The epidemiology of Rift Valley fever in northern Tanzania.

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Abstract

Rift Valley fever is a mosquito-borne viral disease of ruminants, camels and humans. In Tanzania, outbreaks have occurred at intervals of 10 - 20 years with major epidemics reported in 1977, 1997/98 and 2006/2007. Our ability to prevent future epidemics is limited by poor understanding of how the virus circulates between major epidemics. This study aimed to investigate the epidemiology of inter-epidemic RVFV infections in northern Tanzania.

This study involved (a) collection and characterisation of mosquitoes; (b) RVFV serological analysis of serum samples from cattle (n=3582), sheep (n=2586), goats (n=3303) and human populations (n=565) collected through cross-sectional household surveys; (c) analysis of risk factors for livestock and human seropositivity; (d) molecular detection of RVFV in mosquitoes and diagnostic materials collected during investigation of 190 livestock abortion events. Generalised Linear Mixed-Effects Models (GLMMs) were used to examine predictors of vector mosquito abundance, and risk factors for RVFV exposure in livestock and humans. Maximum Entropy (MaxEnt) algorithm was used to model vector mosquito habitat suitability and spatial distribution.

A total of 2224 mosquitoes were collected including *Culex spp* (n = 1123), *Anopheles spp* (n=1006), *Mansonia spp* (n=56), *Aedes spp* (n=34), and *Coquillettidia spp* (n=5) with significant variation in abundance with percentage difference in normalised difference vegetation index (NDVI). No RVFV infections were detected in any of the mosquitoes collected. RVFV seroprevalence was higher in cattle 4.4% (95% CI:3.7-5.1), than in sheep 2.6%, (95% CI: 2.0-3.3) and goats 1.4% (95%CI: 1.0-1.8), with seropositivity in young animals providing evidence of recent virus circulation. Seropositivity in livestock increased with age (OR=1.3, CI: 1.2 - 1.4, p<0.001) consistent with endemic circulation and was associated with a history of abortion in goats (OR=2.5, 95%CI: 1.1 - 5.4, P=0.023) and sheep (OR=2.7, 95%CI: 1.1 - 6.3, P=0.025). Human seroprevalence was 8.5% (95% CI: 6.4 - 11.2) and varied between villages and between households within villages. Handling of aborted material (OR=4.3, 95% CI: 1.7-10.8) and consumption of raw milk (OR=4.1, 95%CI: 1.8 - 9.3, P=0.001) were significant risk factors for human seropositivity. RVFV was detected in a cluster of 14 (7.4%) abortion cases including the milk of three aborting dams. This provides strong evidence for continuous RVFV circulation in livestock between major epidemics in Tanzania and that unboiled milk is an important potential source of infection for people.

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Author's declaration

I declare that, except where explicit reference is made to the contribution of others, that this thesis is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Abbreviations

AIC	Akaike`s information criterion
AUC	Area under the curve
BIC	Bayesian information criterion
BBSRC	Biotechnology and Biological Sciences Research Council
CDC	United States Centres for Disease Control and Prevention
CI	Confidence interval
ELISA	Enzyme-Linked-ImmunoSorbent Assay
ENM	Ecological niche models
ENSO	El Nino-Southern Oscillation
ESRI	Environmental Systems Research Institute
EVI	Enhanced Vegetation Index
FAO	Food and Agriculture Organization
GIS	Geographical Information System
GPS	Geographical Positioning System
HRPO	Horseradish peroxidase
IEP	Inter-epidemic period
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KCMC	Kilimanjaro Christian Medical Centre
KCRI	Kilimanjaro Clinical Research Institute
MaxEnt	Maximum entropy-based niche modelling algorithm
NDVI	Normalized Difference Vegetation Index
NIMR	National Institute for Medical Research
OD	Net optic density
OIE	World Organisation for Animal Health
p	Probability value
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PP	Percentage positivity
RNA	Ribonucleic acid
ROC	Receiver operating characteristic curves
RT-PCR	Real Time-polymerase chain reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SEBI-TZ	Supporting Evidence Based Interventions to Achieve Agricultural Development Goals in Tanzania
SEEDZ	Social, Economic and Environmental drivers of Zoonoses
Spp	Species
TAWIRI	Tanzania Wildlife Research Institute
TCID	Tissue Culture Infective Dose
UK	United Kingdom
VNT	Virus Neutralization Test
WHO	World Health Organization
^{0}C	Celsius or Degree centigrade
%	Percent
<	Less than sign
>	Greater than sign

≤	Less than or equal to sign
≥	Greater than or equal to sign

Chapter One

1 Introduction and Literature review

1.1 Background

Rift Valley fever (RVF) is an arthropod-borne viral disease of ruminants, camels and humans (Gerdes, 2004). The Rift Valley Fever virus (RVFV) can be transmitted through mosquito bites or by exposure to infectious blood and bodily fluids (Balkhy and Memish, 2003). Unprotected handling of the afterbirth, aborted material (Fontenille et al., 1998), and drinking raw, unpasteurized milk from infected animals have been suggested as potential routes of transmission (Balkhy and Memish, 2003). The disease in humans is often asymptomatic, but clinical signs in severe cases can manifest as flu-like illness (Davies et al., 1985), haemorrhagic disease with liver involvement (Gerdes, 2004) and ocular or neurological lesions (Gerdes, 2004; Pepin *et al.*, 2010). In ruminants, RVF may be inapparent in non-pregnant adults (Davies et al., 1985) but outbreaks are characterised by the onset of abortions and high neonatal mortality (Swanepoel and Paweska, 2011b).

Rift Valley Fever was first identified in 1931 on a farm in the Rift Valley of Kenya (Daubney et al., 1931). Since then, outbreaks have been reported largely in sub-Saharan Africa, West Africa, North Africa (Seufi and Galal, 2010) and in 2000 in the Arabian peninsula (Bird et al., 2007) (Figure 1.1). The disease is considered to be endemic in sub-Saharan African countries, with periodic major outbreaks, associated with episodes of heavy rainfall and flooding (Davies et al., 1985, Nderitu et al., 2010). Outside sub-Saharan Africa, RVF epidemics have been confirmed in Egypt in 1977 (El-Akkad, 1978), Mauritania and Senegal in West Africa (Fontenille et al., 1998, Diallo et al., 2005), Saudi Arabia and Yemen in the Arabian Peninsula (Jupp et al., 2002), and Madagascar and the Comoros islands in the Indian Ocean (Morvan et al., 1991, Sissoko et al., 2009). The spread of RVFV outside its endemic region raises concerns about threat of RVFV introductions to new geographical areas (Pepin et al., 2010).

Since the 1970s, periodic epidemics of RVF have been reported in an increasing number of countries in Eastern and Southern Africa including Kenya, Somalia, Sudan, Tanzania, Zimbabwe, and South Africa (Nderitu et al., 2010). The RVF epidemic of 1997–1998 that affected Kenya, Somalia, and Tanzania was characterized by outbreaks that started in the North Eastern Province of Kenya in November 1997 and ended with cases reported from the north-central region of

Tanzania in June 1998 (WHO, 2007, CDC, 2007). Another RVF epidemic occurred in 2006–2007 in these three countries, with cases first reported in the North Eastern Province of Kenya and later in Tanzania, where the last livestock and human cases were reported in June 2007 (Mohamed et al., 2010, Munyua et al., 2010). Recent outbreaks in East Africa were reported in 2018 in Kenya, Rwanda and Uganda involving livestock and humans (Anyamba et al., 2018) and in South Sudan (WHO, 2018).

In Tanzania, epidemics over the past five decades have occurred at 10-20 years interval with major epidemics reported in 1977, 1997/98 and 2006/2007 (Mohamed et al., 2010, Karimuribo et al., 2012, Fyumagwa et al., 2012). The RVF epidemics (outbreaks) of 1977 and 1997/98 were largely confined to northern Tanzania but that of 2006/2007 extended to central and southern parts of the country with animal and human cases reported (Mohamed et al., 2010, Fyumagwa et al., 2012). Despite recent reports of RVF in Kenya, Rwanda and Uganda until early September 2018, no human or animal cases were officially documented in Tanzania since 2007. Due to RVF reports in the region, in June 2018 an RVF alert was issued by the Ministry of Livestock and Fisheries (http://www.xinhuanet.com/english/2018-06/19/c_137263241.htm).

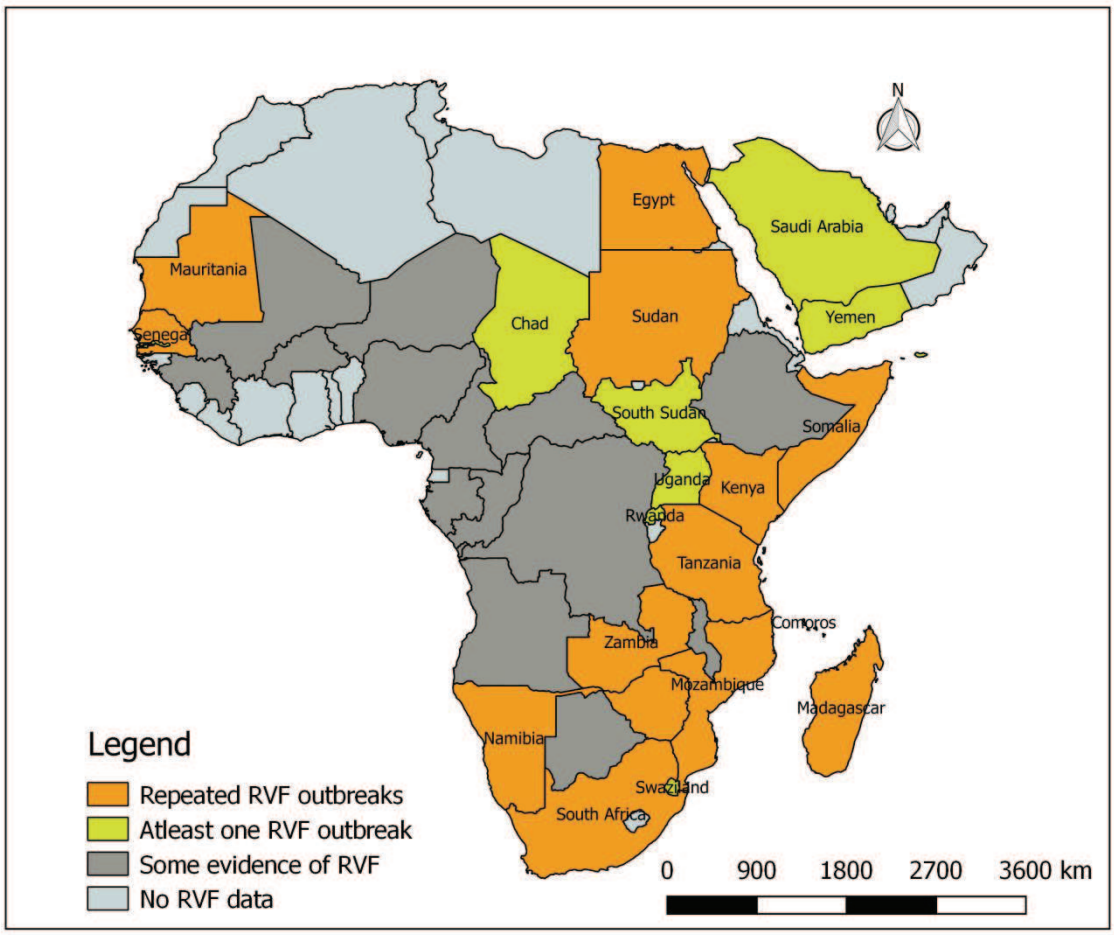

Figure 1.1 Map of Africa showing countries with history of RVF outbreaks and those with reported evidence of RVFV circulation. Map recreated in QGIS 2.14.0 -Essen, 2016 using data from (Mansfield et al., 2015, Kenawy et al., 2018) and shape files for country administrative boundaries available at www.arcgis.com

1.2 Rift Valley fever virus

Rift Valley fever virus (RVFV) is an enveloped spherical (80 - 110 nm) particle with tri-segmented negative-sense single-stranded RNA virus belonging to the order *Bunyavirales*, genus *Phlebovirus* within the *Phenuiviridae* family (Adams et al., 2017, Bird et al., 2009). Several members of the *Phenuiviridae* family are responsible for fatal hemorrhagic fevers: Rift Valley fever virus (*Phlebovirus*), Crimean-Congo hemorrhagic fever virus (*Nairovirus*), Hantaan, Sin Nombre and related viruses (*Hantavirus*), and recently Garissa, now identified as Ngari virus (*Orthobunyavirus*) (Flick and Bouloy, 2005). The RVFV viral genome (approx 11.9 kilobases) consists of three segments designated large (L), medium (M) and small (S), which encode the viral RNA-dependent RNA polymerase, the viral glycoproteins Gn and Gc and non-structural protein NSm, and the viral nucleocapsid protein N

and the non-structural virulence protein, NSs, respectively (Havranek et al., 2019, Bird et al., 2009, Bouloy and Weber, 2010) (**Figure 1.2**). These viral proteins, in conjunction with host proteins, ensure that the virus can replicate its genome during a productive infection (Havranek et al., 2019, Bouloy and Weber, 2010). All the replication steps occur in the cytoplasm of infected cells and virions mature by budding in the Golgi compartment (King et al., 2012). During the replication cycle, each segment is transcribed into mRNA and is replicated through a process involving synthesis of the exact copy of the genome, called complementary RNA (cRNA) or antigenome. The cRNA representing the copy of the S ambisense segment serves as a template for the synthesis of the NSs mRNA. Since the S cRNA is present in the input virus, the protein is expressed early, a good indication that it has an important role during infection (Pepin et al., 2010, Ikegami et al., 2005). During the viral cycle, the glycoproteins play an essential role for the penetration of the virus and their proper processing is crucial for the maturation and budding of the virion (Liu et al., 2008, King et al., 2012). The glycoproteins, being the most exposed components of the virus during infection, are recognized by the immune system and induce the production of neutralizing antibodies, which play a predominant role in protection (Filone et al., 2006, Pepin et al., 2010).

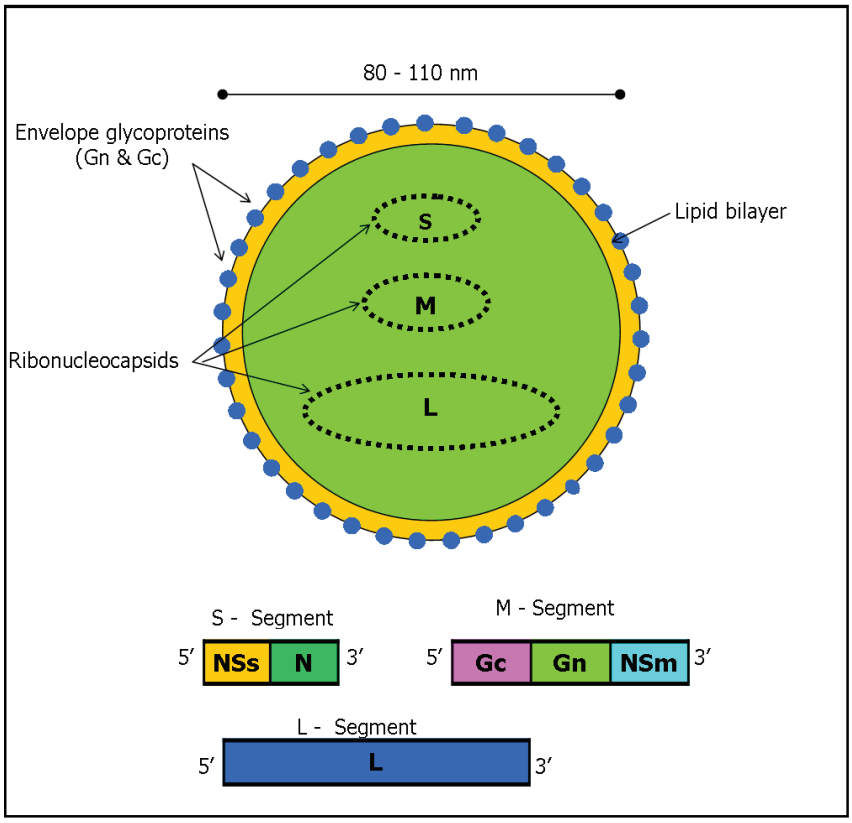

Figure 1.2 Schematic illustration of RVFV genome containing small (S), medium (M), and large (L) RNA segments and surface glycoproteins (Gn and Gc) incorporated into the lipid bilayer. Schematic representation of RNA segments and

respective proteins encoded by each segment, L = L protein; NSm = non-structural protein M; glycoproteins Gn, Gc; N = nucleoprotein; NSs = non-structural protein S.

1.3 Rift Valley Fever virus transmission

Rift Valley Fever virus transmission usually occurs in epizootics/epidemics associated with periods of heavy rainfall (Davies et al., 1985). It is now known that the development of epidemic-associated transmission is dependent on large-scale weather events such as the warm El Niño Southern Oscillation (ENSO), which can lead to heavy precipitation over eastern and southern Africa (Bird et al., 2009). Such heavy rainfall triggers the emergence of large numbers of mosquito vectors through creation of aquatic larval habitats (Fontenille et al., 1998). Mosquitoes are thought to be the only biological vectors of RVFV (Linthicum et al., 1985, Bird et al., 2009).

Rift Valley fever virus (RVFV) has been isolated from at least 40 species of mosquitoes (Meegan, 1979, Fontenille et al., 1998, Turell et al., 2008) belonging to six taxa: *Aedes, Culex, Anopheles, Eretmapodites, Mansonia,* and *Coquillettidia* (Bird et al., 2009). It has been widely suggested that RVFV is maintained through transovarial transmission in the floodwater mosquitoes of the *Aedes* genus (Davies et al., 1985, Lancelot et al., 1990, Gerdes, 2004). *Aedes* mosquitoes are regarded as primary vectors due to their ability of transovarial transmission of RVFV which provides the virus with a potential mechanism of persistence of infected eggs in dry conditions for several years during inter-epidemic periods (Logan et al., 1991, Gerdes, 2004). During periods with normal (non-excessive) amounts of rainfall, RVFV is likely maintained by low-level enzootic/endemic (locally present infections) activity within the mosquito vector population involving transovarial transmission with occasional infection and amplification of virus in wild animals such as African buffaloes (*Syncerus caffer*) or susceptible livestock including cattle, sheep and goats (Bird et al., 2009). Then excessive flooding following heavy rains allows for massive mosquito emergence and an increase in the number of both primary (*Aedes*) and secondary (*Culex*) vectors (Sang et al., 2010, Davies et al., 1985). Transovarially-infected primary mosquito vectors include *Ae. mcintoshi, Ae. (Neomelaniconion) circumluteolus* (Theobald, 1908), *Ae. albopictus,* (Sang et al., 2010, Pepin et al., 2010, Tesh and Shroyer, 1980) and *Ae. aegypti* (Cruz et al., 2015) that feed on susceptible ruminants (Coetzer, 1977). In turn, infected ruminants can infect secondary bridge mosquito vectors such as *Culex spp*, *Mansonia spp* or *Anopheline spp* (Coetzer, 1977, Bird et al., 2009, Sang et al.,

2010) which pick up the virus, and amplify transmission to generate an outbreak (Davies et al., 1985, Sang et al., 2010). The transmission cycle is summarised in schematic diagram in Figure 1.3.

RVFV was isolated from *Culex spp* following the 1998-1999 outbreak in Mauritania and Senegal (Diallo et al., 2005) and from *Aedes spp* and *Culex spp* following the outbreak of year 2000 in Saudi Arabia (Jupp et al., 2002). *Aedes spp, Culex spp, Mansonia spp* and *Anopheles spp* were implicated with the RVF outbreak 2006/2007 in Kenya (Sang et al., 2010, LaBeaud, 2011), whereas *Anopheles spp*, *Culex spp* and *Mansonia spp* were found naturally infected in Madagascar (Ratovonjato et al., 2011). So far, RVFV in mosquitoes has mostly been detected following outbreak periods and unusual heavy rainfall and floods. The role of vectors or other transmission routes (infected bodily fluids) in maintaining RVFV circulation between epidemics is not fully understood largely because detection and/or isolation of the virus during the inter-epidemic period (IEP) has not been achieved (Lichoti et al., 2014a). In addition, epidemics in the region have been generally irregular which could be attributed to climate variability (Martin et al., 2008), emphasising the need for continuous surveillance and exploration of all possible factors contributing to disease epidemics.

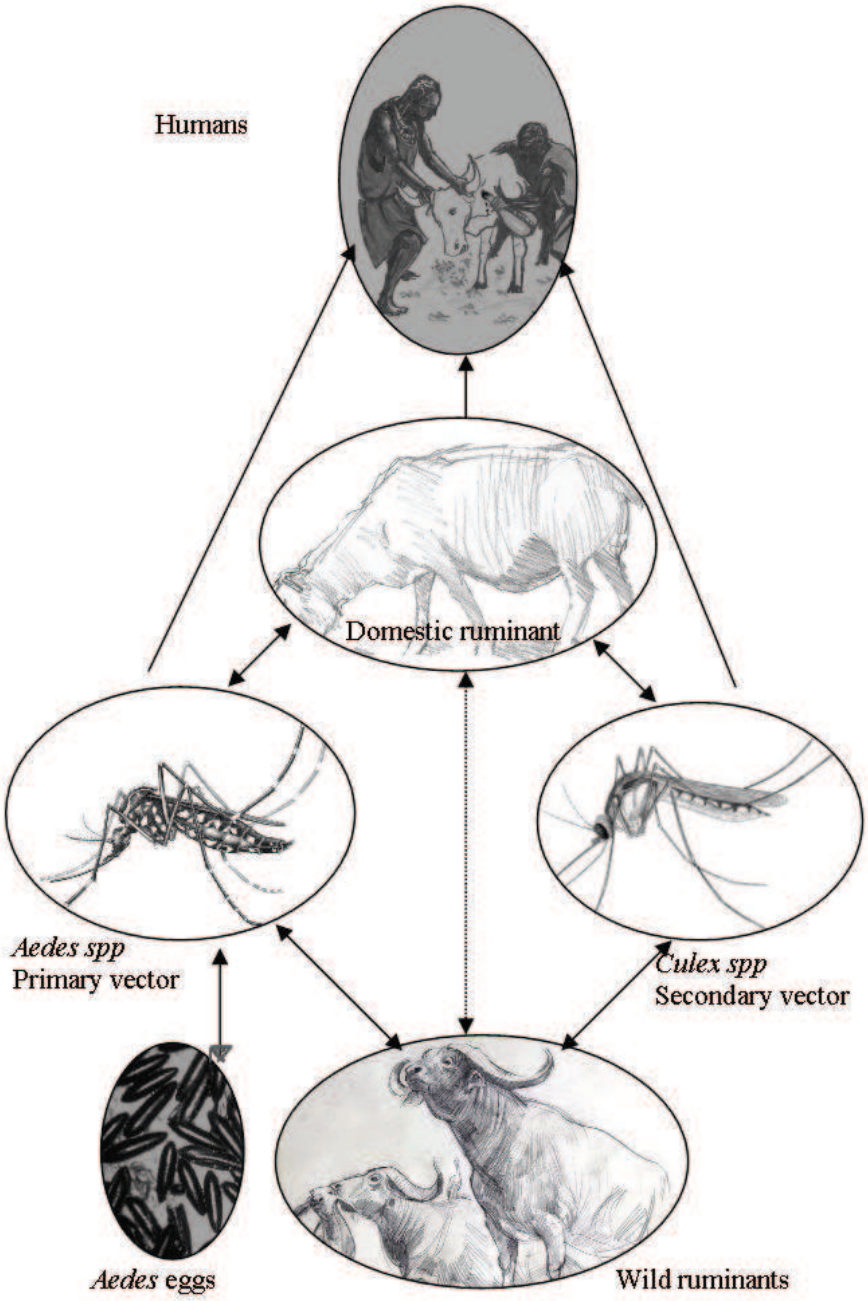

Figure 1.3 Schematic diagram showing potential RVFV transmission routes involving vectors and host species. Dotted lines indicate uncommon but potential horizontal transmission between wild and domestic ruminants.

