosquito collection and identification

Adult mosquitoes were collected in May 2013 using CDC light traps baited with carbon dioxide. Three traps were set approximately 1.5m above the ground in proximity to potential breeding sites and cattle kraals. The traps were set at 17:30 hrs in the evening and retrieved the following morning at 06:00 hours. Mosquitoes were killed by freezing; sorted into sex and abdominal status (fed, unfed, semi gravid, gravid), and were stored in 1.5 mL labelled Eppendorf tubes. Mosquitoes were identified to genera and species levels using conventional taxonomic keys (Edwards, 1941; Gillies & De Meillon, 1968; Service, 1990). They were then preserved and transported in a liquid nitrogen gas to the laboratory at Amani Medical Research Centre in Tanga. The *Anopheles gambiae* s.l. was identified further to species level using the standard polymerase chain reaction (PCR) technique (Scott *et al*., 1993).

One leg from each *An. gambiae* s.l. mosquito was placed in a 1.5 ml Eppendorf tube, 30µl of 1X Tris – EDTA buffer solution was added in each tube. The leg was grounded thoroughly using a sterile pestle, the mixture was then short-span (5 seconds) to catch the DNA template down the tube. The PCR procedure included an initial cycle of denaturation at 95°c for 5 minutes followed by 30 cycles of denaturation at 94°c, 72°c and 50°c for 30 seconds, and a final extra extension step at 72°c for 10 minutes using a Hybrid thermocycler. The resulting amplified Deoxyribonucleic Acid (DNA) was run on an ethidium bromide stained 2.5% agarose gel and photographed under ultraviolet light illumination as described by Scott *et al.* (1993).

Viral RNA extraction

RNA was extracted from female and male mosquitoes using a column purification kit (QIAGEN, Valencia, CA, USA). Pools of mosquitoes were put into a microcentrifuge tube containing 150µL of RNeasy lysis buffer and finally ground with a disposable RNase free pestle. Each pool had 10 mosquitoes. After homogenization, samples were processed according to established protocol (Qiagen RNeasy Mini kit).The molecular detection of the virus was performed using the real time RT- PCR (Applied Biosystems 7500 Fast Real – Time PCR System). The primers Forward: S432 (5'ATGATGACATTAGAAGGGA 3') and Reverse: NS3m (5'GATGCTGGGAAGTG ATGAG 3') and the TaqMan probe CRSSAr (5' ATTGACCTGTGCCTGTTGCC 3') targeting 298 base pair fragment of the S- segment of the RVF virus.

Data analysis

Data was entered in Microsoft Excel spread sheet and imported into STATA version 12 (Statacorp, College Station, TX, USA) for analysis. Mosquito abundance (calculated as the number of mosquitoes collected per village) was compared between the study villages using chi-squared test. However, when the number of mosquitoes was below 5, the Fisher's exact test was applied.

Results

A total of 2,094 adult mosquitoes were collected and identified to nine species belonging to three genera. Mosquito abundance and diversity differed significantly between the study villages ($p<0.0001$). All of the morphologically identified *An. gambiae* s.l. were genotyped as *An. arabiensis*. Overall, *Cx. pipiens* complex was the predominant mosquito species (46.8%) followed by *An. pharoensis* and *An. arabiensis* (14.4%) (Table 1).

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Table 1: Overall mosquito species composition in the study villages

Species	Number	%
Anopheles arabiensis	302	14.4
Anopheles pharoensis	408	19.5
Anopheles squamosus	269	12.8
Culex pipiens complex	981	46.8
Culex cinereus	53	2.5
Culex antenattus	24	1.1
Culex tigripes	19	0.9
Culex annulioris	17	0.8
Mansonia uniformis	21	1.0

Meshili accounted for the majority (87.5%) of the mosquito collected in NCA, followed by Malambo (8.2%) and Osinoni (4.0%) villages. No mosquitoes were collected in Endulen and Nainokanoka Villages during the study period (Table 2). In Malambo, *An. arabiensis* was the most abundant species (48.5%) followed by *Cx. pipiens* complex (43.3%) and *An.pharoensis* (8.2%). On the other hand, *Cx. pipiens* complex was the most abundant species (48.7%) in Meshili, followed by *An. pharoensis* (19.7%), *An. squamosus* (13.3%), *An. arabiensis* (10.9%), *Cx. cinereus* (2.9%), *Cx. antennatus* (1.4%), *Mansonia uniformis* (1.2%), *Cx. tigripes* (1%) and *Cx. annulioris* (0.9%). In Osinoni, *Anopheles pharoensis* predominated (37.4%), followed by *An. squamosus* (28.5%), *An. arabiensis* (19.8%) and *Cx. pipiens* complex (14.3%) (Table 2).

Table 2: Total number of mosquito vectors collected in each village

Species	Malambo N (%)	Meshili N (%)	Osinoni N (%)	Enduleni N (%)	Nainokanoka N (%)
An. arabiensis	83(48.5%)	201(10.9%)	18(19.7%)	0	0
An. pharoensis	14(8.1%)	360(19.6%)	34(37.3%)	0	0
An. squamosus	0	243(13.2%)	26(28.5%)	0	0
Cx. pipiens	74(43.2%)	894(48.7%)	13(14.2%)	0	0
Cx. cinereus	0	53(2.8%)	0	0	0
Cx. antennatus	0	24(1.3%)	0	0	0
Cx. tigripes	0	19(1%)	0	0	0
Cx. annulioris	0	17(0.9%)	0	0	0
Ma. uniformis	0	21(1.1%)	0	0	0
Total	**171**	**1,832**	**91**	**0**	**0**

A total of 960 of these were sorted into 96 pools according to their site of collection, species, and sex and were tested for RVFV RNA. The RVFV RNA was not detected in any of 96 tested mosquito pools.