1.4 Potential Rift valley fever virus (RVFV) vectors in northern Tanzania

Although a number of RVF epidemics have been reported in Tanzania, the relative importance of different vector species implicated in epidemic and inter-epidemic transmission is still uncertain, and no surveys have yet detected RVFV in mosquitoes in Tanzania. An entomological study by Mweya et al. (2015) in

Ngorongoro district in northern Tanzania reported the occurrence of potential vectors *Aedes aegyti* and *Culex pipiens* complex (Mweya et al., 2015). Other species recorded in area include *Culex antennatus*, *Culex tigripes*, *Culex annulioris*, *Culex cinereus*, *Anopheles arabiensis*, *Anopheles squamosus*, *Anopheles pharoensis* and *Mansonia uniformis* (Mhina et al., 2015). These studies suggests associations of previous RVF epidemics with abundance and distribution of *Aedes spp* and *Culex spp* in Ngorongoro district (Mweya et al., 2015, Mweya et al., 2013). Both of these studies (Mweya et al., 2015, Mhina et al., 2015) were carried out in the same (Ngorongoro) district, and were unable to detect presence of RVFV in mosquitoes. Surveillance of mosquito vectors from other areas including those which experienced repeated RVF outbreaks will expand understanding of the ecology and distribution of potential vector species, and guide identification of which groups may be primarily responsible for RVFV circulation in the area.

1.5 Ecology and feeding preference of potential RVFV Vectors

The distribution of RVFV vectors in endemic regions were previously found to be associated with the ecology and habitats of the regions (Tantely et al., 2015, Sang et al., 2017). In the Arabian Peninsula outbreaks have been linked to *Aedes spp* and *Culex spp* found in areas suitable for vector larval development including temporarily flooded agricultural fields or pasture land, and permanent or semi-permanent aquatic habitats (Jupp et al., 2002, Elfadil et al., 2006). In West Africa, *Aedes spp* and *Culex spp* linked to the outbreaks in Senegal and Mauritania in the Sahelian and Sudano-Sahelian region are found in temporary ponds where livestock concentrate during the rainy season (Diallo et al., 2005, Biteye et al., 2018). The abundance and composition of vectors varied with rainfall, season, land use, proximity to water bodies, and drainage and/or soil properties (Jupp et al., 2002, Gerdes, 2004, Diallo et al., 2005, Sang et al., 2010, Glancey et al., 2015, Biteye et al., 2018). Mosquitoes (*Aedes, Culex Mansonia* and *Anopheles*) implicated in RVF outbreaks in Kenya, East Africa (Logan et al., 1991, Sang et al., 2010) were associated with semi-arid areas (Sang et al., 2010, Sang et al., 2017), which experience sporadic rainfall with occasional torrential storms that cause extensive flooding in the lowlands (Sang et al., 2010, Sang et al., 2017). In South Africa, the outbreaks between 2008 and 2011 were associated with above average rainfall, in shrubland, low fynboes, grassland, and agricultural areas in close proximity to water bodies, including rivers and drainage areas (Glancey et al., 2015). These

studies suggest a range of ecological characteristics where RVF and vectors have been recorded previously in other endemic countries.

The distribution and abundance of mosquito vectors is also influenced by feeding ecology. The role of livestock in the feeding ecology of mosquitoes has been the subject of extensive study in relation to mosquito vectors (Diatta et al., 1998, Deichmeister and Telang, 2011, Lutomiah et al., 2014). However, little is known about how the presence, abundance or density of livestock might affect RVF transmission risk. The presence of livestock could influence mosquito vector species abundance and distribution as previously demonstrated (Mayagaya et al., 2015a, White, 1971, Besansky et al., 2004, Costantini et al., 1998, Diatta et al., 1998). It has also been suggested that zooprophylaxis, the diversion of disease carrying insects from humans to animals, may reduce transmission of mosquito-borne diseases (MacDonald, 1957, WHO, 1982, Service, 1976) and has therefore been recommended as a potential environmental strategy to reduce transmission of mosquito borne diseases (Saul, 2003). Studies involving *Anopheles spp* (Mahande et al., 2007, Muriu et al., 2008) and similar studies involving *Aedes* and *Culex* spp (Hess and Hayes, 1970) suggest that domestic animals would have the highest potential values for zooprophylaxis against RVFV vectors. Conversely, other studies have shown that, in situations where livestock are kept close to humans, animals may actually increase the risk of individual humans being bitten (zoopotentiation) by attracting mosquitoes to the household environment (Bouma and Rowland, 1995, Schultz, 1989, Hasyim et al., 2018), suggesting that keeping livestock in the household contributes more to risk than prophylaxis (Mayagaya et al., 2015b, Hasyim et al., 2018). Investigating the influence of livestock on local abundance and distribution of potential RVF vectors at household level will broaden our understanding of risk factors for RVFV transmission in livestock and human populations in different agro-ecological settings.

Host odours play a major role in the orientation of nocturnal mosquitoes towards their hosts (Takken and Kline, 1989). The animal breath and skin odour influences mosquito host-seeking behaviour. Adult female mosquitoes use host-emitted odour to locate hosts to obtain blood meals (Takken and Kline, 1989, Takken and Verhulst, 2013). When searching for hosts for blood meals, some mosquitoes express preferential feeding behaviour (Takken and Verhulst, 2013). Most known RVFV vector species have zoophagic behaviour, feeding mostly on animals (cattle, sheep, and goat), and some of them are described as opportunistic anthropophagic

feeders, feeding mostly on humans (White, 1971, Tantely et al., 2013). A study in Madagascar suggests cattle were the main domestic host for five species (*An. squamosus*, *An. coustani*, *Cx. antennatus*, *Cx. univittatus* and *Cx. pipiens*), and that vectors preferred these to other domestic animals (Tantely et al., 2013). Similar observations were reported in Kenya where *Aedes spp*, *Culex spp*, and *Mansonia spp* prefered cattle to other livestock species (Tchouassi et al., 2016). Another similar study in Senegal observed that *Culex spp.* fed most often on humans, *Aedes spp* fed almost equally on all animal species tested (cattle, goats, sheep and chickens), while *Anopheles spp* preferred cattle over humans, goats and sheep. In Mauritania *An. funestus* and *An. pharoensis* were observed to feed on human and ovine hosts respectively (Dia et al., 2009). These studies demonstrate different feeding behaviour and host preference of the vector species suggesting potential different exposure risks of different host species. To better understand the risk of RVFV transmission associated with having livestock at a household, it is important to examine the occurrence, abundance and distribution of the RVFV vector species at households with and without livestock.

1.6 Serological survey for RVFV infection in Tanzania

Rift Valley fever cases in livestock and humans are usually reported during large outbreaks. However, the maintenance and transmission of the virus to livestock and humans during the inter-epidemic period (IEP) when there is low or no disease activity is not well understood (Lancelot et al., 1990, Gerdes, 2004, Martin et al., 2008, Sang et al., 2010, Mroz et al., 2017). It is possible that RVF cases are passing undetected due to inadequate surveillance in livestock and human populations during inter-epidemic periods. This may occur due to an assumption that cases can only occur during periods of extreme rainfall (Beechler et al., 2015, Sindato et al., 2015). Wild ruminants are suggested as potential reservoirs of the virus, playing a role in inter-epidemic maintenance of the virus (Beechler et al., 2015, Evans et al., 2008). This is supported by detection of neutralizing antibodies in buffaloes from the Kruger National Park and Hluhluwe-iMfolozi Park in South Africa (Beechler et al., 2015, Fagbo et al., 2014), buffaloes in Botswana (Jori et al., 2015) and seven wildlife species in Kenya, including African buffalo, black rhino, lesser kudu, impala, African elephant, kongoni, and waterbuck including animals born during the 1999–2006 IEP (Evans et al., 2008). In Egypt, RVFV antibodies were detected in buffaloes born after the last Egyptian RVF epidemic in 2003 (Mroz et al., 2017).

These studies suggest that wildlife could be playing an important role in the inter-epidemic circulation of RVFV.

There is some evidence of inter-epidemic RVFV infections on the basis of serological surveys in the region including in sheep and goats in Mozambique (Blomström et al., 2016), cattle, sheep, and goats in Kenya (Lwande et al., 2015, Mbotha et al., 2018, Lichoti et al., 2014a) and Madagascar (Jeanmaire et al., 2011). Inter-epidemic seroprevalence in humans has been reported in Gabon (Pourrut et al., 2010), Saudi Arabia (Mohamed et al., 2014), Kenya (Cook et al., 2017), and Central African Republic (Nakouné et al., 2016). In Tanzania, antibodies against RVFV have been detected in cattle, and small ruminants (Sumaye et al., 2013, Wensman et al., 2015, Sindato et al., 2015). Serological evidence for human exposure to RVFV infections in the inter-epidemic period has been reported in Morogoro and the Mbeya regions of south-western Tanzania (Heinrich et al., 2012a, Sumaye et al., 2015) and the in the Serengeti ecosystem, northern Tanzania (Ahmed et al., 2018). Although all these studies show some level of (inter-epidemic) RVFV circulation in livestock and humans in Tanzania, little is still known about spatial patterns, risk factors and outcomes of inter-epidemic infection in northern Tanzania. These infections could be driven by either host factors (i.e. species, sex, age, susceptibility) as well as vector ecology and feeding behaviour and further knowledge of these factors will be important for understanding the dynamics of inter-epidemic infection.

1.7 Risk factors for RVFV inter-epidemic infections in livestock and humans

Rift valley fever outbreaks in livestock and humans have been well documented to be associated with unusual heavy rainfall periods coupled with floods and massive breeding of mosquitoes (Davies et al., 1985, Gerdes, 2004). Human infections have been linked with contacting infected animals and/or animal materials (Gerdes, 2004, Evans et al., 2008). The means by which infections are maintained in livestock or human populations in the inter-epidemic periods is not fully understood. To further explore this question it is essential to identify what factors are associated with RVFV infections between epidemics. Identification of risk factors will provide insight into the transmission routes and will advance our understanding of maintenance of RVFV infections which will inform surveillance, prevention and control strategies and programmes.

A wide range of factors have been associated with RVFV infections in livestock in different settings. Animal species, sex and age (young and old) were associated with RVFV seropositivity in Egypt (Mroz et al., 2017), Kenya (Lichoti et al., 2014b), Mauritania (Rissmann *et al.*, 2017) and Tanzania (Sumaye *et al.*, 2013). Older age and heavy rainfall were considered as potential risk factors for RVFV seropositivity among the Sudanese one-humped camel (*Camelus dromedaries*) in Khartoum State, Sudan (Abdallah et al., 2015). In Madagascar, cattle seropositivity was associated with older age, humid environments and high cattle density (Olive et al., 2016). In addition, animal introductions and history of abortions have also been reported as risk factors in a previous study in Tanzania (Sindato et al., 2015). These findings suggest that seropositivity in different livestock species can be influenced by different factors or similar factors in different ways.

Risk factors for seropositivity in humans have been reported in a number of studies and mostly related to behaviours, occupation, household responsibilities and traditions. Studies in different endemic countries have identified factors that are consistent across the region or apply only to local settings. In Madagascar, human exposures to RVFV were found to be associated with the presence of temporary and artificial water points and frequent handling/consumption of raw milk (Olive et al., 2016). In Kenya, human seropositivity was found to be associated with being male and a herdsperson, handling aborted animal foetus, consuming or handling products from sick animals (Anyangu et al., 2010), consuming raw milk (Nicholas et al., 2014) and birthing an animal (Woods et al., 2002, Anyangu et al., 2010). Whereas in Uganda RVFV seropositivity were greater in participants who were butchers and those who reported handling raw meat (Nyakarahuka et al., 2018). Understanding factors that drive RVFV infection in local settings will help inform appropriate intervention strategies.

Livestock abortions have been associated with RVF outbreaks following periods of unusual rainfall and floods, but unreported abortions in the inter-epidemic periods and their association with RVFV infections in livestock and humans have not been well studied. It is also worth investigating other potential factors for RVFV infections in the inter-epidemic period. Factors related to management of livestock in different agro-ecological settings which have not been well studied in relation RVF, including farming systems (small-holder, agro-pastoral and pastoral), seasonal movement following pasture and water in dry seasons, and animal introductions. It is important therefore, to examine and identify potential risk factors associated

with RVFV infections in livestock and humans in small-holder, agro-pastoral, pastoral communities in Tanzanian in order to inform appropriate control strategies.

1.8 Detection of Rift Valley Fever virus infections

Rift Valley fever virus infections or exposure status can be detected using various techniques, including virus isolation, viral nucleic acid detection, antigen detection and detection of specific antibodies (Pepin et al., 2010, Swanepoel et al., 1986). RVFV infection is generally associated with a high and early humoral response. The immune response developed following infection, involves the production of detectable neutralizing antibodies from the 4th–8th day after infection (Hubbard et al., 1991, Morrill et al., 1987, Pepin et al., 2010). These antibodies, which are primarily directed against the viral glycoproteins, Gn and Gc, are also accompanied by the production of IgM and IgG antibodies raised against the nucleoprotein, N, and the non-structural protein, NSs (McElroy et al., 2009). The detection of IgM and IgG antibodies coupled with the results of molecular (RT-PCR) assays is critical to accurately stage the time since infection (Pepin et al., 2010).

1.8.1 Serological detection of RVFV infections

The presence of immunoglobulin M (IgM) is usually interpreted as an indicator of recent infection (Morvan et al., 1992). Immunoglobulin M antibodies are associated with a primary immune response which provide first line defence and are frequently used to detect acute exposure to an immunogen or pathogen in 5-8 days of being infected (Schroeder and Cavacini, 2010). In most cases IgM antibodies do not persist beyond the 50th day after infection (Paweska et al., 2003b) (**Figure 1.4**). On the other hand, immunoglobulin G (IgG) are the predominant antibodies found in the body picking up after IgM as secondary antibodies and have the longest serum half-life of all immunoglobulins (Schroeder and Cavacini, 2010). They may provide protection against specific pathogens for several years. Immunoglobulin G are the only antibodies that can pass from mother to foetus via the placenta and are also secreted into the mother's milk and is taken up from the gut of the neonate into the blood, providing protection for the newborn against infection (Alberts et al., 2002). Anti-RVFV IgM antibodies do not persist beyond the 50th day after infection and confirms recent infections (Paweska et al., 2003b, Morvan et al., 1992). This is supported by a study involving 195 naturally infected cattle in Madagascar including 37 aborted females and 158 other infected animals (Morvan et al., 1992). Only 27%

of animals had detectable IgM two months post infection, and no animals had detectable IgM after 6 months.

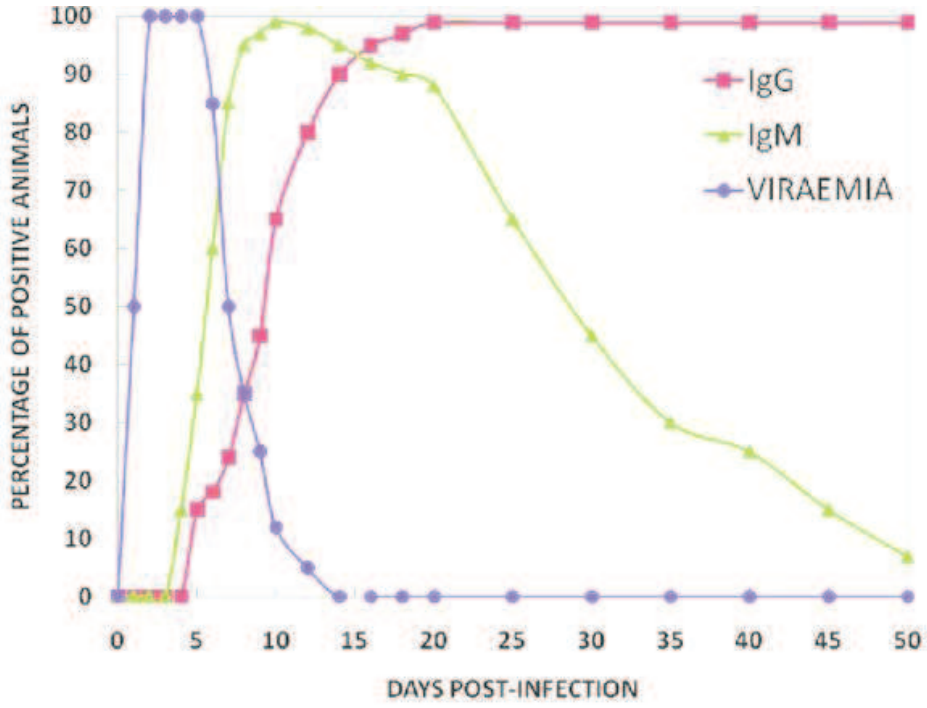

Figure 1.4 Schematic representation of time course viraemia and antibody response against RVFV in experimentally infected animals. Diagram adapted from Pepin et al., 2010.

1.8.1.1 Virus Neutralization Test

The virus neutralization test (VNT) regarded as the gold standard serological assay for RVF (Schreur et al., 2017), is a specialized type of immunoassay because it does not detect all antigen–antibody reactions. It only detects antibodies that can block (neutralize) virus replication, which is important because closely related groups of viruses may share common antigens, but only a fraction of these antigens are targets of neutralizing antibody. The VNT is the only assay available to measure the presence of virus neutralizing antibodies (VNAb) (Monath and Vasconcelos, 2015). The test protocol involves mixing dilutions of test sera with a fixed amount of virus and incubating to allow VNAb to neutralise the virus. The serum/virus mix is then added to a monolayer of permissive cells and incubated for a period sufficient for the virus to infect cells and produce a cytopathic effect (CPE). Cells are then viewed under the microscope, the presence of VNAb is determined by the absence of CPE, as VNAb in the serum have successfully neutralised the virus. For viruses which do not produce CPE, labelled antibodies directed against the virus can be

used to detect virus in the cell culture. Although the VNT is considered the gold standard for detection of antibody against RVFV, its use in most of the endemic countries is limited by being expensive, requires high-containment facilities for working with live virus, requires the use of cell culture, and involves incubation for 5-7 days for completion (Pepin et al., 2010, Paweska et al., 2003b). Additionally, the test is laborious and few samples can be investigated simultaneously (Mather et al., 2013). This can limit serosurveillance efforts for such viruses as RVFV in regions where such facilities are not available.

1.8.1.2 Enzyme-linked immunosorbent assay (ELISA)

Antibody detection by enzyme-linked immunosorbent assay (ELISA) can be used to confirm presence of specific IgG and IgM antibodies. These assays are useful for epidemiological surveillance and control programmes, import/export veterinary certification, early detection of infection, and for monitoring of immune response in vaccinated animals (Paweska et al., 2003b). An ELISA can be used to detect either the presence of antigens or antibodies in a sample, depending on how the test is designed (Ma and Shieh, 2006, Gaastra, 1984). To detect antibodies using the ELISA test (**Figure 1.5**), antigen is coated onto a 96-well plate and test sera are added. Secondary antibody coupled to an enzyme, specific to the host species being tested, is then added to the plate wells. Enzyme substrate is then added allowing a colour change to be determined by measuring the optical density measured in a spectrophotometer. Depending on the ELISA platform used, either the presence or absence of colour indicates the presence of antibodies (Ma and Shieh, 2006). IgM or IgG antibodies can be detected using isotype-specific secondary antibodies. The antibodies detected may be either neutralising or non-neutralising and the tests cannot differentiate between the two.

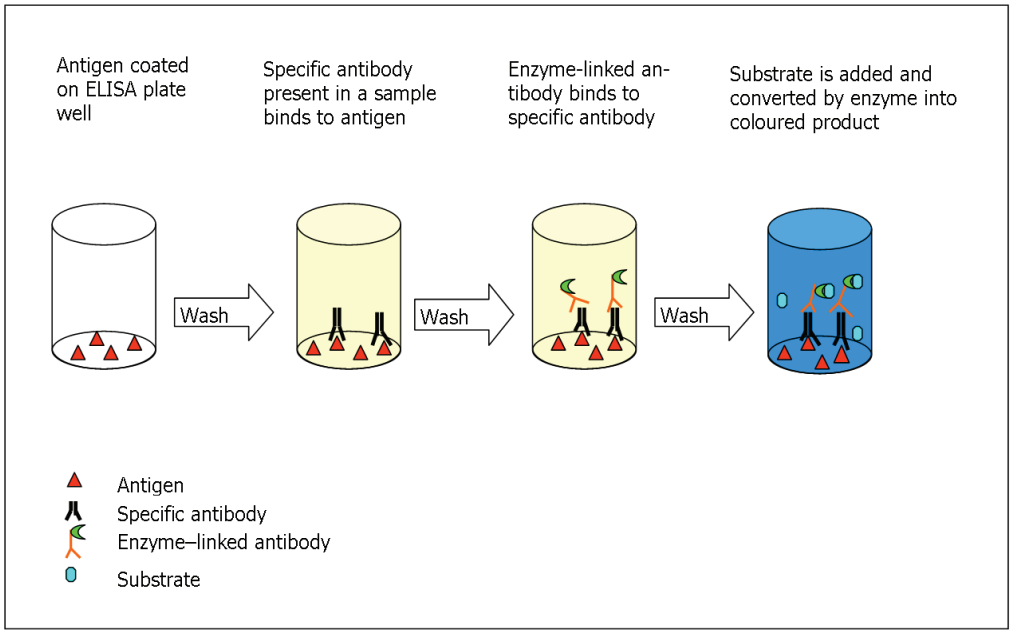

Figure 1.5 Schematic representation of the ELISA test and procedure involved in detecting specific antibodies in a given sample.

When testing for anti RVFV antibodies, non-specific background has been observed using ELISAs (Faburay et al., 2013) suggesting antibody cross-reactivity, as antibodies raised against other members of the family *Phenuiviridae* and genus *Phlebovirus* have shown to cross-react (Szymczak et al., 2015, Xu et al., 2007), which make results interpretation difficult. In addition, most of the available ELISAs have raised concerns with their performance, sensitivity and specificity (Paweska et al., 2003a). However, ELISAs remain the most commonly and widely utilised method for sero-epidemiology of RVFV (Mansfield et al., 2015) in endemic areas. Commercial RVFV ELISAs based on the viral nucleoprotein are available and detect IgM and/or IgG. These ELISAs allow for large scale sero-surveillance outside of high containment facilities and are much more convenient for 'in field' testing (Van Vuren and Paweska, 2010). ELISAs based on recombinant N-protein, which can perform as well as VN and HI tests (Fafetine et al., 2007), have the potential to complement the traditional assays for serological detection of RVFV infections. Advantages of the N-protein are its safety, stability and cost-effectiveness in use and production (Fafetine et al., 2007) and the RVFV-N-based ELISAs can be useful in large-scale epidemiological investigations. It is imperative therefore, to develop a cheap, safe, thermal stable, and easy to use ELISA that can be used in endemic countries. In this study, the performance an inhouse RVFV-N based ELISA developed by the University of Glasgow was evaluated using commercially available ELISA as a reference.

1.8.2 Molecular detection of RVFV infections

Rift Valley fever virus (RVFV) is a member of the family *Phenuiviridae* and as such is an enveloped virus that has a negative stranded RNA genome consisting of three segments. The M (medium) and L (large) segments are of negative orientation, whereas the S (small) segment has an ambisense polarity. The M segment encodes the two glycoproteins Gn and Gc and the non-structural protein NSm, while the L segment encodes the RNA-dependent RNA-polymerase. The small segment encodes the nucleocapsid (N) protein and a non-structural protein NSs using an ambisense coding strategy (Paweska, 2014, Pepin et al., 2010). The N protein is highly conserved and it is one of the most immunodominant viral proteins among members of the *Phenuiviridae* family (Pepin et al., 2010)

Detection of nucleic acids of RVFV has been shown to be useful in field diagnostics (Drosten et al., 2002). Molecular methods are increasingly used for a rapid and accurate detection of viral nucleic acid in blood, tissue and mosquito samples (Drosten et al., 2002). Molecular techniques such as reverse-transcriptase polymerase chain reaction (RT-PCR) and other newly developed techniques allow for a rapid and accurate detection of RVFV (Escadafal et al., 2013). The RT-PCR is often used as a method of detection for a great number of arboviruses, which facilitates phylogenetic studies (Garcia et al., 2001). Other molecular methods that have been developed recently include: single-tube RT-PCR, quantitative RT-PCR (qRT-PCR) and more recently real-time reverse-transcription loop-mediated isothermal amplification (RT-LAMP) (Le Roux et al., 2009) and recombinase polymerase amplification assays (RPA) (Euler et al., 2012). Despite some limitations in terms of costs and the level of expertise required of technicians, RT-PCR is a rapid, sensitive, specific, and reliable assay for detection of RVFV infection (Sall et al., 2002). RT-PCR has shown to detect viral infections in the very early phase of the disease, before the appearance of IgM antibodies and the decline of viremia (Burt et al., 1998). The RT-PCR is therefore recommended as an efficient diagnostic tool for the investigation of enzootic circulation of the RVFV. It allows the detection of low viral RNA loads adapted for the investigations of reservoirs or specific epidemiological situations such as during inter-epidemic periods (Maquart et al., 2014).

1.9 Rationale for the study

1.9.1 Problem Statement

Available serological evidence suggests that, across many countries and settings in Africa, RVFV continues to circulate in livestock and humans in the periods between large epidemics. However, little is still known about patterns and risk factors of inter-epidemic infection in northern Tanzania where repeated large outbreaks have occurred previously. While no cases have ever been reported in Tanzania between the large RVF epidemics, it is possible that RVF cases are going undetected or are not being reported during inter-epidemic periods, either because of limited surveillance and awareness of the possibility of cases during this periods and/or because inter-epidemic infections present differently, for example with only low-level clinical or subclinical signs.

1.9.2 Study Justification

Understanding of how the virus circulates in livestock and humans between epidemics, as well as the risk factors and outcomes of these infections in different agro-ecological settings will help and inform intervention strategies to control and prevent future epidemics.

1.10 Objectives

1.10.1 General Objective

This study sought to investigate patterns of RVFV infection in livestock and humans in the inter-epidemic period in northern Tanzania, in order to inform surveillance, preparedness, and control strategies and/or programmes.

1.10.2 Specific Objectives

This study specifically sought to:

a) Determine the occurrence, abundance, distribution and infection status of potential RVFV mosquito vectors in northern Tanzania;

b) Investigate inter-epidemic seroprevalence and risk factors for RVFV infections in livestock and human populations in northern Tanzania; and

c) Investigate RVFV as a possible cause of livestock abortions during inter-epidemic periods in northern Tanzania.

Chapter Two

2 Core Methods

The methods described here are the general methodology for the study including field sample collection, laboratory work and statistical analyses. Detailed statistical analyses for specific study components are described under specific chapters of this thesis. Samples and data described here were collected as part of three study projects, namely Social, Economic and Environmental Drivers of Zoonoses (SEEDZ), Supporting evidence based interventions to achieve agricultural development goals in Tanzania (SEBI-TZ) and an earlier study on bacterial zoonoses (BacZoo). Therefore, a number of people have contributed in different study activities as summarised in Table 2.1.

Table 2.1 Activities that relates to completion of my PhD along with the roles of people who participated in various activities.

Activities	**Concept Development**	**Field implementation**	**Laboratory and data analyses**
Mosquito surveys	**M.J. Nyarobi** H. Ferguson W. de Glanville	**M.J. Nyarobi**	**M.J. Nyarobi** W. de Glanville H. Ferguson P. Johnson
Livestock sero-epidemiological studies	W. de Glanville S. Cleaveland **M.J. Nyarobi**	W. de Glanville SEEDZ & BZ field teams **M.J. Nyarobi**	**M.J. Nyarobi** W. de Glanville P. Johnson
Human sero-epidemiological studies	W. de Glanville S. Cleaveland **M.J. Nyarobi**	W. de Glanville SEEDZ & BZ field teams **M.J. Nyarobi**	**M.J. Nyarobi** W. de Glanville P. Johnson
ELISA assay studies	B. Willet **M.J. Nyarobi**		**M.J. Nyarobi**
VNT assay	B. Willet **M.J. Nyarobi**		**A.** Szemiel
Mosquito PCR assay	**M.J. Nyarobi** H. Ferguson		**M.J. Nyarobi** R. Carter K. Allan
Livestock PCR assays	S. Cleaveland W. de Glanville	T. Kibona SEBI field teams	**M.J. Nyarobi** R. Carter K. Allan

Activities	Concept Development	Field implementation	Laboratory and data analyses
			K. Thomas
Mosquito habitat suitability analyses	W. de Glanville S. Cleaveland **M.J. Nyarobi**		**M.J. Nyarobi** W. de Glanville

2.1 Study Area

The study involved samples and data collected as part of the three epidemiological studies in three regions of northern Tanzania namely Arusha, Kilimanjaro and Manyara. The study area covered six districts in Arusha region (Arusha, Ngorongoro, Longido, Monduli, Karatu, Meru), four districts in Kilimanjaro region (Hai, Moshi Municipality, Moshi rural and Rhombo) and three districts in Manyara region (Simanjiro, Mbulu, and Babati), (Figure 2.1). These districts were selected as part of the project on Social, Economic and Environmental Drivers of Zoonoses (SEEDZ), between February and November 2016. The SEEDZ project aimed at examining and assessing the drivers, risks and impacts of a wider range of zoonotic diseases, including brucellosis, Q fever and Rift Valley Fever, that affect livestock and human health, livelihoods and poverty in pastoral, agro-pastoral and peri-urban communities in Tanzania. In addition to SEEDZ study districts, other samples included in the current study were collected in 2013 as part of other studies on bacterial zoonoses (BacZoo) and 'Supporting evidence based interventions to achieve agricultural development goals in Tanzania (SEBI-TZ)'. The SEBI study sought to extend and broaden the range of diseases investigated under the SEEDZ project with a particular focus on investigating the causes of abortions in livestock. The aim of the SEBI project was to develop effective and sustainable intervention strategies that will result in a reduction in abortion losses in cattle, sheep and goats. The study area has a high level of interaction between livestock, wildlife and humans (Prins, 1992, Newmark et al., 1994); and cases of RVF were reported during outbreaks in1977, 1998 and 2006/2007 (Mohamed et al., 2010, Fyumagwa et al., 2011, Chengula et al., 2013). The study included pastoral, agro-pastoral and small-holder communities. In pastoral communities such as Maasai and Barbaig, livestock (cattle, goats and sheep) are kept in animal enclosures (bomas) made of thorny acacia tree logs and branches within household compounds. Young animals (e.g. kids and lambs) often sleep in the same house as people. In agro-pastoral or

peri-urban areas, livestock are kept in sheds made of tree branches and mud or cow dung, thatched with grass or iron sheets within household compounds. In some cases, animals have a partition in the same house as people.

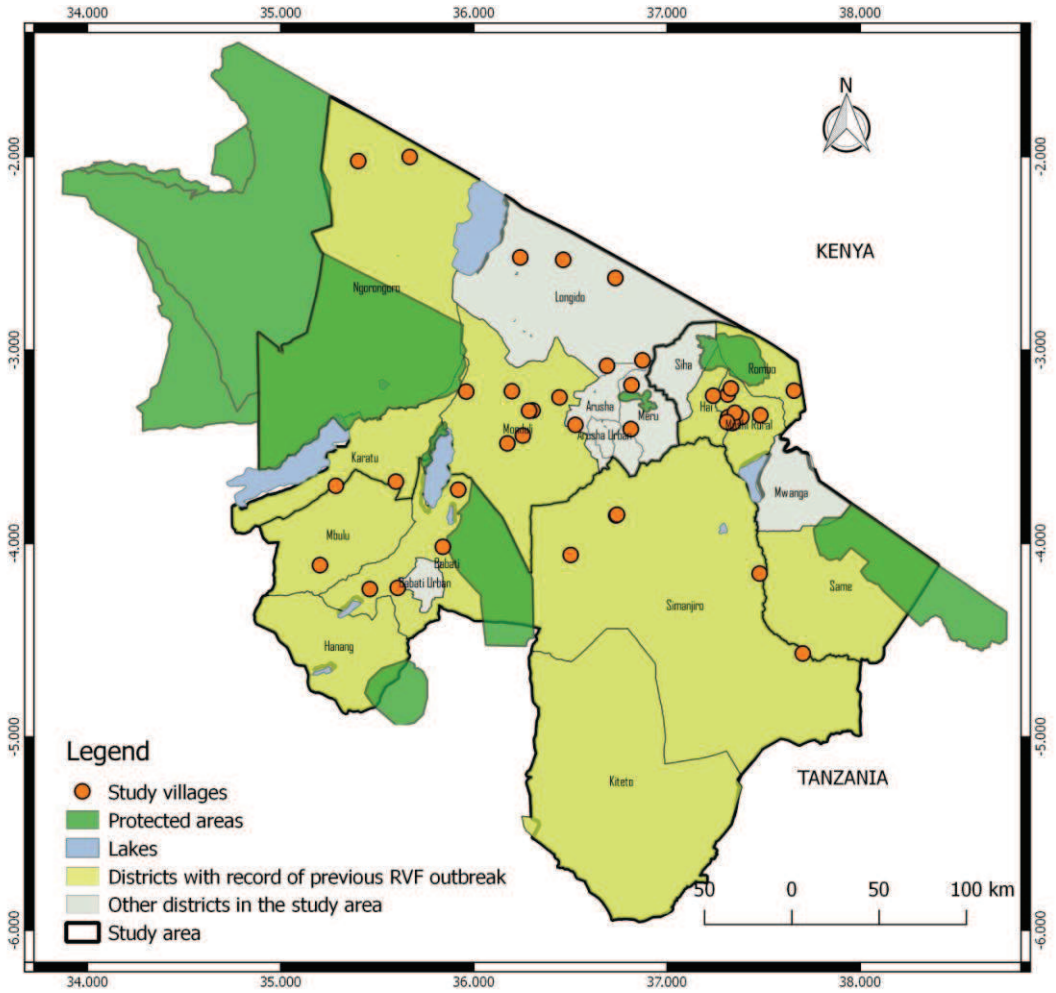

Figure 2.1 Map of northern Tanzania showing districts in the study area including those with record of previous RVF outbreaks and location of villages where the study was carried out. Map created in QGIS 2.14.0 -Essen, 2016 using RVF outbreaks data from (Sindato et al., 2014) and shape files for country administrative boundaries available at www.diva-gis.org

2.2 Village and household selection

For administrative, governance and provision of social services in Tanzania, there are different levels of regional and local government authorities apart from the national government. In the order of highest to lowest local government administrative unit, they include region, district, ward, village, sub-village (in rural settings) or *mtaa*/street (in urban settings). The SEEDZ study involved 20 villages and BacZoo involved 15 villages which were randomly selected. Villages were

selected using a Generalised Random Tessellation Stratified (GRTS) sampling approach (McDonald, 2004, Stevens Jr and Olsen, 2004) in order to achieve a spatially representative sample. Villages in the study area contained four or more sub-villages. Two sub-villages per village were randomly selected for inclusion in the SEEDZ study. Within sub-villages, households were randomly selected. Here livestock keepers were invited to a central point (e.g. dip tank, crush) where livestock sampling was conducted. The sub-village chairperson was notified at least three days in advance of the sampling event, and a meeting arranged with the chair person and cell (10 households) leaders at least a day before the event. The "Supporting Evidence Based Interventions in Tanzania" (SEBI-TZ) study established a real-time surveillance platform covering 16 randomly selected sentinel wards in Arusha, Manyara and Kilimanjaro Regions of Tanzania. Livestock (cattle, sheep and goats) samples were collected in response to reported abortion events from the SEBI study villages.

2.3 Ethical Clearance

The SEEDZ protocols, questionnaire tools and consent and assent procedures were approved by the ethical review committees of the Kilimanjaro Christian Medical Centre (KCMC/832), National Institute of Medical Research (NIMR/2028), and Commission for Science and Technology (2015-244-ER-2005-141) in Tanzania, and in the UK by the ethics review committee of the College of Medical, Veterinary and Life Sciences, University of Glasgow (Ref: 200140152). Approval for the animal elements of the study was provided by the Clinical Research Ethics Committee at the University of Glasgow School of Veterinary Medicine (39a/15). The BacZoo protocols were approved by NIMR, protocol number: NIMR/HQ/R.8a/Vol.IX/1499) and the Tanzanian Wildlife Research Institute (TAWIRI). Ethical approval for human sampling and testing was granted from the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, NIMR and the Institutional Review Boards of Duke University Medical Center and the United States for the Centers for Disease Control. In the UK, the University of Glasgow College of Medicine, Veterinary Medicine and Life Sciences Ethics Committee (protocol number: 200140152).

2.4 Sample collection and preparation

2.4.1 Animal Sampling

Animal sampling was performed by the respective project field teams led by a registered veterinarians. Livestock keepers from different bomas (households) were encouraged to bring cattle, goats and sheep of different ages and sex at the central point (identified sampling point) for sampling. Animals from different households (bomas) were brought to the central point for sampling. At the central sampling point, the aim, purpose and benefits of the study were explained to livestock keepers, and written consent was obtained before sampling animals from a selected boma. Animals were sampled in relative proportion to the size of the herd. The target sample size for livestock sampling was 10 bomas from a sub-village, and 10 each of cattle, sheep and goats selected per boma. In cases where a boma had less than 10 animals in a herd or flock, all animals would be sampled. From all animals, a 10ml blood sample was collected by jugular venepuncture following appropriate restraint in an existing or temporarily constructed livestock crush. About 2-5 mls of sera was obtained from each blood sample through centrifugation, and transferred into screw capped cryovials and stored at -80 °C for further serological analysis. For the BacZoo study, a list of all livestock keeping households in each village was generated and up to six households randomly selected. Households were visited and, where numbers allowed, 12 of each of cattle, sheep and goats sampled.

Under the SEBI study samples were collected following the report of an abortion or peri-natal mortality event, by recruited Livestock Field Officers (LFOs) or members of the study field team who visited the dam/ewe/doe(s) to collect samples within 72 hours of the abortion/still birth event. In addition to basic farm level data, the following samples were collected: (i) Blood samples from the cow/ewe/doe; (ii) Milk samples; (iii) Vaginal swabs from the cow/ewe/doe; (iv) Tissue from the placental inter-cotyledonary space; (v) Placental cotyledon; (vi) Foetal organs (liver, lung and kidney, thymus); and (vii) Foetal stomach contents. Only four sample types were used in the current study, namely vaginal swabs from the aborted dam, swabs from aborted foetus, placenta cotyledon tissue samples and milk samples. Samples were double packed according to UN3373 protocols and transported to the project laboratory at the Kilimanjaro Clinical Research Institute (KCRI) in the town of Moshi via a project vehicle or by courier.

2.4.2 Human sampling

Human diagnostic samples were collected from selected livestock-owning households from which animals were sampled. Questionnaire surveys were conducted to gather data on residents (e.g. demographics, livestock interaction practices, and clinical history) and household characteristics. An adult head of household from each household was asked to provide informed written consent for questionnaire data gathered. In addition, human blood sampling was carried out if there were consenting participants in a household. Participants for blood sampling were approached on an individual basis for consent for individual specific data collection. The individual themselves or head of household (in the case of minors aged <18 years) provided informed written consent. Blood sampling was performed by a Practitioner Nurse from Kilimanjaro Christian Medical Centre (KCMC) using vacuum extraction method as per World Health Organization guidelines (WHO, 2010).

2.4.3 Mosquito Sampling

2.4.3.1 Selection of mosquito sampling sites

Mosquito sampling was carried out in 12 villages randomly selected from the 20 SEEDZ study villages. The target was sampling two sub-villages from a village, and four households from each sub-village. However, there were logistical challenges coupled with size of some villages (some with larger geographical coverage) with large distance between household locations, and limited number of non-livestock keeping households in some villages hence uneven number of sampled sub-villages and households respectively. Mosquitoes were sampled from households with and without livestock in both agro-pastoral and pastoral settings. Livestock households were randomly selected from a list of animal sampled households. Non livestock keeping households were randomly selected from the list provided by the village and sub-village authorities. Numbers based on the list of households, were written in bottle tops, placed in a plastic container, shuffled each time a household number was selected.

In addition to households, potential mosquito breeding habitats (Figure 2.2) such as dambos (flooded depressions), rivers, open wells, swamps, ponds, rice field and animal water points (Fontenille et al., 1998, Gerdes, 2004, Davies et al., 1985, Diallo et al., 2005, Jupp et al., 2002) were purposefully identified for mosquito

trapping. This was carried out to identify mosquito species emerging from the breeding sites and that could be tested for RVFV infection status as obtained from trans-ovarial infection and were also compared with those sampled in and around households to determine which species were most likely to feed on infected livestock and humans.

Permission of community leaders and informed consent were sought from each head of household where trapping were carried out. Aims of the study, methods and potential benefits of participation were explained to the head of the household in Swahili or translated into the local language. Geographic co-ordinates of each selected household or mosquito trapping site were captured using a Garmin eTrex® 10 Handheld GPS.

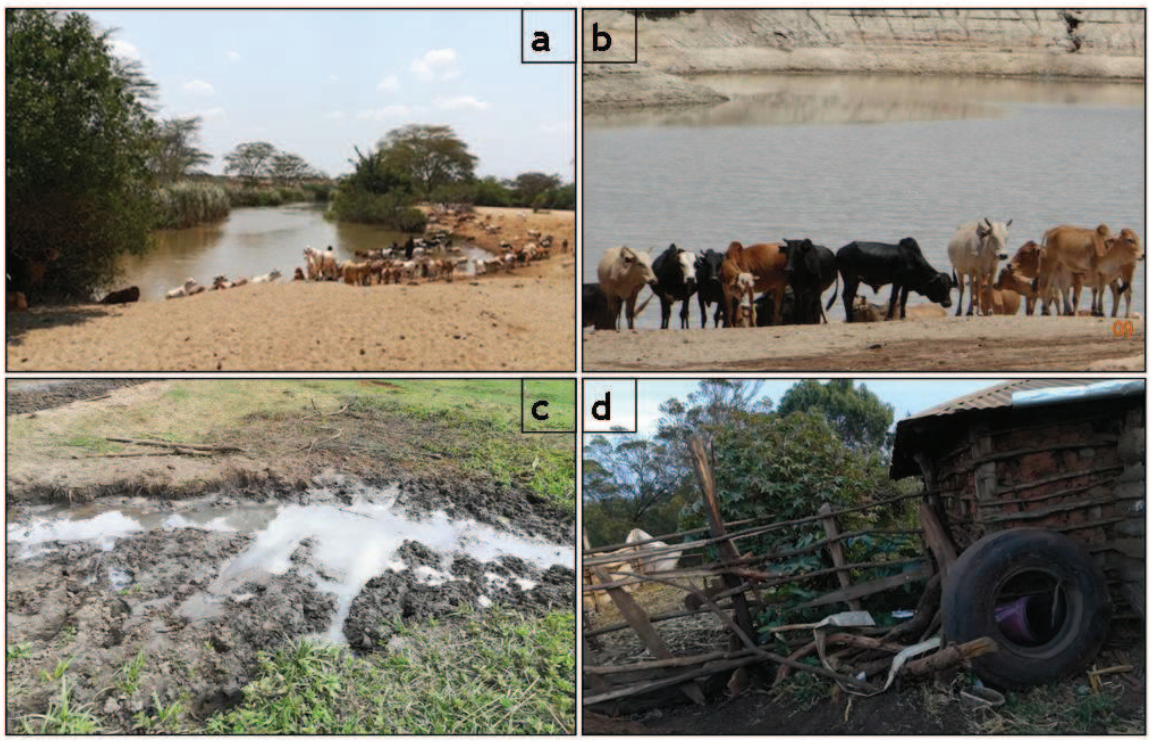

Figure 2.2 Pictures of different potential mosquito breeding habitat that were considered for trapping in the study area. (a) annual river near a village, (b) pond flooded by rain water, (c) stagnant water on ground, and (d) old tyre near animal enclosure

2.4.3.2 Mosquito collection methods

For mosquito collection, four trapping methods were tested to assess the risk of biasing collection data when using one trap over another. The following mosquito trapping methods (Figure 2.3) were used at each household: (1) BG Sentinel trap (BG) (Biogents AG, Regensburg, Germany) to target outdoor host seeking

mosquitoes. BG-Sentinel traps were used in combination with the BG-Lure, a dispenser which releases emanations such as those found on human skin (lactic acid, ammonia, and caproic acid) to attract host seeking species (Maciel-de-Freitas et al., 2006). This trap has been shown to be effective for collecting *Aedes spp* (Bhalala and Arias, 2009); (2) Mosquito Magnet traps (MM) (Woodstream Corporation, Pennsylvania, USA) to target outdoor host seeking mosquitoes particularly *Aedes spp, Ochlerotatus spp, Culex spp, Mansonia spp, and Anopheles spp* (Bell et al., 2005). The trap uses propane gas and produce CO_2, warmth and moisture to attract mosquitoes; (3) CDC Light traps (LT) (John W. Hock Company, Florida, USA) to target night time indoor host seeking species such as *Anopheles spp* and *Culex spp* (Mboera et al., 1998, Davis et al., 1995); and (4) Resting Bucket (RB) locally made from (20l) plastic buckets bought from the local shops, and lined with black cloth on the inside to target outdoor resting mosquitoes as previously described (Kreppel et al., 2015). This method has been previously evaluated for malaria vectors in Tanzania, and was shown to be effective for sampling *Anopheles* and *Culicinae* (*Aedes, Culex* and *Mansonia*) mosquitoes (Kreppel et al., 2015).