Discussion

Assessment of abundance and diversity of RVF vectors, and their infectivity to RVFV provides important entomological features for the identification of potential high risk areas for RVF occurrence, which can provide guidance in the design of appropriate prevention and control measures. The findings of this study have shown that the abundance and diversity of potential RVF mosquito vectors vary between the study villages, suggesting the spatial variations in the risk of RVF occurrence in animals and humans within NCA. The high abundance and diversity of mosquitoes in Meshili suggests the potential suitability of environments in this village during the study period for the mosquito species collected. Although Malambo and Meshili are both located at lower altitudes than other study villages, the presence of seasonal pond at Meshili (which was filled with water during the study period) is likely to partly explain for the higher catches of mosquitoes in this village. This seasonal pond was also observed to be one of the obvious

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potential breeding sites in a malariometric survey conducted in the area about a decade ago (Mboera *et al.*, 2005). It is worth noting further that compared with Osinoni and Nainokanoka villages; Meshili, Malambo and Endulen have been persistently affected by past RVF outbreaks which were reported mainly during the period of prolonged heavy rainfall (Sindato *et al*., 2014).

The potential role of prolonged rainfall and mass emergence of mosquitoes have been reported as risk factors for RVF epidemics (Ngulu *et al*., 2010). Although it is not very clear what causes the differential abundance and diversity of mosquito vectors between the study villages, it is likely that high altitude provide less suitable habitat for mosquito breeding and survival compared with lower lying areas which are likely to be more susceptible to water stagnation during the rainy season (Bodker *et al.*, 2003). This observation and the sampling season may partly explain why no single mosquito was collected in Endulen and Nainokanoka villages during the study period.

Cx. pipiens complex, *An. arabiensis* and *An. pharoensis* were the common mosquito species collected in Malambo, Meshili and Osinoni villages, and all these species have been reported as potential vectors of RVF virus in Kenya (Sang *et al*., 2010) and Mauritania (Nabeth *et al*., 2007). In a recent study in Ngorongoro district Mweya and others (2013) found that *Cx. pipiens* complex was the most abundant mosquito species followed by *Aedes aegypti*. *An. arabiensis* and *An.pharoensis* have been reported as vectors of RVFV in Sudan and Mauritania (Digoutte & Peters, 1989; Faye *et al*., 2003; Nabeth *et al*., 2007; Seufi & Galal, 2010). *Cx. antennatus*, *Cx. annulioris*, *Cx. tigripes* and *Ma. uniformis* which were collected in Meshili have been associated with the transmission of RVFV in Madagascar (Ratovonjato*et al*., 2011; Balenghien *et al*., 2013), Nigeria and Kenya (Easterday *et al*., 1962; Sang *et al.,* 2010).The vector competence of *Cx. antennatus* has also been demonstrated in laboratory studies (Easterday *et al*., 1962). While *Cx. tigripes* was reported as a potential vector of RVFV in Marigat and Ijara districts in Kenya (Tchouassi *et al*., 2012) *Ma. uniformis* was found infected with RVFV in Baringo District in the same country (Sang *et al*., 2010). *An. squamosus* was only collected in Meshili and Osinoni villages; this species has also been implicated with the transmission of RVFV in a study in Garisa District in Kenya (Sang *et al*., 2010) and Madagascar (Ratovonjato*et al*., 2011).

Despite the fact that RVFV RNA was not detected in the collected mosquitoes in our study, RVFV RNA has been detected in cattle, sheep and goats sampled during the same period from Meshili and Malambo villages (A. Mhina *et al*., 2015 unpubl). Similar to our findings, a recent study in Ngorongoro district by Mweya et al. (2013) did not detect RVFV activity in the potential mosquito vectors collected during the IEP. While the salient reasons for this observation are not known, it should be noted that compared with IEP; during the outbreak phase infected animals develop high levels of viraemia, making it easy for mosquitoes that bite these animals to become infected with RVFV. An entomological study that was conducted during the last RVF outbreak in Kenya in 2006/2007 detected RVFV in *Ae. mcintoshi*, *Ae. ochraceus*, *Ae. pembaensis, Ma. uniformis, Cx. poicilipes*, *Cx. bitaeniorhynchus, An. squamosus*, *Ma. africana, Cx. quinquefasciatus* and *Cx. univittatus* (Sang *et al.*, 2010).

It is important to note that the findings of our study are likely to have been affected by sampling technique, choice of sampling sites, duration of our study, season of sampling and sample size. Further studies targeting the periods of high mosquito activity with range of sampling techniques and sampling sites would improve our understanding on the abundance and diversity of the RVF potential mosquito vectors in the study area.

This study reports the presence of potential mosquito vectors for RVF within Ngorongoro Conservation Areas. The abundance and diversity of these potential vectors were significantly higher in Meshili village, suggesting that the risk of RVF occurrence is higher in this village than other study villages. These findings should inform the design of appropriate prevention and control measures.

Doi: http://dx.doi.org/10.4314/thrb.v17i4.3

Acknowledgements

The authors would like to thank Joseph Myamba and Bernard Batengana of Amani Medical Research Centre for their technical assistance in mosquito identification. This study was funded by the Wellcome Trust Grant (WT087546MA) to the Southern African Centre for Infectious Diseases Surveillance.

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