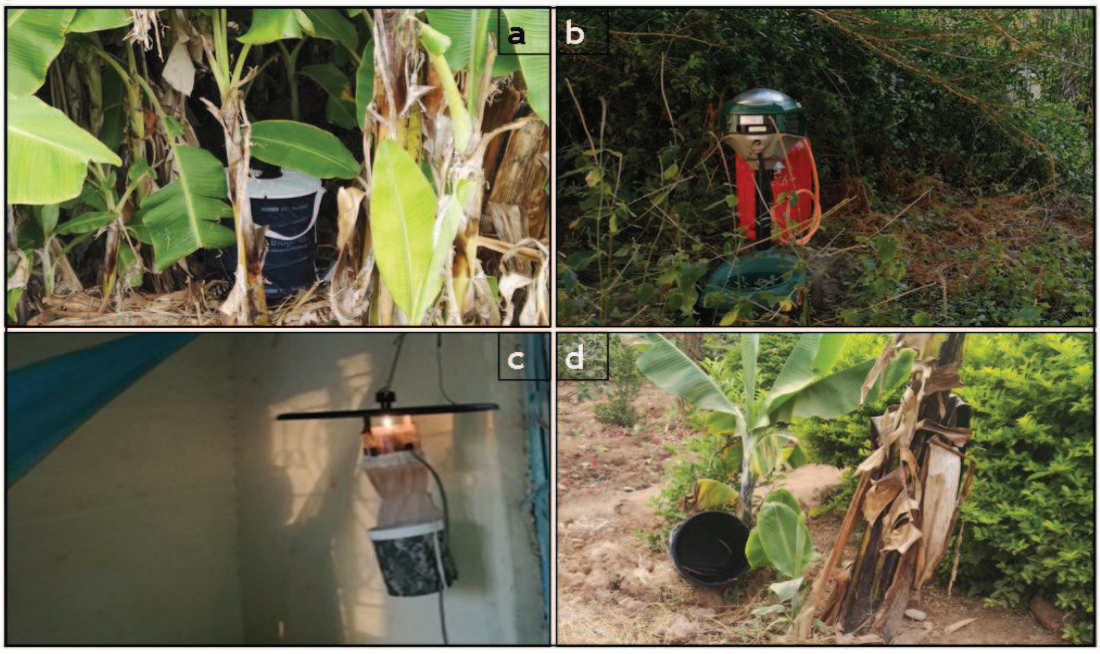

Figure 2.3 Different types of mosquito traps that were used for mosquito collections. (a) BG Sentinel trap, (b) Mosquito Magnet trap, (c) CDC Light trap (d) Resting Bucket trap.

2.4.3.3 Mosquito trap setting

In each sub-village one day and one night of sampling was carried out near the identified mosquito breeding habitat sites, and one day (8 am - 6 pm) and night

(7pm - 6am) at each of the four selected households (2 households per day and night trapping). Except for the light traps that were set to collect mosquitoes during night time, all other traps were set for day and night catches including one Mosquito Magnet trap, one BG Sentinel trap and 10 Resting Buckets were placed in the outdoor environment of each household.

Light traps were suspended at approximately 1.5 m above the floor on the foot side of the bed where a person is protected by mosquito net (Bhalala and Arias, 2009, Davis et al., 1995), or well above the reach of the animals in an animal room. No light trap was used outside, instead other outdoor traps were used. The Mosquito Magnet traps were placed outside in a shaded place within the household compound but about 5m away from the houses or livestock shelter/enclosure. BG-Sentinel traps were placed in shaded or partially shaded locations (where applicable) under vegetation outside (Cilek and Hallmon, 2005, Hiwat et al., 2011) in the household compound or the selected breeding site. In order to avoid interference among the traps (BG Sentinel and Mosquito Magnets) they were placed in different locations (15 m distance apart) of the household compound as suggested by (Brown et al., 2014) depending on the size and orientation of the household compound.

When setting the Resting Bucket traps, the inside of traps was sprinkled with water to make the lining cloth wet and increase humidity. Traps were placed haphazardly within a 5 m range of the house or animal enclosure/shelter in relatively shady areas, ideally next to or under vegetation (Bidlingmayer, 1971, Burkett-Cadena et al., 2008). Bucket traps were placed on their sides with the opening facing the nearest structure, a house or livestock enclosure/shelter. Mosquitoes resting inside the resting buckets were collected using a CDC backpack aspirator by aspirating for 10–20 seconds at the open end of the bucket. Mosquito catches from all traps were collected between 6:00 am and 7:00 am in the morning, and between 6:00 pm and 7:00 pm in the evening.

2.4.3.4 Mosquito sorting

Mosquitoes from all trapping methods were anesthetized by asphyxiation with chloroform before sorting and identification. All mosquitoes caught were morphologically identified to genera or species level using morphological identification keys (Gillies and Coetzee, 1987, Huang, 2001, Jupp, 1996). Using a magnifying hand lens (X5) and forceps, mosquitoes from each trap were sorted according to genera, sex, trap type, site and date of collection, in pools of 1-25

mosquitoes and preserved in labelled 2 ml cryo vials containing Trizol reagent (Figure 2.4). Pools of mosquitoes were kept in a car fridge initially for 4-5 days and then transported to the laboratory at Kilimanjaro Clinical Research Institute (KCRI) for storage in the -80^{0}C freezer for further RNA extraction at a later stage.

Figure 2.4 Plate showing mosquito sorted from other insects using magnifying hand lens and forceps, morphologically identified and stored in labeled cryovials.

2.4.3.5 Environmental data

In addition to mosquito collections, data on vegetation cover of the area was essential as a potential determinant of mosquito abundance and distribution. Data on normalized difference vegetation index (NDVI) which quantifies vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs) was obtained from https://earthexplorer.usgs.gov/. NDVI was MOD12A3 data for each month with a spatial resolution of 0.05 decimal degrees by 0.05 decimal degrees. NDVI ranges from -1 to +1. Negative values indicates that it's highly likely that it's water and NDVI value close to +1 indicates that there's a high possibility that it's dense green vegetation. To provide control for potential differences in rainfall between sampling days, the percentage difference in NDVI at the household between the month of the visit to the household and NDVI for that household over the whole study period (i.e. between 2015 and 2017) was calculated. A positive percentage difference could be expected to reflect a "greener" than average time of year and

to therefore broadly represent of a wet period. A negative percentage difference would reflect a "browner" than average time of year, and therefore broadly represent a dry period. It could be expected that more mosquitoes would be trapped during a wetter (greener) time of year than during a dryer (browner) time of year.

2.5 Laboratory analyses

2.5.1 Serological detection of RVFV antibodies in livestock and human sera

Considerable challenges remain in the reliable detection of RVFV antibodies, particularly in relation to the ability of tests to distinguish exposure to RVFV from other closely related phleboviruses that may be circulating in the East African region. In this study, livestock sera were analysed for presence of anti-RVFV antibodies (IgG/IgM) using two enzyme linked immuno-sorbent assays (ELISAs), with results from a subset of samples compared using the virus neutralization test (VNT). A recombinant nucleocapsid-based in-house ELISA developed at the University of Glasgow was first used as screening test, with further tests carried out using a commercial ID Screen® Rift Valley fever multi-species competitive ELISA (ID.Vet Innovative Diagnostics, Grabels, France), and an in-house VNT. Due to limited number of commercial ELISA kits, all in-house ELISA positive (goat and sheep) sera and 10% of randomly selected negative sera samples were repeated by commercial ID Screen® Rift Valley fever multi-species competitive ELISA. All cattle sera samples were tested by the commercial cELISA.

To test for any recent infections in livestock based on anti-RVFV immunoglobulin M (IgM), a sub-set (14% dictated by the number of test kits available) of positive and negative (goat and sheep) sera samples were randomly selected and tested by commercial ID Screen® IgM capture ELISA as described by the manufacturer (ID.Vet Innovative Diagnostics, Grabels, France) as IgM antibodies to RVFV are produced first before IgG and can only be detected up to two months after infection (Morvan et al., 1991, Paweska et al., 2003a). All human sera samples were tested by commercial ID Screen® Rift Valley fever multi-species competitive ELISA. ELISA results were validated by virus neutralization test (VNT) of a subset of livestock and human randomly selected positive and negative samples.

2.5.1.1 In-house ELISA

The assay used a recombinant nucleocapsid-based in-house ELISA developed at the University of Glasgow. Briefly, 6His-tagged recombinant N protein was expressed in E. coli and purified using nickel-affinity chromatography. The RVFV recN protein was used as an antigen coated on the plates to detect presence of anti-RVFV antibodies in sera samples. The protein (RVFV recN) was genorously prepared by Dr. Ping Li, University of Glasgow.

2.5.1.1.1 Preparation of reagents and working dilutions

PBS (Phosphate Buffered Saline) 0.001M, pH 7.4 was prepared by dissolving 1 sachet of PBS powder in 1L of distilled water. Wash buffer (PBS Tween 0.1%) was prepared by dissolving 1 ml of Tween 20 in 1 L PBS. Marvel powdered skimmed milk (Premier Foods, Thame, UK) was used for preparation of diluent and blocking buffers. Diluent buffer was prepared by adding 2% of skimmed milk in PBS (2 g milk in 100 ml PBS), where as blocking buffer was prepared by adding 10% of skimmed milk in PBS (10 g milk in 100 ml PBS). Carbonate bicarbonate buffer (for protein dilutions) was prepared by adding 8.4 g sodium bicarbonate ($NaHCO_3$) and 3.56 g anhydrous sodium carbonate (Na_2CO_3) in 1L distilled water. Proteins were diluted in carbonate bicarbonate buffer to 500ng/ml. RVF-N protein (12.5µl) was diluted in 10 ml carbonate bicarbonate buffer.

2.5.1.1.2 Assay Procedure

Plates were coated by adding 100 µl (50µg) diluted RVFV-N protein to each well of the ELISA plates, covered by plate sealers and incubated at 4^0C overnight. The next day protein was removed and the plate washed 3 times with wash buffer. Two hundred microliters of blocking buffer (10% skimmed milk in PBS) was added to each well, covered and incubated for 1 hour at room temperature. During the blocking stage, control and test sera were diluted to 1:400 in diluent buffer. After incubation, blocking buffer was removed and plates washed 3 times with wash buffer. Then 100µl diluted positive control sera was added to wells A1-2 (in duplicate), negative control sera to wells B1-2 of the plate, followed by 100µl of each of the test sera added to the remaining wells in duplicate, covered and incubated at room temperature for 2 hours. After washing plates 3 times with wash buffer, 100µl of horseradish peroxidase (HRP)-conjugated anti-species (cattle, goat or sheep) antibody (Thermo Fisher Scientific, Massachusetts, USA) diluted to

1:1000, was added to each well, covered and incubated at room temperature for 1 hour. After incubation plates were washed 5 times with wash buffer and then 100µl of TMB (3,3',5,5'-Tetramethylbenzidine) was added to each well and the plates kept in the dark for 15 minutes to allow colour change. Plates were read on a Multiskan Ascent ELISA plate reader (MTX Lab Systems, Florida, USA) at 650nm. Arrangement of controls and test sera on a plate is shown in Figure 2.5.

	1	2	3	4	5	6	7	8	9	10	11	12
A	C+	C+	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
B	C-	C-	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
C	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
D	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
E	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
F	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
G	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45
H	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	S46	S46

Figure 2.5 In-house plate layout showing the arrangement of control and test samples in plate wells. C+ = positive control, C- = negative control, S1-S46 = test samples.

2.5.1.1.3 Interpretation

A specific activity of each test serum was calculated by subtracting the background activity in the wells with the negative control from the specific activity in wells with positive control. The raw absorbance (OD) values were expressed as a percentage positive (PP) of the positive control, using the following formula: (Mean OD of test samples/Mean OD of positive control - mean OD of negative control) x 100. A percent positive (PP) value of ≥30% was considered to be positive.

2.5.1.2 ID Screen® Rift Valley Fever Competition Multi-species ELISA

A commercial, indirect competition ELISA (cELISA) kit (ID Screen Rift Valley fever multi-species ELISA; ID.Vet Innovative Diagnostics, Grabels, France) was used according to the manufacturer's instructions for detecting RVFV specific

antibodies. Given that it is reported to have a validated high diagnostic sensitivity and specificity (Comtet et al., 2010, Kortekaas et al., 2013), the assay was used as reference for evaluating the In-house ELISA.

2.5.1.2.1 Preparation of reagents and working dilutions

The ID.Vet kits contained ready to use reagents which were diluted to the recommended concentration. Wash Buffer (1X) was prepared by adding 50 ml of Wash Concentrate (20X) to 950 ml distilled water. Anti-nucleoprotein-horseradish peroxidase (Anti-RVF-NP-HRP Conjugate 1X) was prepared by adding 1 ml of Anti-RVF-NP-Po conjugate (10X) to 9 ml Dilution Buffer 19. Other reagents that were ready to use without dilution include Dilution Buffer 19, Substrate Solution, Stop Solution and Control sera (Positive and Negative Control sera).

2.5.1.2.2 Assay procedure

Sera samples were diluted in dilution plates, where 50 µl of dilution buffer was added to each well of the dilution plates, followed by 50 µl of either the control sera or the test sera. All samples were analysed in duplicate. Next, 100µl per well of the diluted samples were transferred to pre-coated test plates using a multichannel pipette. The plates were incubated for 1 hour at 37^0C and then washed three times with 300 µl per well of washing buffer. The anti-RVF-NP-HRP conjugate was diluted in dilution buffer and 100 µl added per well. The plates were then incubated for 30 min at room temperature and washed as before, after which 100 µl of ready-to-use TMB substrate (ID.Vet Innovative Diagnostics) was added to each well and incubated in the dark for 15 min. Then, 100 µl of stop solution was added per well and the absorbance (OD) was read at 450 nm.

2.5.1.2.3 Interpretation

The results were calculated as percentage inhibition (competition), using the following formula: Suspect or negative (S/N) = (OD Sample/OD Negative control) x 100. A suspect or negative (S/N) value of ≤ 40% was considered to be positive, otherwise negative.

Comparing the two ELISA assays (Figure 2.6), a sample was confirmed positive if it was tested positive by both tests (In-house and cELISA) or tested positive by the commercial cELISA only.

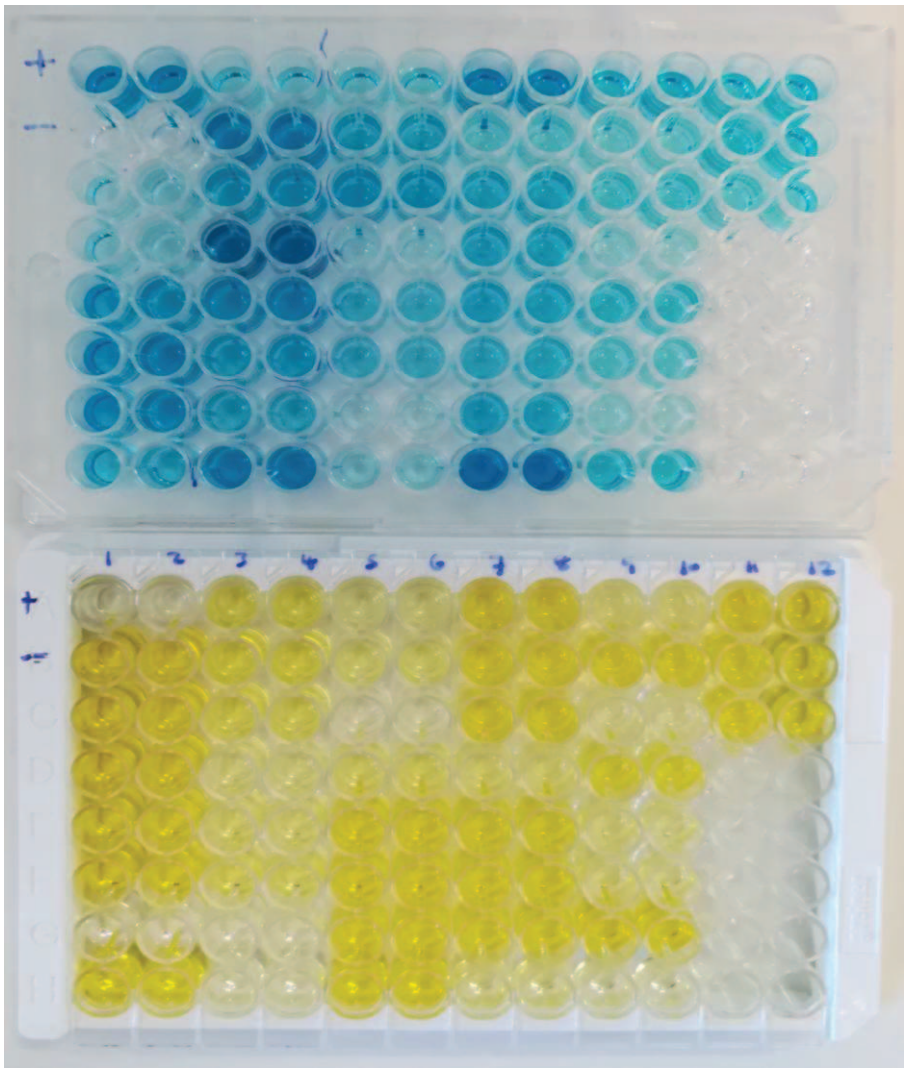

Figure 2.6 ELISA plates showing comparison of two assays, In-house ELISA (blue) and ID Screen Multi-species Competitive ELISA (yellow). Deep blue color on the top plate indicates specific antibody activity and no color or faint color indicates background activity. On the bottom plate no color or faint color indicates specific antibody activity whereas yellow color indicates background activity.

2.5.1.3 ID Screen® Rift Valley Fever IgM Capture

Immunoglobulin M (IgM) appears early in infection (5-7 days), rises rapidly in the disease course and can be detected within 6-8 weeks after infection (Morvan et al., 1991), and is usually less virus cross-reactive than IgG (Martin et al., 2000). IgM Capture ELISA is an important assay for detection of recent viral infections. A commercial IgM Antibody Capture ELISA (MAC-ELISA) designed specifically to detect IgM antibody was used in this study to detect IgM antibodies directed against the Rift Valley Fever (RVF) nucleoprotein (N) in bovine, ovine and caprine serum or plasma. Antibody capture ELISAs use two types of antibody specific for different epitopes of the antigen molecule. The primary antibody (known as capture

antibody) is coated to the wells followed by adding the sample solution. Then a secondary enzyme linked antibody (known as detection antibody) added to detect the presence of the captured antigens in the sample.

2.5.1.3.1 Preparation of reagents and working dilutions

Wash Solution(1X) was prepared the by diluting the Wash Concentrate(20X) in distilled water (50 µl Wash Concentrate 20X added to 950 µl distilled water). RVF Nucleoprotein 10X was prepared by adding 1ml of Reconstitution Buffer to the Freeze-dried RVF Nucleoprotein vial and mixed well to homogenize the solution. The reconstituted RVF Nucleoprotein (10X) was then diluted to 1X concentration by adding 1 ml RVF Nucleoprotein to 9 ml Dilution Buffer 13. Anti-nucleoprotein-horseradish peroxidase (Anti-RVF-NP-HRP Conjugate 1X) was prepared by adding 1 ml of Anti-RVF-NP-Po conjugate (10X) to 9 ml Dilution Buffer 11. Other ready to use reagents that were supplied in the kit include Reconstitution Buffer, Dilution Buffer 11, Dilution Buffer 13, Dilution Buffer 18, Substrate Solution (TMB), Stop Solution (0.5 M) and Control sera (Positive and Negative Controls).

2.5.1.3.2 Assay Procedure

All reagents were mixed by inversion or vortexing. Plates were already pre-coated with anti-bovine-ovine-caprine IgM polyclonal antibodies. Samples were deposited in duplicate in adjacent even and odd-numbered wells (Figure 2.7). Forty microlitres of Dilution Buffer 18 was added to each well. Thereafter, 10 µl of the Negative Control was added to wells A1, B1 and A2, B2. Next, 10 µl of the Positive Control was added to wells C1, D1 and C2, D2. After that 10 µl of each sample to be tested in duplicate was added to the remaining wells (each sample was deposited twice in adjacent even and odd numbered wells). The plate was then incubated for 1 hour at 37°C. After incubation, plates were washed three times with approximately 300 µl per well of the Wash Solution. Then 50 µl of the RVFV nucleoprotein 1X was added to the even-numbered plate columns only and 50 µl of Dilution Buffer 13 to the odd numbered columns and incubated for 1hour at 37°C. Next, each well was washed three times with approximately 300 µl of the Wash Solution. Then 50 µl of the Conjugate 1X was added to each well, incubated for one hour at 37°C, after which each well was washed three times with approximately 300 µl of the Wash Solution. Then 100 µl of the Substrate Solution was added to each well, incubated for 15 min at room temperature (21°C ± 5°C) in the dark to allow coloration, after which 100 µl of the Stop Solution was added to each well in

order to stop the reaction. The absorbance was read by a plate reader and recorded the OD at 450 nm.

	D13	RVFV-NP	D13	RVFV-NP	D13	RVFV-NP	D13	RVFV-NP	D13	RVFV-NP	D13	RVFV-NP
A	C-	C-	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	C-	C-	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	C+	C+	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	C+	C+	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

Figure 2.7 Plate layout showing how controls, test samples and RVFV-nucleoprotein were added to plate wells. C+ = positive control, C- = negative control and S1-S44 = test samples

2.5.1.3.3 Interpretation

Results were determined by the net OD using the following formula:

Net OD = OD even well – OD odd well.

The test was validated if: the mean value of the net Positive Control OD (net OD PC) > 0.350 or the ratio of the mean values of the net Positive and Negative Control ODs (net ODPC and net OD NC) > 3. Results were interpreted by the S/P percentage (S/P %): S/P % = net OD sample/net OD PC x 100. Samples presenting a S/P percentage (S/P %) less than or equal to 40% were considered negative, between 40% and 50% were considered as intermediate and greater than or equal to 50% were considered positive.

2.5.1.4 Virus Neutralization Test

Although ELISAs are convenient diagnostic tools, the virus neutralization test (VNT) is regarded as superior and is therefore considered the gold standard serological

assay due to its high specificity (Schreur et al., 2017). It is a specialized type of immunoassay because it does not detect all antigen–antibody reactions. It only detects antibodies that can block (neutralize) virus replication, which is important because closely related groups of viruses may share common antigens, but only a fraction of these antigens are targets of neutralizing antibody. In this assay a recombinant live attenuated MP-12 strain of RVFV (rMP-12) was used. The MP-12 attenuated vaccine strain is the only RVFV strain used outside high containment laboratories and can be handled at BSL-2 in USA facilities (Lokugamage et al., 2012) and CL-3 in the UK. Strain MP-12 is different from its parental pathogenic RVFV strain, strain ZH548, because of the presence of 23 mutations (Ikegami et al., 2015). The neutralisation assay presented here was performed by Dr. Agnieszka Szemiel (Centre for Virus Research, University of Glasgow, Glasgow, UK)

2.5.1.4.1 Preparation of reagents and dilutions

The overlay was prepared by mixing 1.2% Avicel solution with 2XMEM (minimum essential medium, Gibco) containing 4% FCS (foetal calf serum) v/v) in 1:1 ratio. 2X MEM was made by combining 20% (v/v) 10X Modified Eagle's Medium (MEM) (Gibco), 2% (v/v) L-glutamine, 0.435% (v/v) NaHCO3, diluted in distilled water. Crystal Violet stain was prepared by mixing 10 ml Methanol and 200 ml Ethanol absolute and 1 g Methyl Violet (Crystal Violet), mixed well by shaking to dissolve the methyl violet. Then added 100 ml Formaldehyde solution (41%) and added distilled water up to 1L.

2.5.1.4.2 Assay procedure

The Virus Neutralisation Test (VNT) was performed using Vero E6 cells,seeded a day prior to infection in 12 well plates at a cell density of 1.5 x 10^5 cells per well. The next day, 4-fold dilutions (from 1/32 to 1/32768) of the sera samples were prepared in a 96 well plate in DMEM (Dulbecco's modified eagle medium, Gibco) supplemented with 2% FCS (150ul per well). Then 150ul of DMEM containing 100 pfu of rMP-12 virus was added to diluted sera into each well and plates were incubated at 37^0C for 1h. Next the media was removed from the cells. Thereafter, 200ul of each mixture of serum and rMP-12 virus was added to infect confluent monolayers of Vero E6 cells in 12-well plates. After 1h incubation at 37^0C the supernatant was removed, then 1ml overlay (0.6% Avicel, 2x MEM, 2% FCS) was added per well, incubated at 37^0C for 4 days, after which the cells were fixed with 1ml of 8% formaldehyde in PBS (v/v) per well and incubated for at least 1h at room

temperature. Formaldehyde and avicel overlays were removed and plates washed in water, then stained with crystal violet stain followed by washing the plates with water and left to dry. Then the plaques were counted.

2.5.1.4.3 Interpretation

Counts from replicate wells were averaged, and the average was multiplied by the dilution factor of the inoculum, which produced that number, and the volume of inoculum plated to calculate the plaque forming units (PFU) per mL of the original stock virus preparation. Results were calculated using this formula: the average number of plaques in replicate wells × dilution factor ÷ virus inoculum volume (in mL) = titer in PFU/mL.

2.5.2 Molecular detection of Rift Valley fever virus in Mosquitoes, aborted materials and milk

Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used as previously described (Drosten et al., 2002) when the starting material is RNA. In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase. The cDNA is then used as the template for the qPCR reaction.

2.5.2.1 RNA Purification from mosquito samples

Mosquito samples collected in the field were preserved in Trizol in pools (each pool containing 1-25 mosquitoes). Samples stored in cryovials were transferred into Lysing Matrix, impact-resistant tubes containing 1.4 mm ceramic beads (MP Biomedicals, CA, USA) The samples were disrupted by bead beating at 10,000 x g for 1min and homogenised by centrifugation. Transferred the supernatant into labelled RNase-free tubes. Then 300 µl of ethanol (100%) was added to each sample lysed in a previous step and mixed thoroughly. Next, the mixture was transferred into a Zymo-Spin™ IIICG Column2 in a Collection Tube (provided in the kit) and centrifuged at 12000 x g for 30 seconds. The column was then transferred to a new collection tube and the flow-through discarded. 400 µl of RNA Wash Buffer was added to the column and centrifuged for 30 seconds, followed by adding 80 µl DNase I, incubated at room temperature (20-30°C) for 15 minutes. The column was washed two times by adding 400 µl Direct-zol™ RNA PreWash to the column and centrifuged for 30 seconds and the flow-through discarded. Then 700 µl RNA Wash Buffer was added to the column and centrifuged for 2 minutes to ensure complete

removal of the wash buffer and the column carefully transferred into RNase-free tube. RNA was eluted by adding 100 µl of DNase/RNase-Free Water directly to the column matrix and centrifuged for 30 seconds. RNA quantity (concentration) was measured by NanoDrop™ 2000 Spectrophotometer (Thermo Scientific) and RNA was stored frozen at -80°C.

2.5.2.2 RNA preparation from swabs, placenta tissue and milk using RNeasy® Mini Kit

The RNA preparation process described here is based on the Qiagen RNeasy Mini kit with slight modification. Four volumes of 100% ethanol were added to Buffer RPE for a working solution, then RNA shield stabilized tissue/swabs were removed from the reagent using forceps. Next, 30mg of each tissue sample was added to sterile 2ml micro centrifuge tubes with glass beads and 600µl RLT buffer (OR 200µl of milk sample into 600µl RLT buffer). The tissue samples were disrupted using a bead-beater at 10000 x g for 1 minute. Milk samples were homogenised directly without bead beating. Next, the supernatant was pipetted to new micro centrifuge tubes, then 590 µl RNase free water and 10 µl Proteinase K were added and incubated at 55^0 C for 10 minutes. After cooling to room temperature, the samples were centrifuged at 10000 x g for 3 minutes then supernatant transferred to new Eppendorf tubes, 0.5 volumes of 96-100% ethanol to the lysate (if 700 µl were transferred, then 350 µl ethanol were added and mix well by pipetting. The 700µl of lysate and ethanol mixture were then transferred to spin column, centrifuged for 3 min at maximum speed and then the supernatant was removed by pipetting. One volume of 70% ethanol was added to the lysate, and mixed well by pipetting, then 700 µl of the sample, including any precipitate were transferred to an RNeasy Mini spin column 16 placed in a 2 ml collection tube, lid closed and centrifuged for 15 s at ≥8000 x g and discarded the flow-through. Next, 700 µl Buffer RW1 was added to the RNeasy spin column and centrifuged for 15 s at ≥8000 x g, the flow-through discarded followed by adding 500 µl Buffer RPE to the RNeasy spin column and centrifuged for 15 s at ≥8000 x g discarded the flow through and added 500 µl of Buffer RPE to the RNeasy spin column, and centrifuged for 2 min at ≥8000 x g.

Then the RNeasy spin column was placed in a new 1.5 ml collection tube and 50 µl RNase-free water was added directly to the spin column membrane, the lid was then closed and the tube centrifuged for 1 min at ≥8000 x g to elute the RNA.

2.5.2.3 Positive and negative control RNA

Positive and negative control RNA were generously offered by Dr. Agnieszka Szemiel (Centre for Virus Research, University of Glasgow, Glasgow, UK). The MP-12 viral RNA and mosquito total RNA from clean uninfected *Aedes* mosquitoes from the Centre for Virus Research, University of Glasgow, were used as positive and negative controls respectively. These RNA samples were extracted by the Trizol method as described previously (Rio et al., 2010). Briefly, RVFV (MP-12) virus-infected cell cultures were centrifuged at 3000 x g for 15 min and RNA was extracted from 100 µl aliquots of the supernatant by the Trizol method (Gibco BRL, Gaithersburg, MD). Mosquitoes were homogenized in 1 ml of Trizol reagent, and 100 µl of the homogenates were processed for RNA extraction by the Trizol method.

2.5.2.4 PCR Mastermix preparation

The PCR mastermix was prepared in a designated RNA/DNA-free room. Reagents were homogenised before preparing mastermix by brief pulse-vortexing and centrifuging. Master mix was prepared as indicated in Table 2.2.

Table 2.2 PCR Master Mix components, concentrations, and volumes used in the test

Component	Concentration	µl per reaction	Final concentration	Mix for 72 rxns (36 duplicate rxn) (µl)
QuantiNova™ Probe RT-PCR Master Mix	2 x	10 µl	1x	720 µl
QN Probe RT-Mix		0.2 µl	1x	14.4 µl
20x primer–probe mix 1		1 µl	0.8 µM forward primer 1 0.8 µM reverse primer 1 0.2 µM TaqMan probe 1	72 µl 72 µl 72 µl
RNase-free water		Variable	-	
RNA template		5 µl		

Component	Concentration	µl per reaction	Final concentration	Mix for 72 rxns (36 duplicate rxn) (µl)
Total reaction volume		20 µl	-	

To minimise risk of contaminating stock reagents, aliquots of appropriate volumes were made and working aliquots stored at ~3°C. Stock reagents were stored at -20°C until when further aliquots were required.

2.5.2.5 Real time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Detection of RVFV by one-step quantitative reverse-transcription real-time PCR (RT-qPCR) using RNA samples was carried out as previously described by Drosten et al., 2002. One-step assays combine reverse transcription and PCR in a single tube, using a reverse transcriptase along with a DNA polymerase and utilizing sequence-specific primers. The procedure detailed here is for the Qiagen Rotor-Gene Q/6000 qPCR platform and associated Rotor-Gene Q Series Software for analysis. Primers and dual-labelled probe for RVFV Gc gene were used for this study. Sequences are detailed in Table 2.3.

Table 2.3 Sequences of Primers and Probe used in the PCR reaction

Virus (GenBank accession no.)	Sense primer, antisense primer, probe (sequence [position])	Genomic target region	Amplicon length (bp)
RVFV (AF134508)	RVS (AAAGGAACAATGGACTCTGGTCA [349-371]) RVAs (CACTTCTTACTACCATGTCCTCCAAT [443-417]) RVP (FAM-AAAGCTTTGATATCTCTCAGTGCCCCAA-BHQ1 [388-416])	Gc gene	94

The reaction was set by adding 15µl of the master mix into each of the strip-tube wells using sterile filter pipette tips, while inside the laminar flow hood followed

by adding 5µl of sample RNA (template) in duplicate to each well to obtain a final reaction volume of 20µl. The negative extraction control (NTC) well, and then positive control (RVFV- MP-12 supernatant RNA) well were added last and all caps secured. A 72 well rotor disc was used for the runs in the Rotor-Gene machine and the cycling conditions involved reverse transcription at 45°C for 10 min, initial denaturation at 95°C for 5 min, and 40 cycles with 95°C for 5 s and 60°C for 30 s. Fluorescence was read at the combined annealing-extension step at 60°C. Curves produced were read in 'linear scale' and Ct values < 40 was considered positive.

2.6 Statistical Analysis

Details of specific statistical analyses for different study components are described in Chapters 3,4,5 and 6. Regression analysis was performed to explore determinants of mosquito abundance in each household, focusing on exploring differences between livestock and non-livestock keeping households. The response variable was the count (abundance) of mosquitoes in each genera collected per household. Type of household (livestock keeping or non-livestock keeping) and NDVI (relative green-ness) were included as fixed effects. Random effects were included at the household and village level in all models. Regression analysis was performed in R version 3.5.3 (R Core Team, 2018) using the *glmmTM*B package, and negative binomial regression models were used for these count data. A likelihood ratio test was used to assess the contribution of each fixed effect to model fit. Mosquito species distribution modelling was performed by Maximum Entropy (MaxEnt) algorithm (Phillips et. al., 2008) and QGIS (QGIS 2.14.0 -Essen, 2016) using mosquito occurrence data and environmental variables to predict habitat suitability and the spatial distribution of potential RVFV vectors as described previously (Elith et. al., 2011). All Maps were produced using QGIS (QGIS 2.14.0 -Essen, 2016).

In assessing the determinants of RVFV seropositivity in livestock and humans the binomial Generalised Linear Mixed Models (GLMMs) was used to allow modelling of random and fixed effects of the explanatory variables (Bolker et al., 2009). Village and households were considered as random effects, RVFV seropositivity was the response variable. In livestock (cattle, goats or sheep) fixed effects included sex, age, farming classification (small holder, agro-pastoral or pastoral), herd/flock size, animal introductions, seasonal movements/camps, history of abortions, presence/history of standing water/flood, history of animal deaths related to disease, animal management (confining cattle with small ruminants) and vector

habitat suitability derived from species distribution modelling detailed in Chapter three. Variables considered as fixed effects on seropositivity in human populations included occupation, sleeping in the same house as domestic ruminants, engaging in milking animals, birthing animals, handling placenta, handling aborted materials, handling animal carcasses, slaughtering animals, consuming raw meat or milk and seropositivity in livestock. Purposeful selection was used in variable and model selection as previously described (Hosmer Jr et al., 2013).

Chapter Three

3 The ecology of RVFV mosquito vectors in northern Tanzania

3.1 Introduction

Rift Valley fever virus was first discovered and characterised in the Rift Valley of Kenya in 1931 (Daubney et al., 1931), but outbreaks probably occurred before this time (Gerdes, 2004). The virus is transmitted through mosquito bites or by exposure to infectious blood and bodily fluids (Balkhy and Memish, 2003). This arthropod-borne infection affects a wide range of vertebrates, but clinical disease is limited to domestic ruminants and humans (Gerdes, 2004).

The virus can be transmitted by mosquitoes of at least six genera, including *Aedes*, *Culex*, *Anopheles, Eretmapodites, Mansonia*, and *Coquillettidia* (Bird et al., 2009) with over 30 different species shown to be competent vectors (Turell et al., 2008). Earlier studies suggest that RVFV is transmitted transovarially from females to eggs in some mosquito species of the *Aedes* genera (Logan et al., 1991, Gerdes, 2004). *Aedes spp* emerge in huge numbers from flooded depressions in soil and other habitats where oviposition has occurred. These eggs can survive desiccation for many years between flooding periods and re-emerge as infected adult mosquitoes to cause major epidemics during periods of extreme rainfall (Sang et al., 2010, Davies et al., 1985).

While the emergence of *Aedes* has been widely reported to initiate RVF outbreaks, virus amplification typically also involves secondary vectors such as *Culex*. So far, all evidence implicating mosquito vectors in RVFV transmission comes from periods of epidemics following heavy rainfall and flooding. The role of vectors in maintenance of RVFV between epidemics is not well understood largely due to lack of evidence of detection and/or isolation of the virus from mosquitoes during the inter-epidemic period (IEP) (Lichoti et al., 2014a). Examining the ecology and role of vectors in inter-epidemic transmission is crucial for understanding RVFV epidemiology and control.

In order to further understand the role of mosquitoes in inter-epidemic RVFV circulation, the study sought to: (1) assess the abundance, and diversity of mosquito vectors during an inter-epidemic period in northern Tanzania, (2) assess the role of household livestock ownership on the abundance of potential RVF

vectors, and (3) use environmental characteristics to predict mosquito vector distribution.

3.2 Methods

This section briefly describes the methods used for purposes of this chapter. Further details are provided in the core methods chapter (Chapter Two).

3.2.1 Study Area

The study was carried out in Arusha and Manyara Regions of northern Tanzania (Figure 3.1) where mosquitoes were sampled in November 2015, between March and September 2016, and between July and October 2017. Sampling included 12 of the 20 SEEDZ villages selected in districts across northern Tanzania, as described in Chapter Two.

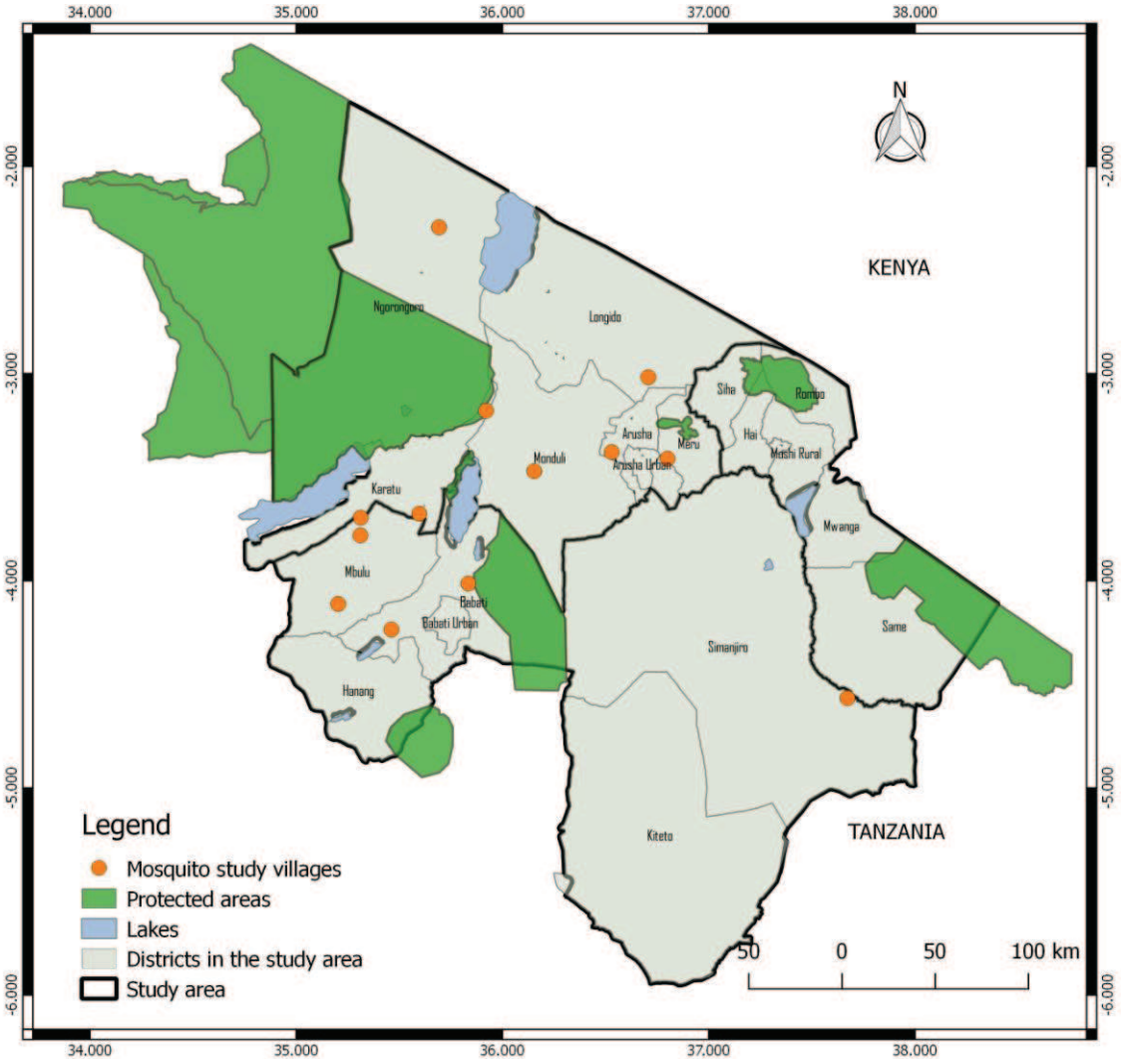

Figure 3.1 Map of northern Tanzania showing districts and locations of study villages (orange circles) where mosquitoes were collected. Map created in QGIS 2.14.0 -Essen, 2016 using shape files for country administrative boundaries

available at www.diva-gis.org and geo coordinates for study sites recorded during the study.

3.2.2 Mosquito Sampling

3.2.2.1 Selection of sampling sites

Mosquitoes were sampled from households with and without livestock in both agro-pastoral and pastoral settings in the study area. Livestock households were randomly selected from a list of households selected for animal sampling by the SEEDZ study within a particular sub-village. Non-livestock keeping households were randomly selected from a list of all non-livestock keeping households in the sub-village generated by the village and sub-village authorities. In addition to households, potential mosquito breeding habitats were also purposefully identified for mosquito trapping. Permission from community leaders and informed consent were sought from each head of household where mosquito trapping was carried out. Geographic co-ordinates of each selected household or mosquito trapping site were captured using a Garmin eTrex® 10 Handheld GPS.

3.2.2.2 Mosquito collection

In each sub-village, one day (8 am - 6 pm) and one night (7pm - 6am) of sampling was carried out at each of the selected households or near the identified mosquito breeding habitat sites. Four mosquito trapping methods were used for mosquito collection: (1) BG Sentinel trap (BG) (Biogents AG, Regensburg, Germany) to target outdoor host seeking mosquitoes; (2) Mosquito Magnet traps (MM) (Woodstream Corporation, Pennsylvania, USA) to target outdoor host seeking mosquitoes; (3) CDC Light traps (LT) (John W. Hock Company, Florida, USA) to trap indoor host seeking mosquitoes; and (4) locally made Resting Bucket traps (RB) to sample outdoor resting mosquitoes as previously described (Kreppel et al., 2015). All mosquitoes collected were morphologically identified to genera level using morphological identification keys (Gillies and Coetzee, 1987, Huang, 2001, Jupp, 1996).

3.2.2.3 Environmental data

Household co-ordinates were used to extract data on the normalized difference vegetation index (NDVI) from satellite imagery of the area surrounding the household at the time of the sampling visit. The NDVI quantifies the amount of

vegetation cover by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs). NDVI data (MOD12A3) were obtained from https://earthexplorer.usgs.gov/ for each month between 2015 and 2017. These data have a spatial resolution of 0.05 decimal degrees by 0.05 decimal degrees (around 900 x 900 metres in the study area). Household-level NDVI values were extracted from rasters covering the study area in R statistical software version 3.5.3 (R Core Team, 2018) using the *raster* package.

3.2.3 Data analysis

3.2.3.1 Determinants of mosquito vectors abundance

Regression analysis was performed to explore determinants of mosquito abundance in each household, with a particular focus on exploring differences between livestock and non-livestock keeping households. Here the response variable was the count (abundance) of mosquitoes in each genera collected. Mosquitoes of a particular genus collected from different trapping methods were pooled per household. Type of household (livestock keeping or non-livestock keeping) were included as fixed effects. Percentage difference in NDVI was also included as a fixed effect to control for the relative "green-ness" of the household environment, and therefore the expected levels of rainfall preceding the study visit. For this, the percentage difference in NDVI at the household was estimated by comparing the NDVI for the month of the visit with the average NDVI value at that household over all study years (i.e. between January 2015 and December 2017). A positive percentage difference could be expected to reflect a "greener" than average time of year and therefore represent a sampling visit during a relatively wet period. A negative percentage difference would reflect a "browner" than average time of year, and therefore represent a sampling visit during a relatively dry period.

Regression analysis was performed in R using the *glmmTM*B package (Brooks et al., 2017). Poisson and negative binomial regression models were used for these count data. Performance of the latter compared to the former was assessed using a likelihood ratio test. Random effects were included at the household and village level in all models. A likelihood ratio test was used to assess the contribution of each fixed effect to model fit and the output from the full model (i.e. with all fixed effects) is presented.

3.2.3.2 Mosquito vectors distribution

Prediction of mosquito vector distribution using environmental characteristics was performed on the basis the Maximum Entropy (MaxEnt) algorithm (Phillips et. al., 2008) in QGIS (QGIS 2.14.0 -Essen, 2016). There are a range of potential species distribution modelling methods (Elith et. al., 2011), with MaxEnt found to perform particularly well (Elith et al., 2011, Peterson, 2006, Huerta and Peterson, 2008). MaxEnt is a maximum entropy-based machine learning programme that estimates the probability distribution for a species' occurrence based on environmental constraints (Phillips et al., 2006). It requires only species presence data and environmental variable layers for the study area and generates an estimate of the suitability of a particular location for the occurrence of a species that varies from 0 to 1, with 0 being the lowest and 1 the highest suitability.

In this study, adult mosquito occurrence data were used to model the vector distribution. Occurrence records and/or locations (Figure 3.2) of potential RVFV vectors were obtained from the study described above and unpublished data from an additional study that was performed in the Serengeti ecosystem between 2012 and 2013 (Nyarobi et al.).

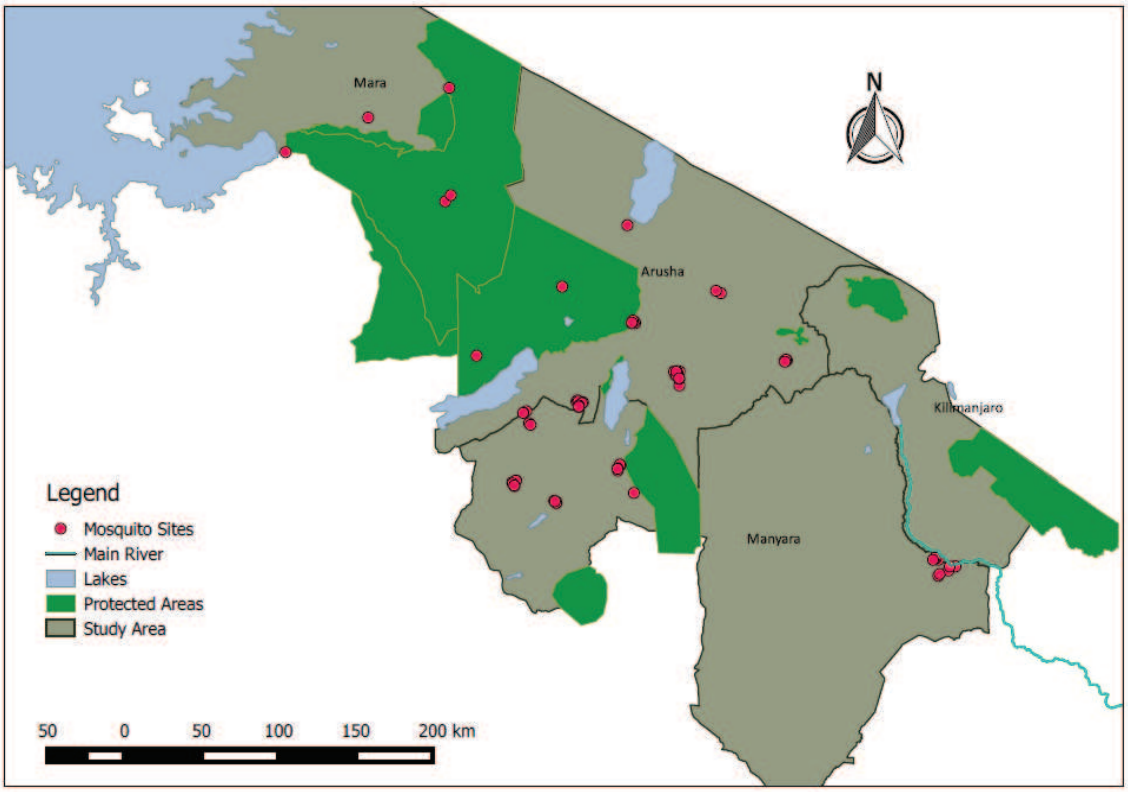

Figure 3.2 Map of northern Tanzania showing mosquito occurrence locations used in species distribution modeling. Map created in QGIS 2.14.0 -Essen, 2016 using shape

files for country administrative boundaries available at www.diva-gis.org and geo coordinates of the mosquito occurrence records.

Twenty-four environmental variables were considered as potential predictors of the RVFV vector species habitat distribution. These variables were chosen based on their biological relevance to mosquito species distributions (Phillips et al., 2006, Pearson et al., 2007). Nineteen bioclimatic variables related to temperature and rainfall generated from an interpolation of average monthly climate data from stations around the world (Hijmans et al., 2005) were obtained from WorldClim dataset (http://www.worldclim.org/bioclim.htm). Elevation (Digital Elevation Model (DEM)) data were also obtained from the WorldClim website (at 1 km spatial resolution), and soil layers (sand, clay and silt) obtained from the World Soil Information (https://www.isric.org/projects/soil-and-terrain-soter-database-programme). The variables included in the MaxEnt procedure were:

1) Bio1 = Annual Mean Temperature
2) Bio2 = Mean Diurnal Range (Mean of monthly (max temp – min temp))
3) Bio3 = Isothermality (P2/P7)*(100)
4) Bio4 =Temperature Seasonality (standard deviation*100)
5) Bio5 = Max Temperature of Warmest Month
6) Bio6 =Min Temperature of Coldest Month
7) Bio7 =Temperature Annual Range (P5-P6)
8) Bio8 =Mean Temperature of Wettest Quarter
9) Bio9 =Mean Temperature of Driest Quarter
10) Bio10 =Mean Temperature of Warmest Quarter
11) Bio11 =Mean Temperature of Coldest Quarter
12) Bio12 =Annual Precipitation
13) Bio13 =Precipitation of Wettest Month
14) Bio14 =Precipitation of Driest Month

15) Bio15 =Precipitation of Seasonality (Coefficient of Variation)

16) Bio16 =Precipitation of Wettest Quarter

17) Bio17 =Precipitation of Driest Quarter

18) Bio18 =Precipitation of Warmest Quarter

19) Bio19 =Precipitation of Coldest Quarter

20) Elevation

21) EVI - Enhanced vegetation index

22) Clay soil

23) Sand soil

24) Silt soil

All the 24 predictor variables were processed using QGIS (QGIS 2.14.0 -Essen, 2016). The processing steps included clipping raster data to the extent of the study area and re-sampling all the predictors layers to the same spatial resolution (1 km) and file types (TIF/ACS). All the variables were tested for multicollinearity by examining cross-correlations (Pearson correlation coefficient, r) based on individual mosquito genera occurrence records and the randomly generated background. Initially 11 temperature related variables were examined, followed by eight rainfall related variables. Collinearity between each pair of the eight precipitation predictor variable layers and pairs of eleven temperature layers (Figures 3:6 - 3:8) was assessed using Pearson correlation analyses in R. Selection of variables from a set of highly cross-correlated variables ($r > 0.75$) was based on the potential biological relevance of each of a correlated pair to the occurrence and distribution of the genera, and the ease of interpretation (Merow et al., 2013).

3.2.3.3 Modelling procedure

MaxEnt software (Version 3.4.1) was used (Phillips et al., 2017) http://biodiversityinformatics.amnh.org/open_source/maxent/. Distribution modelling focused on *Aedes* and *Culex* as important primary and secondary vectors of RVFV in the study area. The distribution of these mosquitoes was compared with the distribution of *Anopheles spp*. Each model was run with the selected

temperature and rainfall variables in addition to soil, elevation and vegetation variables. Since the sample size was moderately low for the vector genera, only linear and quadratic features were used and other settings were maintained as default (Phillips et al., 2004). The regularization multiplier was set to default 1 and number of replications set to 10 and a maximum of 5000 iterations at a convergence threshold of 0.00001, with cross validation replicate type in order to limit model over fitting. The output was set to a logistic format, so that the predictions of habitat suitability would assume probability scores between 0 and 1 (Elith et al., 2006, Huerta and Peterson, 2008, Phillips et al., 2006).

Due to the limited number of the vector occurrence localities, default settings were maintained without data partitioning to allow MaxEnt to employ data used to develop the model (also called training data) and to also test the model. The performance of the predictor represented by the average training gain over the 10 replicate runs was normalized to percentages. The higher the percentage contribution of the predictor, the greater the importance of that particular variable in predicting habitat suitability for RVFV vector genera occurrence (Warren et al., 2010). The jacknife approach was also used to assess the contribution of individual variables on the basis of regularised training gain (Phillips et al., 2017). For this, variables were removed one at a time, and a habitat suitability model created using all remaining variables. Each variable was also used on its own to create a habitat suitability model. The training gain reflects how well the resulting models fit the data. MaxEnt also generates the receiver operating curve (ROC) curve of the full model predicting suitability of a particular vector genera. The area under the curve (AUC) values allow comparison of performance of one MaxEnt model with another. An AUC value of 0.5 indicates that the performance of the model is no better than random, while values closer to 1.0 indicate an excellent model performance.

3.3 Results

3.3.1 RVFV vector species abundance and composition

A total of 2224 mosquitoes were sampled from 132 households in 12 villages (summarized in Table 3.1) representing five different genera including *Aedes*, *Anopheles*, *Culex*, *Coquillettidia* and *Mansonia*. The mosquitoes collected were morphologically identified as *Aedes spp*, *Culex spp*, *Mansonia spp*, *Anopheles spp* and *Coquillettidia spp*. Of the total mosquitoes collected, the majority were *Culex*

spp (50.5%), followed by *Anopheles spp* (45.0%), *Mansonia spp* (2.5%), *Aedes spp* (1.5%), and *Coquillettidia spp* (0.2%).

Table 3.1 Mosquito species collected across study villages in northern Tanzania

Village	*Aedes spp*	*Anopheles spp*	*Culex spp*	*Coquilletidia spp*	*Mansonia spp*	Total
Endanyawish	0	539	43	0	0	582
Engikareti	0	3	7	0	0	10
Ilkerin	3	7	82	0	0	92
Kansay	6	26	39	0	2	73
Long	0	66	44	0	0	110
Lositete	0	1	1	0	0	2
Maheri	2	5	6	0	0	13
Malambo	2	10	15	1	0	28
Naiti	4	17	34	4	33	92
Nambala	10	136	806	0	2	954
Ruvu-remiti	7	194	33	0	19	253
Sarame	0	2	13	0	0	15
Total	34	1006	1123	5	56	2224

Culex and *Anopheles* mosquitoes were recorded in all sampled villages and were the most abundant mosquitoes. The abundance of these mosquitoes was particularly high in three villages: Endanyawish, Nambala and Ruvu remiti. *Aedes spp* were relatively rarer and recorded in 7 out of 12 sampled villages. *Mansonia spp* were recorded in 4 villages, and *Coquillettidia spp* recorded in 2 villages only.

3.3.2 Predictors of mosquito abundance

Due to missing data, two villages (Engikareti and Lositete) were excluded from the regression analysis. Water points were also excluded. The number of households included in the regression was therefore 132. Regression analysis was also confined to *Culex spp* and *Anopheles spp* due to relatively low numbers of other genera (*Aedes, Coquillettidia and Mansonia*) collected. The data for both genera were over-dispersed and a negative binomial rather than Poisson regression was therefore used for analysis.

Although *Anopheles spp* abundance was higher (n=730) in livestock keeping households than in non-livestock households (n=233), the difference was not statically significant (p=0.142) in the negative binomial regression model (Table 3.2). The difference in *Culex* abundance between livestock households (n=438) and non-livestock households (n=644) was also not statistically significant (p=0.221). While regression analysis was not performed for *Aedes*, it is notable that of the 28 *Aedes* collected at households, most were from livestock keeping households (20 versus 8 at non-livestock households). The abundance of both *Culex and Anopheles spp* was positively and significantly associated with visiting households during a greener than average period (i.e. on the basis of % difference in NDVI) (p<0.001).

Table 3.2 Summary of the Generalized Mixed-effect Model showing factors associated with *Anopheles* and *Culex* abundance in northern Tanzania

Predictors	***Anopheles***			***Culex***		
	Estimates	**95% CI**	**P-value[1]**	**Estimates**	**95%CI**	**P-value[1]**
Type of household						
Livestock	Ref.			Ref.		
Non-livestock	0.65	0.31 - 1.41	0.275	0.71	0.38 - 1.31	0.268
% difference in NDVI	2.32	1.41 - 3.88	0.001	2.15	1.45 - 3.20	<0.001

[1] Estimated from likelihood ratio test.

3.3.3 RVF vector genera distribution modelling

Assessment of co linearity between each pair of the eight precipitation predictor variables (BI012-BIO19) and pairs of eleven temperature variables (BIO1-BIO11) (Figure 3.3, Figure 3.4 and Figure 3.5) demonstrated high levels of cross-correlation.

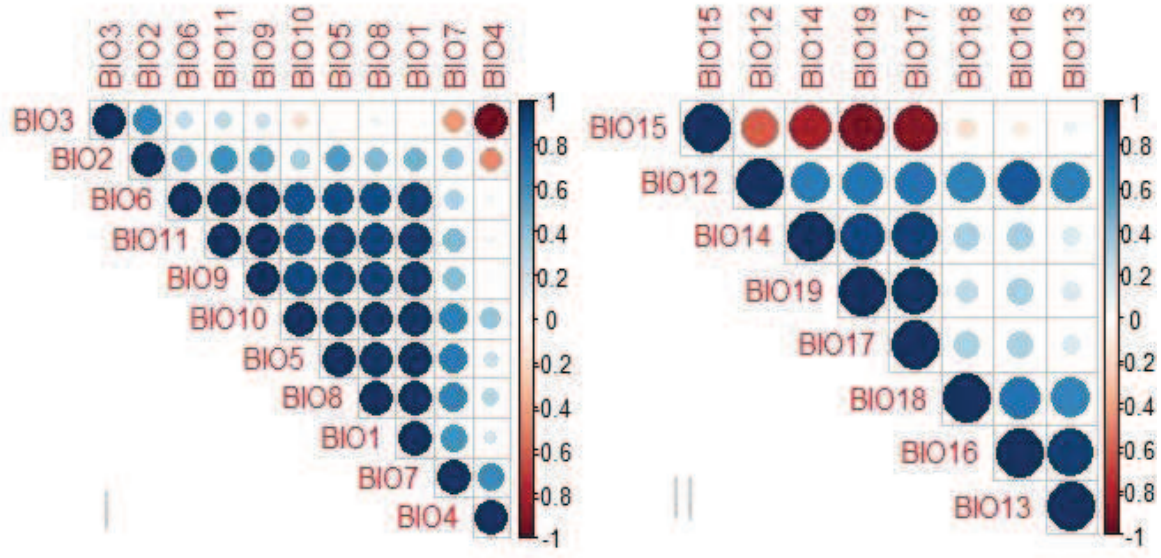

Figure 3.3 Correlation matrices of (I) temperature- and (II) precipitation-related bioclimatic variables related to *Aedes spp* occurrence data. Circle size vary with correlation between variables ranging from 0 to 1 or 0 to -1 indicated by blue-red colour scale.

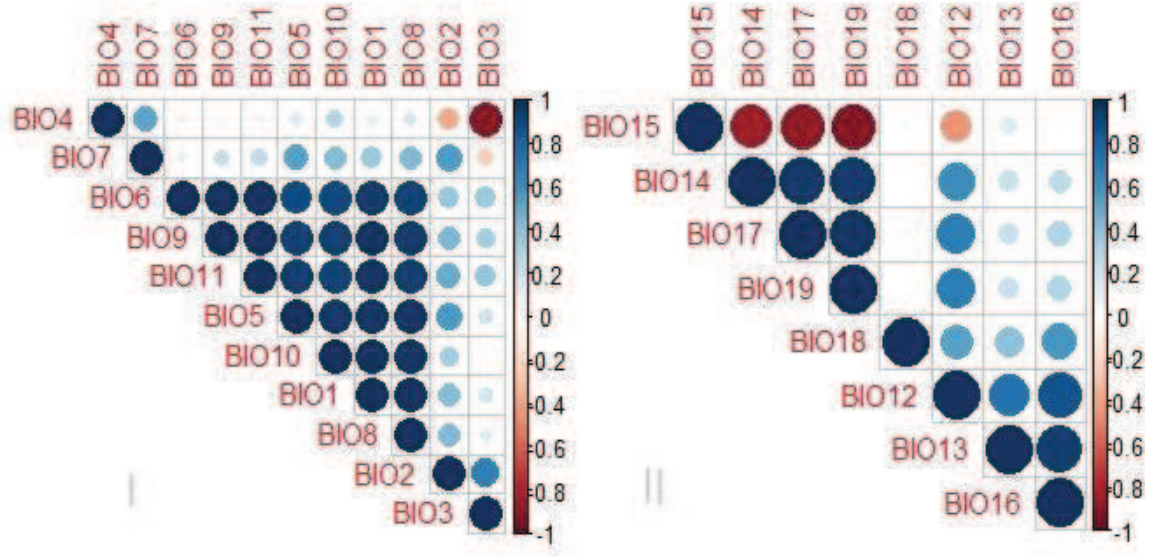

Figure 3.4 Correlation matrices of (I) temperature- and (II) precipitation-related bioclimatic variables related to *Culex spp* occurrence data. Circle size vary with correlation between variables ranging from 0 to 1 or 0 to -1 indicated by blue-red colour scale.

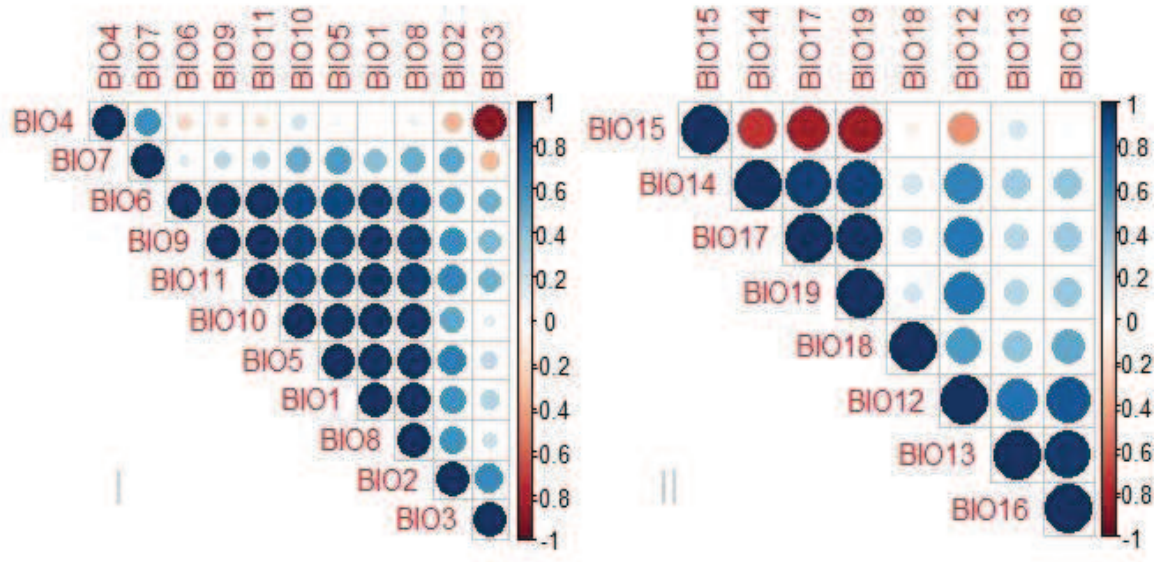

Figure 3.5 Correlation matrices of (I) temperature- and (II) precipitation-related bioclimatic variables related to *Anopheles spp* occurrence data. Circle size vary with correlation between variables ranging from 0 to 1 or 0 to -1 indicated by blue-red colour scale.

In modelling mosquito distribution, the included environmental variables had variable influence on the prediction of suitability of all the three vector species (Table 3.3**).** Precipitation of wettest month made the greatest contribution in predicting *Aedes spp* distribution, while elevation was most important in predicting *Culex spp* distribution. Enhanced vegetation index (EVI) had the greatest contribution to predicting the distribution of *Anopheles spp*.

Table 3.3 Environmental variables used in modeling species distribution and their percent predictive contribution of each variable as generated by MaxEnt.

Variable	Percent contribution
Aedes spp	
BIO13 = Precipitation of Wettest Month	28.8
BIO3 = Isothermality	23.1
Elevation	21.1
EVI = Enhanced Vegetation Index	17.6
Clay soils	9.5
Culex spp	
Elevation	26.1
BIO4 = Temperature seasonality	23
Clay soils	19.9
BIO18 = Precipitation of Warmest Quarter	18.7

Variable	Percent contribution
EVI = Enhanced Vegetation Index	12.4
Anopheles spp	
EVI = Enhanced Vegetation Index	53.8
Clay soils	25.7
BIO4 = Temperature seasonality	9.7
Elevation	6
BIO18 = Precipitation of Warmest Quarter	4.8

3.3.4 Jackknife test of regularized training gain for RVF vectors species habitat suitability

The results of the jackknife regularized training gain for *Aedes spp* indicated that the environmental variable with highest gain when used in isolation was precipitation of the wettest month (BIO13). Removal of isothermality (BIO3) resulted in the largest decrease in gain, suggesting this variable had the most information that wasn't present in the other variables (Figure 3.6).

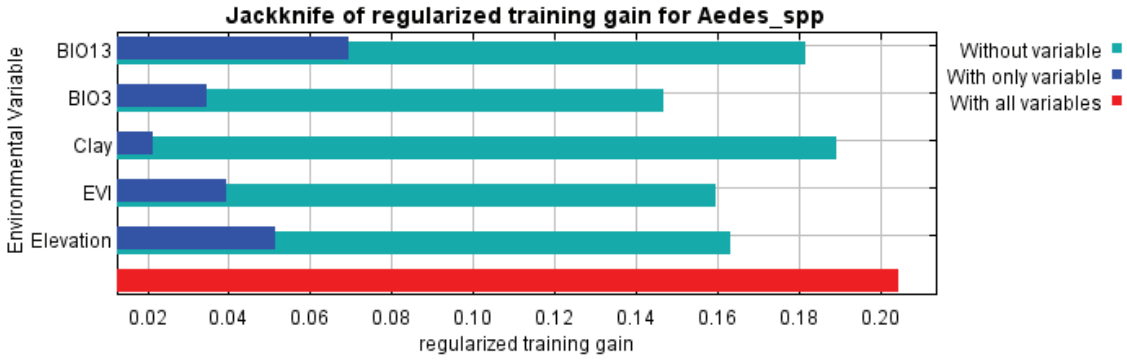

Figure 3.6 Jackknife test of variable importance for *Aedes spp* distribution prediction

For *Culex spp*, elevation had the highest gain when used in isolation and therefore appeared to have the most useful information by itself, while temperature seasonality (BIO4) decreased the gain the most when omitted (Figure 3.7).

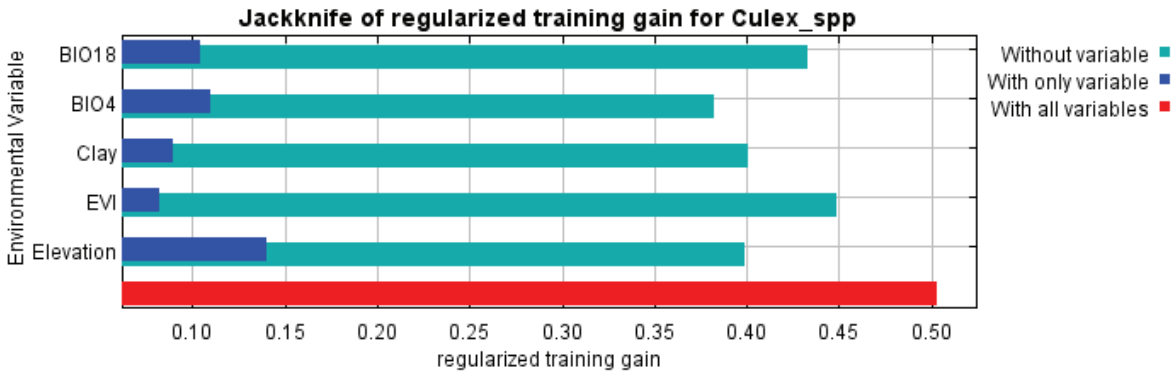

Figure 3.7 Jackknife test of variable importance for *Culex spp* distribution prediction

For *Anopheles spp*, the environmental variable that showed highest gain when used in isolation was enhanced vegetation index (EVI), which had the most useful information by itself and the same variable decreased the gain the most when omitted (Figure 3.8).

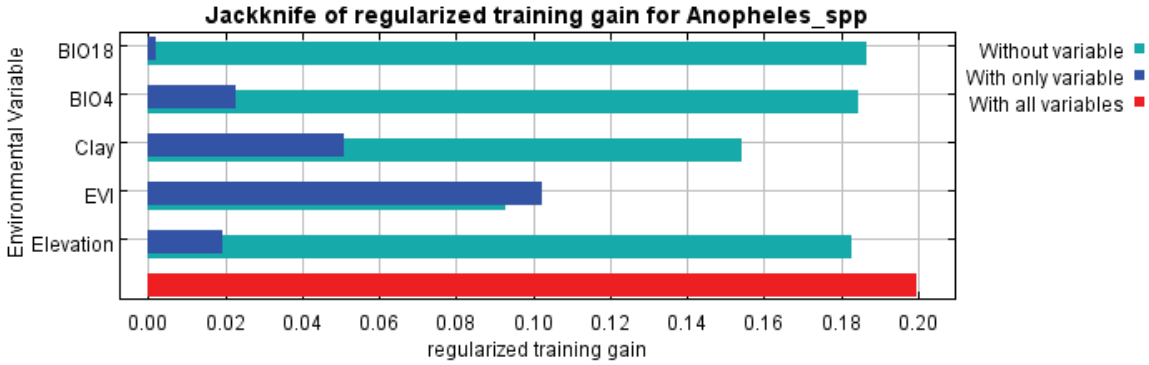

Figure 3.8 Jackknife test of variable importance for *Anopheles spp* distribution prediction

3.3.5 Evaluation of Model performance

The predictive performance of the three models based on AUC values was considered good (Figure 3.9, Figure 3.10 and Figure 3.11). The mean test AUC and standard deviation for 10 replicate runs of the three vector species were 0.681 ± 0.155 for *Aedes spp*, 0.765 ± 0.071 for *Culex spp*, and 0.675 ± 0.120 for *Anopheles*.

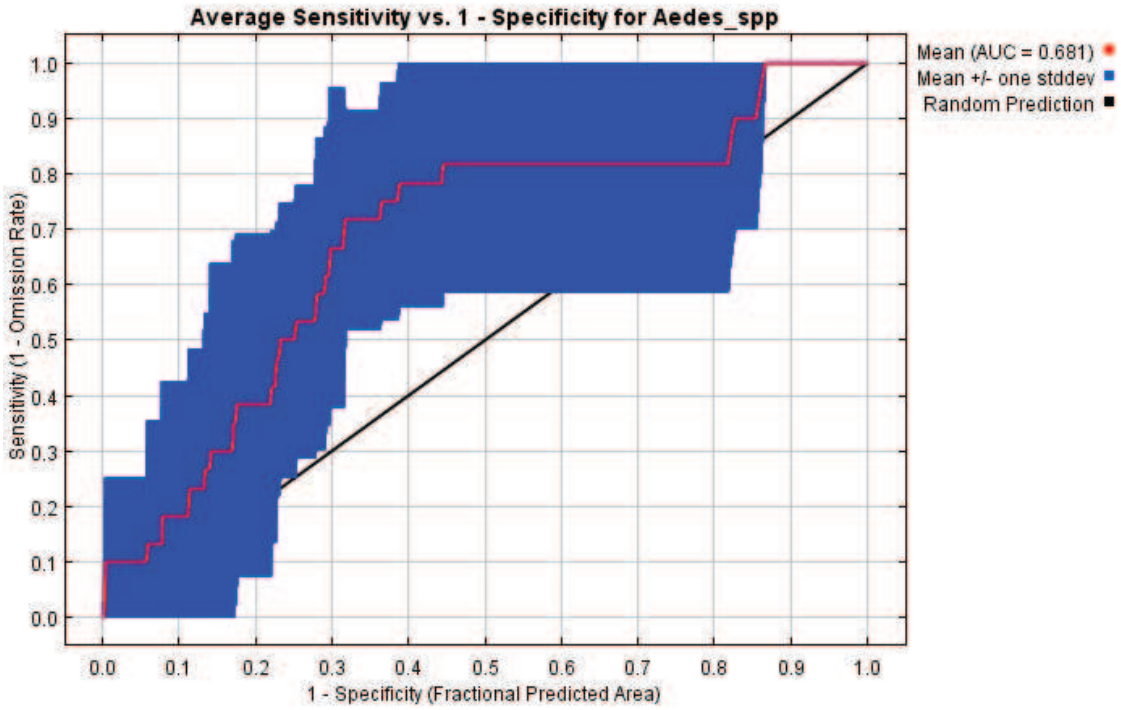

Figure 3.9 The Receiver Operating Characteristic (ROC) Curve or AUC for *Aedes spp* showing the mean (red) and standard deviation (blue) averaged from the 10 replicate runs

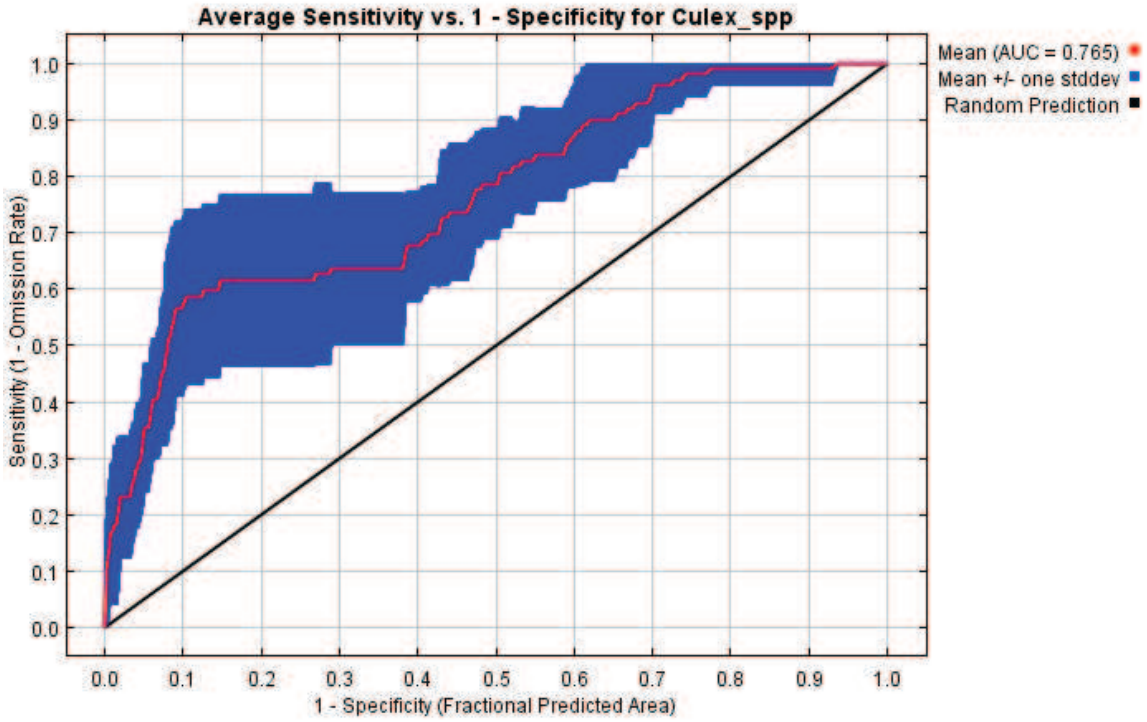

Figure 3.10 The Receiver Operating Characteristics (ROC) Curve or AUC for *Culex spp* showing the mean (red) and standard deviation (blue) averaged from 10 replicate runs

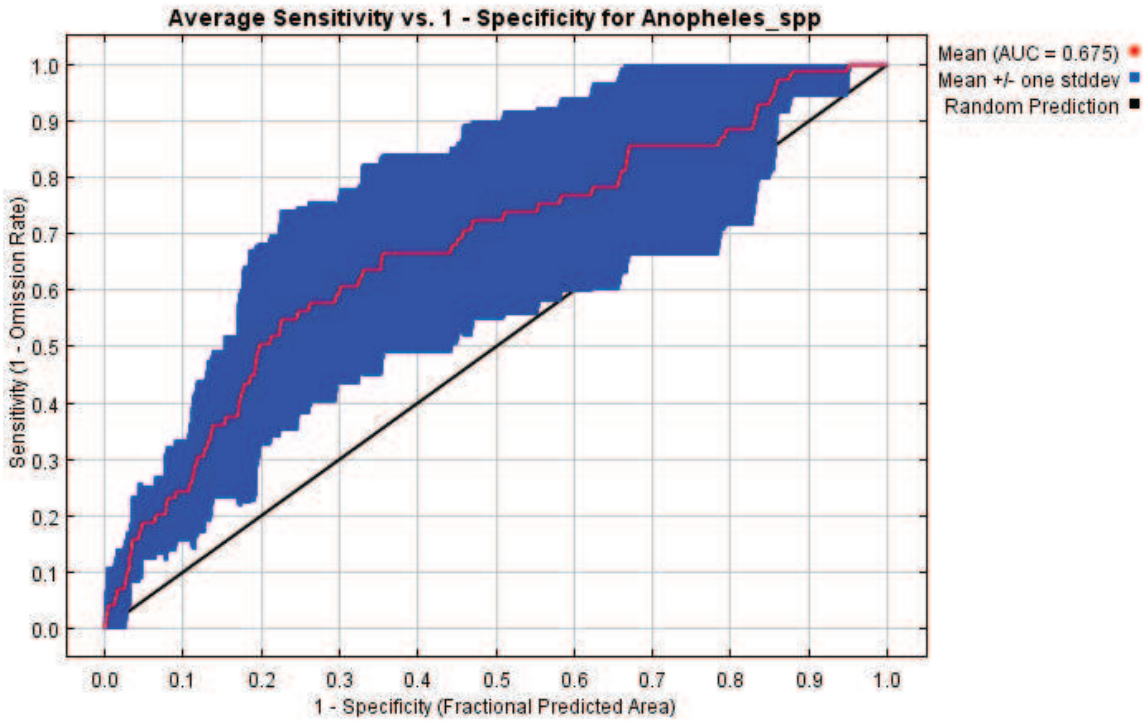

Figure 3.11 The Receiver Operating Characteristic (ROC) Curve of AUC for *Anopheles spp* showing the mean (red) and standard deviation (blue) averaged from 10 replicate runs

3.3.6 Habitat suitability maps for RVFV vector species

Habitat suitability maps (Figure 3.12, Figure 3.13 and Figure 3.14) show heterogeneous distribution of habitat suitability for the three vector species. While there are overlaps in many parts, the distribution of *Aedes spp* and *Culex spp* appears to be fragmented, whereas the distribution of *Anopheles spp* is more spread out across the study area. It is also noted that the predicted distribution of *Aedes spp* covers most parts of the Serengeti National Park and districts in western Serengeti, Northern and Southern parts of the Ngorongoro Conservation Area, and most parts of Arusha Region. Other suitable areas include Southern and Eastern parts of Kilimanjaro Region.

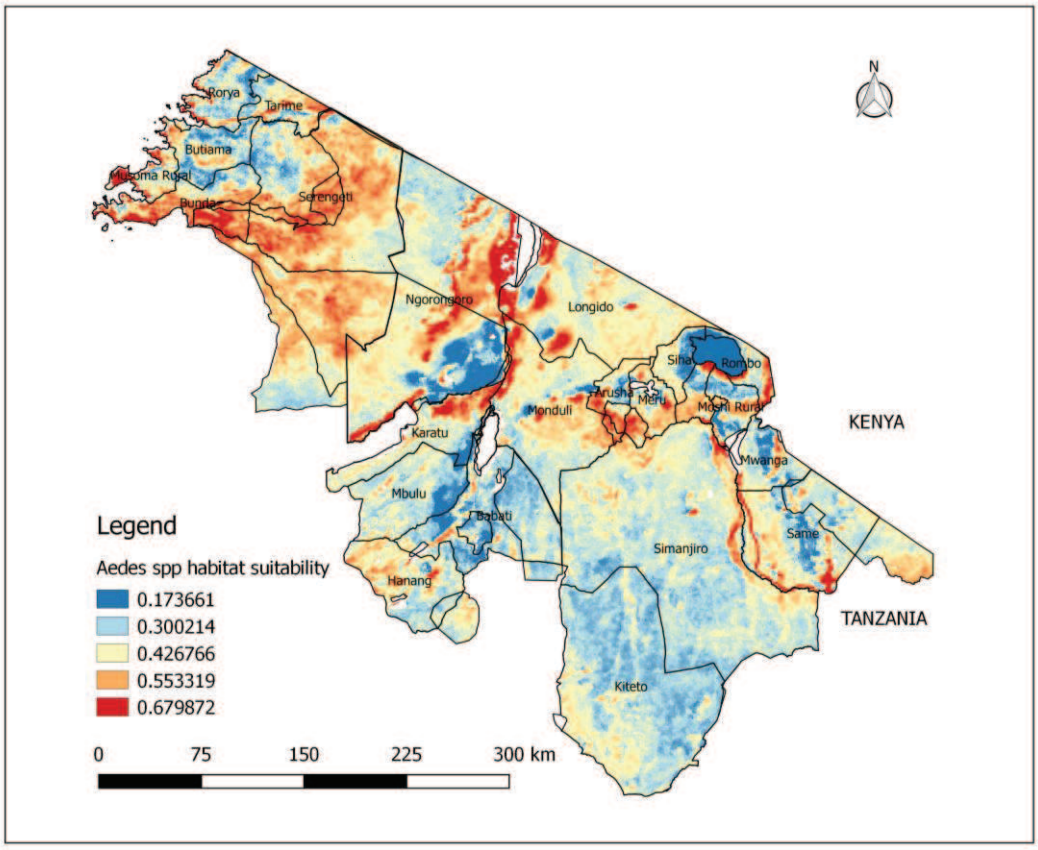

Figure 3.12 Predicted habitat suitability for *Aedes spp* in Northern Tanzania. Blue color indicates low suitability and red indicates high suitability.

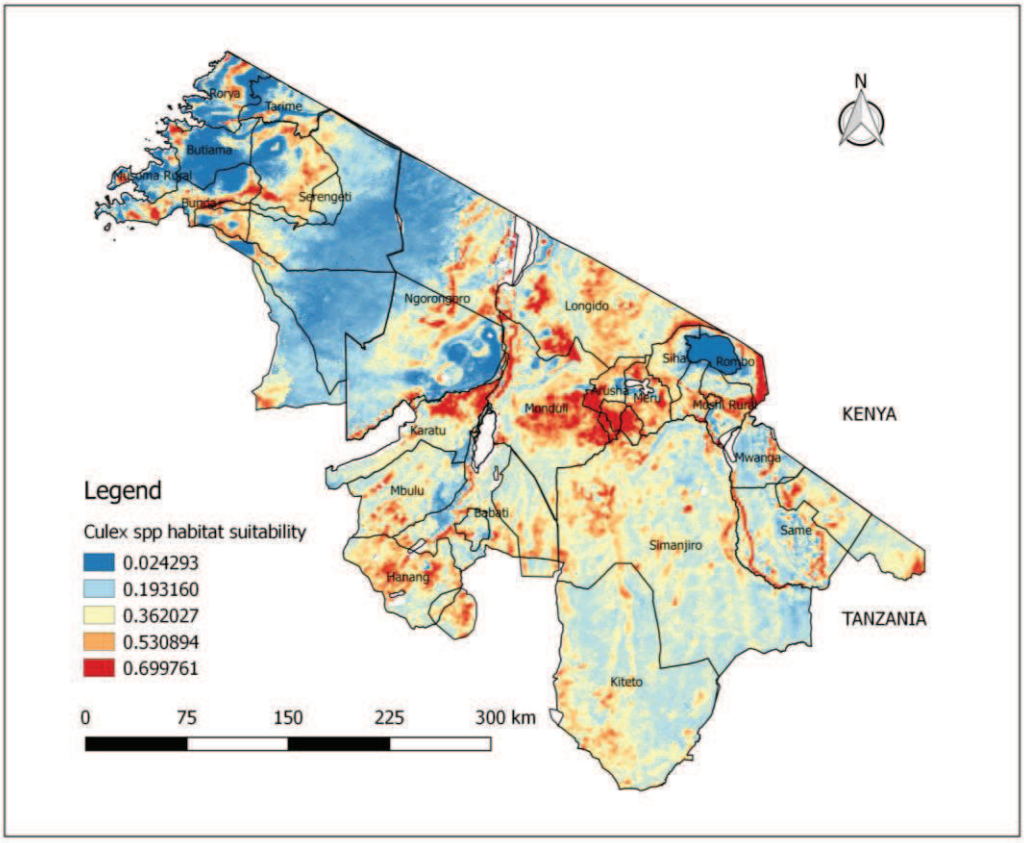

Figure 3.13 Predicted habitat suitability for *Culex spp* in Northern Tanzania. Blue color indicates low suitability and red indicates high suitability.

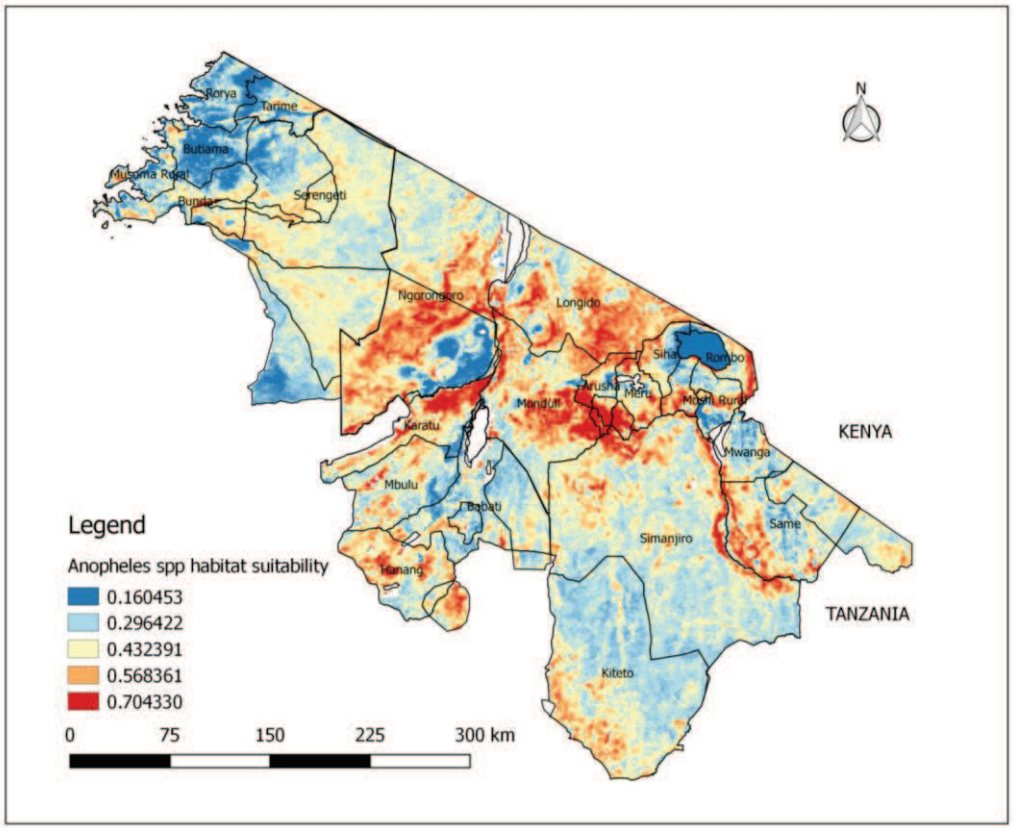

Figure 3.14 Predicted habitat suitability for *Anopheles spp* in Northern Tanzania. Blue color indicates low suitability and red indicates high suitability.

3.4 Discussion

Overall small numbers of mosquitoes were collected in this study which were morphologically identified as belonging to five genera, *Aedes*, *Anopheles*, *Culex*, *Mansonia* and *Coquillettidia*. The identified mosquitoes include those known as primary and secondary vectors for RVFV and which have been implicated in RVF outbreaks in Kenya (Sang et al., 2010, LaBeaud, 2011), Madagascar (Ratovonjato et al., 2011), Mauritania and Senegal (Diallo et al., 2005) and Saudi Arabia (Jupp et al., 2002). The low abundance recorded in this study is likely to reflect the period of sampling that mainly included dry season periods, which is known limit mosquito abundance and distribution (Van Peenen et al., 1972, Minakawa et al., 2002, Deichmeister and Telang, 2011). However, vectors can be detected in many areas of northern Tanzania, including during the dry season.

In the current study, the abundance of mosquitoes collected varied across villages. Although the difference was not statistically significant, more *Anopheles* were collected in livestock keeping households than the non-livestock keeping counterparts. Similar observations were reported previously in studies involving

Anopheles spp carried out in Moshi, Tanzania (Mahande et al., 2007), suggesting that the presence of livestock at a household can attract more host seeking mosquitoes and therefore influence local abundance. This would also pose potential risk for mosquito-borne disease transmission.

The difference in the distribution of vectors across the study villages can be attributed to variation in the environmental characteristics of the study area (Sattler et al., 2005, Minakawa et al., 1999, Sang et al., 2017). In particular, ecologically wetter conditions are likely to increase the number of breeding sites for mosquitoes resulting in an increase in the number of vectors. The most influential environmental predictor variable for *Aedes spp*, *Culex spp* and *Anopheles spp* were precipitation of the wettest month, temperature seasonality, and enhanced vegetation index (EVI), respectively. Other predictors that contributed to prediction of habitat suitability of the three species were clay soils, precipitation of the warmest quarter and elevation. Other studies have reported temperature, rainfall, soil types and vegetation cover (NDVI or EVI) as important predictors for RVFV vector distribution (Anyamba et al., 2009, Sang et al., 2010, Sang et al., 2017, Njenga and Bett, 2019, Minakawa et al., 2002).

Maps developed in this study indicate heterogeneities in the distribution of suitable habitat for *Aedes*, *Culex* and *Anopheles* mosquitoes. However, there was some important areas of overlap in the areas of high suitability for *Aedes* and *Culex*. These may represent areas at particularly high risk of RVFV outbreaks. It appears that different genera of mosquitoes have varied ecological requirements even if they share similar or overlapping habitat. The identified suitable areas include areas within and around the Serengeti ecosystem and districts in northern Tanzania, in which the distribution of RVFV vector species was not known. Some of the highly suitable parts of the study area include those which reported repeated RVF outbreaks hence supporting studies that identify this region as high-risk area for RVF outbreaks (Anyamba et al., 2009, Mweya et al., 2015, Sindato et al., 2015, Sindato et al., 2016, Njenga and Bett, 2019).

This study provides a valuable contribution to our understanding of the distribution of RVFV mosquito vectors in northern Tanzania, with a previous entomological study on RVF vectors limited to one district (Mweya et al., 2015). The current study collected vector samples from 12 districts including areas that have not been sampled before. Although MaxEnt was used for mapping the potential distribution of the mosquito genera using bioclimatic variables, the suitable habitat for RVFV

vectors may be overpredicted in some areas (Pearson et al., 2007). However, the information produced from this analysis has value for example identifying high-risk areas for prioritizing surveillance and disease control interventions. Since the mosquito genera collected include potential vectors of RVFV, other arboviruses, and malaria, the predicted habitat suitability maps can inform further studies, surveillance, and control for these pathogens.

3.5 Conclusions

This study describes the inter-epidemic abundance and composition of potential RVFV vectors in northern Tanzania, including areas that have not been sampled previously. The reported occurrence and predicted distribution identifies areas of potential risk for RVF during inter-epidemic periods. These findings can be used by researchers, policy makers, government agencies to inform surveillance and disease control interventions.

Chapter Four

4 Evaluation of the diagnostic accuracy of an indirect in-house ELISA for detection of Rift Valley fever virus-specific antibodies in small ruminants

4.1 Introduction

A number of serological methods have been used for RVF diagnosis during outbreaks as well as during subsequent surveillance. Commonly used serological diagnosis methods include the virus neutralization test (VNT), the hemagglutination inhibition test (HAI), the complement fixation test (CF), the indirect immunofluorescence assay (IFA), and the enzyme-linked immunosorbent assay (ELISA) (Scott et al., 1986, OIE, 2016, Gerdes, 2004, Schreur et al., 2017). ELISA and VNT are used to assess the serological response to infection and are considered appropriate surveillance tools (Pepin et al., 2010, OIE, 2016) and can detect RVFV IgM and IgG antibodies as early as four to eight days after infection (Morvan et al., 1991, Pepin et al., 2010). The virus neutralization test is highly accurate with little or no cross-neutralization with other phleboviruses (Tesh et al., 1982, Pepin et al., 2010), and is regarded by OIE as the gold standard RVF serological assay. As such it is generally used for vaccine potency determination and is the OIE prescribed test for international trade (Pepin et al., 2010, OIE, 2016). The virus neutralization test is highly specific and, unlike some ELISA based assays in which species-specific detection reagents are used, can be applied to serum from a wide range of host species (Mansfield et al., 2015, Schreur et al., 2017). However, VNT is laborious, expensive, requires five to seven days for completion, and can be performed only when standardized stocks of live virus and tissue cultures are available (Pepin et al., 2010). It can therefore only be performed in suitable biocontainment facilities (Pepin et al., 2010, Van Vuren and Paweska, 2009), which can be found only in highly specialized reference laboratories which are limited in most RVFV-endemic countries. Therefore, there is an increasing demand for high quality and procedurally safe diagnostic tests which do not involve the handling of the virus (Pepin et al., 2010).

Alternatives to the RVFV VNT are being developed and validated. Several RVFV ELISAs have been developed and the performance of some of these compared (Van Vuren and Paweska, 2010, Williams et al., 2011, Kim et al., 2012, Kortekaas et al.,

2013). ELISAs have been developed and widely applied by OIE Reference Laboratories (Kortekaas et al., 2013, Pepin et al., 2010). Several have been developed using either whole cell lysate derived from infected cells or purified nucleocapsid protein as antigen (Fukushi et al., 2012, Paweska et al., 2003a, Paweska et al., 2005). However, some of the available RVFV ELISAs are limited by cross-reactivity between RVFV and other closely related phleboviruses (Pepin et al., 2010) and are commercial assays can be expensive. In an attempt to develop a cheap and simplified assay procedure, the University of Glasgow developed an in-house indirect ELISA based on the recombinant nucleocapsid protein of RVFV for the detection of specific antibodies in ruminant sera. In this chapter, the performance of the University of Glasgow's in-house ELISA was evaluated with reference to the widely used commercial indirect competition ELISA (cELISA) kit (ID Screen Rift Valley fever multi-species ELISA; IDVet Innovative Diagnostics, Grabels, France). Given that it has a validated diagnostic sensitivity and specificity of 100% (Comtet et al., 2010, Ellis et al., 2014), the kit was used as the standard reference for evaluating the in-house ELISA. The virus neutralization test was used as a confirmation test for the ELISA results. The aim of the evaluation was to assess whether or not the in-house ELISA method is good enough to be used for screening RVFV exposure in ruminants.

4.2 Methods

4.2.1 Selection of sera samples

In this study, livestock sera collected in the cross-sectional study (described in Chapter Two) were analysed for anti-RVFV antibodies using two enzyme linked immuno-sorbent assays (ELISAs). These were: (1) a recombinant nucleocapsid-based in-house ELISA developed at the University of Glasgow; and (2) a commercial competition ELISA (IDvet Innovative Diagnostics, Grabels, France) as the reference standard for the purpose of this evaluation. Results from a subset of samples were also confirmed using the OIE gold standard virus neutralization test (VNT) to confirm the reliability of the selected reference ELISA kit. A subset of randomly selected sera samples were screened first using the in-house ELISA beginning with a set of cattle (n=44), goat (n=44) and sheep (n=44) samples. This step was carried out to examine the ability of the in-house assay to test RVFV antibodies in sera samples from three different animal species (cattle, goats and sheep). The assay showed clear distinct (positives and negatives as compared to control sera) results

for goat and sheep sera but more false positives were observed with cattle sera. Therefore, goat and sheep sera were screened by in-house ELISA and retested by commercial cELISA, where as all cattle sera were tested by commercial cELISA. A subset (n=30) of randomly selected ELISA positive and negative sheep and goat sera were further retested with VNT as a confirmation test. Details of the three assays (in-house ELISA, commercial cELISA, and VNT) are described in Chapter Two.

4.2.2 Comparing the diagnostic accuracy between the in-house ELISA and the commercial IDVet cELISA

The aim of this analysis was to assess the performance of the in-house ELISA which is not yet validated. Although gold standard data or knowledge of past exposure of the samples tested was not known, cELISA was considered to be sufficiently reliable to constitute a gold standard or reference test. However, the use of VNT as gold standard was hampered by the expense of testing ~2000 samples. The remaining option was to use VNT first to validate cELISA using 30 samples (13 VNT+ and 17 VNT-), then to use the cELISA to assess the performance of the in-house ELISA with 1861 samples. The confirmation of results of the 30 samples with the VNT was blindly carried out. Results of the tests were presented in a two-by-two contingency table of frequencies with the rows and columns indicating the categories of response as dichotomous outcomes (positive/negative results) for each method. Table 4.1 shows true exposure/infection status as determined by a standard/reference method (cELISA) and rows represent the test outcome as determined by a new index test (in-house ELISA). Test results of sera samples were classified as true positive (TP) or true negative (TN) if they were in agreement with the reference test results; alternatively, they were considered either false positive (FP) or false negative (FN) if they differed from the reference test results. The test accuracy was calculated as the proportion of samples correctly classified by the test using the formula [(TP+TN)/(TP+TN+FP+FN)].

Table 4.1 A two by two contingency table for results of the index test and the reference standard tests.

	Infection status as determined by standard test		
index test	Infection positive	Infection negative	Total
Test positive	True positive (TP)	False positive (FP)	TP + FP
Test negative	False negative (FN)	True negative (TN)	FN + TN

Total	TP + FN	FP + FN	n

4.2.1 Accuracy, sensitivity and specificity of the test

Evaluation of the performance or accuracy of a diagnostic test involves calculating four objective measures of test performance, namely, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The sensitivity of a test refers to the ability of the test to correctly identify those subjects with the disease (Wong and Lim, 2011). Specificity refers to the ability of the test to correctly identify those subjects without the disease (Wong and Lim, 2011). Positive predictive value (PPV) is a measure of how likely is it that the subject has the disease given that the test result is positive, whereas negative predictive value (NPV) shows how likely is it that the subject does not have the disease given that the test result is negative.

The sensitivity and specificity of a quantitative test are dependent on the cut-off value above or below which the test is positive. In general, the higher the sensitivity, the lower the specificity, and vice versa (Lalkhen and McCluskey, 2008). It is therefore recommended to subject samples that are initially positive to a screening test with high sensitivity/low specificity, to a second test with low sensitivity/high specificity or a reference test with high specificity and sensitivity. The selection of a cut-off value allowed test results to be divided into positive or negative categories. The test accuracy, sensitivity, specificity, positive predictive value, and negative predictive value, were calculated using the following formulas.

Accuracy = (True positive + True negative)/Total population

True positive fraction (TPF, sensitivity) = True positives/disease positives

True negative fraction (TNF, specificity) = True negatives/Condition negatives

Positive predictive value (PPV) = True positives/Test outcome positives

Negative predictive value (NPV) = True negatives/Test outcome negatives

4.2.2 Determining the cut-off points for the in-house ELISA

The cut-off points for the in-house ELISA was determined by the receiver operator characteristic (ROC) curve. The ROC curve is a plot of (1−specificity) of a test on

the *x*-axis against its sensitivity on the *y*-axis for all possible cut-off points (Lalkhen and McCluskey, 2008, Hajian-Tilaki, 2013). ROC analysis is used in epidemiology to quantify how accurately diagnostic tests can discriminate between two disease exposure status, typically referred to as "disease positive" and "disease negative" (Green and Swets, 1966, Hajian-Tilaki, 2013). ROC analysis avoids distortion of the accuracy indices by fluctuations caused by the use of arbitrarily chosen decision criteria or cut-offs (Hajian-Tilaki, 2013). The area under the curve (AUC) represents the overall accuracy of a test, with values ranging from 0.5 (purely random discrimination) to 1.0 (perfect discrimination) indicating 100% sensitivity and specificity (Hanley and McNeil, 1982, Hajian-Tilaki, 2013). ROC curve analysis was used to determine diagnostic sensitivity and specificity for a range of cut-off values of the in-house ELISA test. easyROC, a web-tool constructed with the R package shiny for ROC curve analysis (version 1.3.1), was used as described by (Goksuluk et al., 2016). This tool is freely available through www.biosoft.hacettepe.edu.tr/easyROC.

4.3 Results

4.3.1 Confirmation of diagnostic accuracy of the commercial cELISA using VNT as the gold standard

In validating the cELISA against the VNT, results summarised in Table 4.2, the accuracy was 97% (95% CI: 83-100%; one false negative out of 30 samples) indicating high performance of the cELISA at replicating the VNT results. Therefore, cELISA was used as reference test for evaluating the in-house ELISA. Results are summarised in Table 4.3.

Table 4.2 Summary results of the test outcomes of the virus neutralization test (standard) and the commercial competition ELISA

	Virus neutralization test (VNT)		
cELISA	**RVFV positive**	**RVFV negative**	**Total**
Test positive	12	0	12
Test negative	1	17	18
Total	13	17	30

Table 4.3 Test outcomes of the commercial competition ELISA (reference) and the in-house ELISA, where percent positivity, PP >30 is taken as diagnostic of RVFV exposure using the in-house ELISA.

	cELISA		
Inhouse ELISA	RVFV positive	RVFV negative	Total
Test positive	71	1100	1171
Test negative	5	685	690
Total	76	1785	1861

4.3.2 Selection of cut -off point for the in-house ELISA

The cut-off value for an ELISA can be adjusted for different target populations as well as for different diagnostic purposes (Jacobson, 1998). The ROC curve analysis covers the whole range of possible PP values, giving corresponding sensitivity and specificity estimates. The distribution graphs in Figure 4.1 shows the pattern of distribution of the RVFV exposed and not exposed populations as identified by the in-house ELISA with suggested optimum cut-off. Table 4.4 shows the criterion values and coordinates of the ROC curve with PP cut-off values of the in-house ELISA ranging between 20 and 80. Applying the optimum cut-off of 43, the in-house ELISA had a sensitivity of 0.73, 95% CI (0.61 - 0.82), specificity 0.68, 95% CI (0.66 - 0.71), positive predictive value 0.09, 95% CI (0.08 - 0.15), and negative predictive value 0.98, 95% CI (0.97 - 0.99). At the cut-off point of 30 which was initially used for sample screening the sensitivity and specificity of the test was 0.95 and 0.33 respectively.

Table 4.4 Different cut-off points of the in-house ELISA and their corresponding sensitivity, specificity, positive likelihood ratio and negative likelihood ratio.

Cut-off (PP)	**Sensitivity (95%CI)**	**Specificity (95%CI)**	**PLR (95%CI)**	**NLR (95%CI)**
≥20	1.00 (0.95, 1.00)	0.22 (0.20, 0.24)	1.28 (1.25, 1.31)	0.00 (0.00, 0.00)
≥25	0.97 (0.91, 1.00)	0.29 (0.27, 0.31)	1.36 (1.30, 1.43)	0.09 (0.02, 0.36)
≥30	0.93 (0.85, 0.98)	0.38 (0.36, 0.41)	1.52 (1.41, 1.63)	0.17 (0.07, 0.40)
≥35	0.83 (0.73, 0.91)	0.54 (0.51, 0.56)	1.79 (1.60, 2.00)	0.32 (0.19, 0.52)
≥40	0.74 (0.62, 0.83)	0.65 (0.63, 0.67)	2.09 (1.81, 2.43)	0.41 (0.28, 0.59)

Cut-off (PP)	Sensitivity (95%CI)	Specificity (95%CI)	PLR (95%CI)	NLR (95%CI)
≥45	0.67 (0.55, 0.77)	0.73 (0.71, 0.75)	2.48 (2.08, 2.95)	0.45 (0.33, 0.62)
≥50	0.61 (0.49, 0.72)	0.80 (0.78, 0.82)	3.05 (2.49, 3.74)	0.49 (0.37, 0.65)
≥55	0.54 (0.42, 0.65)	0.85 (0.83, 0.87)	3.59 (2.84, 4.55)	0.54 (0.42, 0.69)
≥60	0.45 (0.33, 0.57)	0.89 (0.87, 0.90)	3.97 (3.00, 5.27)	0.62 (0.51, 0.76)
≥65	0.42 (0.31, 0.54)	0.91 (0.89, 0.92)	4.58 (3.39, 6.19)	0.64 (0.53, 0.77)
≥70	0.36 (0.25, 0.47)	0.93 (0.91, 0.94)	4.80 (3.40, 6.78)	0.70 (0.59, 0.82)
≥75	0.30 (0.20, 0.42)	0.94 (0.93, 0.95)	4.91 (3.34, 7.23)	0.74 (0.64, 0.86)
≥80	0.25 (0.16, 0.36)	0.95 (0.93, 0.96)	4.55 (2.95, 7.03)	0.79 (0.70, 0.90)

The ROC curve analysis shows the area under a ROC curve, AUC = 0.77, 95% CI (0.72 - 0.82) which is greater than 0.5 suggesting that the assay performs better than random chance (dotted line in the ROC curve, Figure 4.1). In Figure 4.1 the sensitivity and specificity of the in-house ELISA have been plotted against the whole range of possible PP values. The cross-over point of the two lines, indicate optimal sensitivity and specificity suggesting that a PP value at that point is the best possible cut-off value for minimizing both false positive and false negative results.

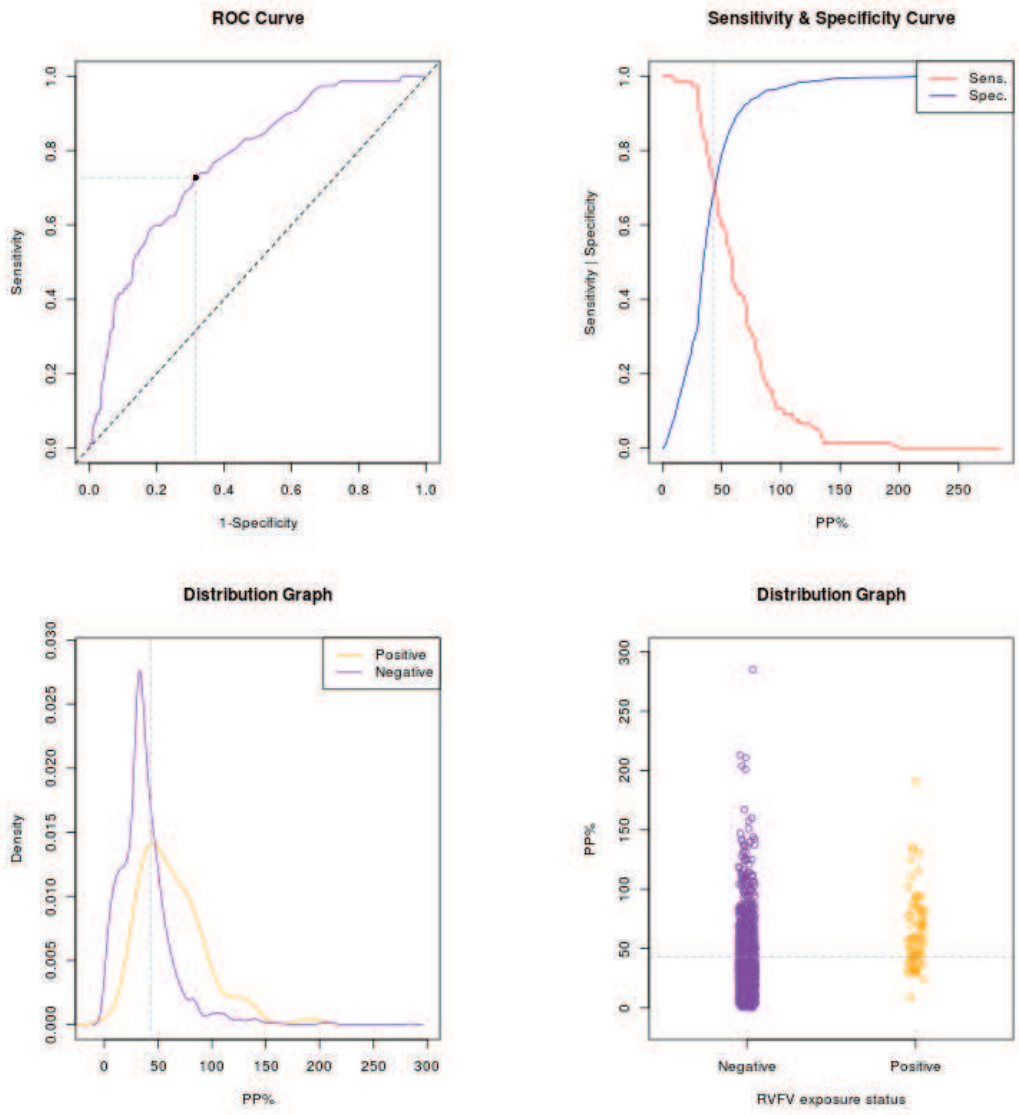

Figure 4.1 The ROC curve and distribution graphs of test results showing the cut-off points, corresponding sensitivity and specificity and distribution of positive and negative samples

4.4 Discussion

Enzyme linked immunosorbent assays (ELISAs) are widely used for RVF serological surveys. However, most of the commercially available ELISA kits are expensive hence the pressing need for a cheap, safe and simplified assay. The University of Glasgow's in-house ELISA the performance of which was assessed in this study in comparison with the commercial ELISA sought to bridge this gap. However, the evaluation results showed the assay to have lower accuracy as compared to that of the commercial cELISA. The low level of agreement between the two assays suggest that the in-house ELISA ability to correctly detect RVFV antibodies in infected subjects does not match up that of the cELISA.

In this study, ROC curve analysis determined a PP cut-off value of 43 for the assay to attain an equilibrium between sensitivity and specificity. The associated area under a ROC curve, AUC of 0.77, which is greater than 0.5 suggests that the assay performs better than random chance but it is not a high-perfoming test. An uninformative test (one no better at identifying true positives or negatives than flipping a coin) would have an AUC of 0.5 (Hajian-Tilaki, 2013).

In addition, the poor specificity of the in-house ELISA, and its inability to distinguish results on cattle samples, suggest that the assay could be detecting cross-reactivity from other possible viral infections in the field collected samples. Livestock in endemic countries including Tanzania could be exposed to many other closely related viral infections. However, the sensitivity of 93% at a cut-off of PP>30 suggest that in cases where large numbers of samples need to be tested, the assay can still be useful as screening test prior to performing a confirmatory test for detecting RVFV specific antibodies to save some cost during high throughput testing.

In comparing the cELISA with VNT, the accuracy of cELISA was 97%, but only a small number of samples (n=30) were used. The high accuracy of the cELISA in relation to the VNT supports the use of the cELISA as a standard reference for assessing the in-house ELISA and its use for epidemiological surveys in Tanzania.

4.5 Conclusion

Although the recombinant RVFV nucleoprotein based in-house ELISA evaluated in this study performs poorly in comparison to the cELISA, particularly in relation to test specificity, the test could have application for high throughput screening of samples from RVF endemic regions.

Chapter Five

5 Inter-epidemic seroprevalence and risk factors for Rift Valley fever virus seropositivity in domestic ruminants and human populations in northern Tanzania

5.1 Introduction

Rift Valley fever epidemics in Tanzania over the past 50 years have occurred at intervals of 10 - 20 years with major outbreaks reported in 1977, 1997/98 and 2006/2007. Although there are some evidence of inter-epidemic RVFV infection in livestock and humans in endemic countries as reported by studies in Tanzania (Sumaye et al., 2015, Sumaye et al., 2013, Ahmed et al., 2018), Rwanda (Umuhoza et al., 2017), Kenya (LaBeaud et al., 2008, Lichoti et al., 2014a), Uganda (Magona et al., 2013, Nyakarahuka et al., 2018), Mauritania (Rissmann et al., 2017) and Egypt (Mroz et al., 2017), it is not fully understood how the virus persist during the inter-epidemic period. There are also questions on the dominant mode of animal-to-animal and animal-to-human transmission between the epidemics. During epidemics, animal infections are attributed mostly to floodwater mosquitoes, which feed on viraemic animals and subsequently transmit RVFV to other animals and potentially to humans. Human infections are also linked to exposure to infectious animal tissues or bodily fluids such as the abortus, birthing fluids, milk, or blood. However, key elements of transmission during the inter-epidemic period are not well understood. Understanding which forms of exposure in both people and animals provide the greatest RVFV transmission risk between the epidemics may be useful for targeting interventions and public health education which can prevent future outbreaks or further spread of the virus to other areas. The aims of this study therefore were to: (1) determine whether inter-epidemic livestock and human RVFV transmission occurs in northern Tanzania, an area that has experienced repeated RVF outbreaks; (2) assess the risk factors associated with inter-epidemic RVFV infection in livestock and humans in northern Tanzania; and (3) evaluate whether seropositivity and risks differ amongst agro-ecological settings in northern Tanzania.

5.2 Methods

5.2.1 Study Area

The study was carried out in three regions of northern Tanzania, namely Arusha Kilimanjaro and Manyara Regions, with samples collected between 2013 and 2016 as part of the Social, Economic and Environmental Drivers of Zoonoses project (SEEDZ) and an earlier study on bacterial zoonoses ("The Impact and Social Ecology of Bacterial Zoonoses in Northern Tanzania" project (BacZoo)). Sampled villages included pastoral, agro-pastoral and small-holder communities. Details of these studies are described in Chapter two. Briefly, the SEEDZ study involved 20 villages randomly selected in Arusha and Manyara Regions using a Generalised Random Tessellation Stratified (GRTS) sampling approach (McDonald, 2004, Stevens Jr and Olsen, 2004). The BacZoo samples and data used in this study were collected from 15 villages randomly selected from villages in the Kilimanjaro and Arusha Regions (BacZoo). Selected villages from both studies are shown in Figure 5.1.

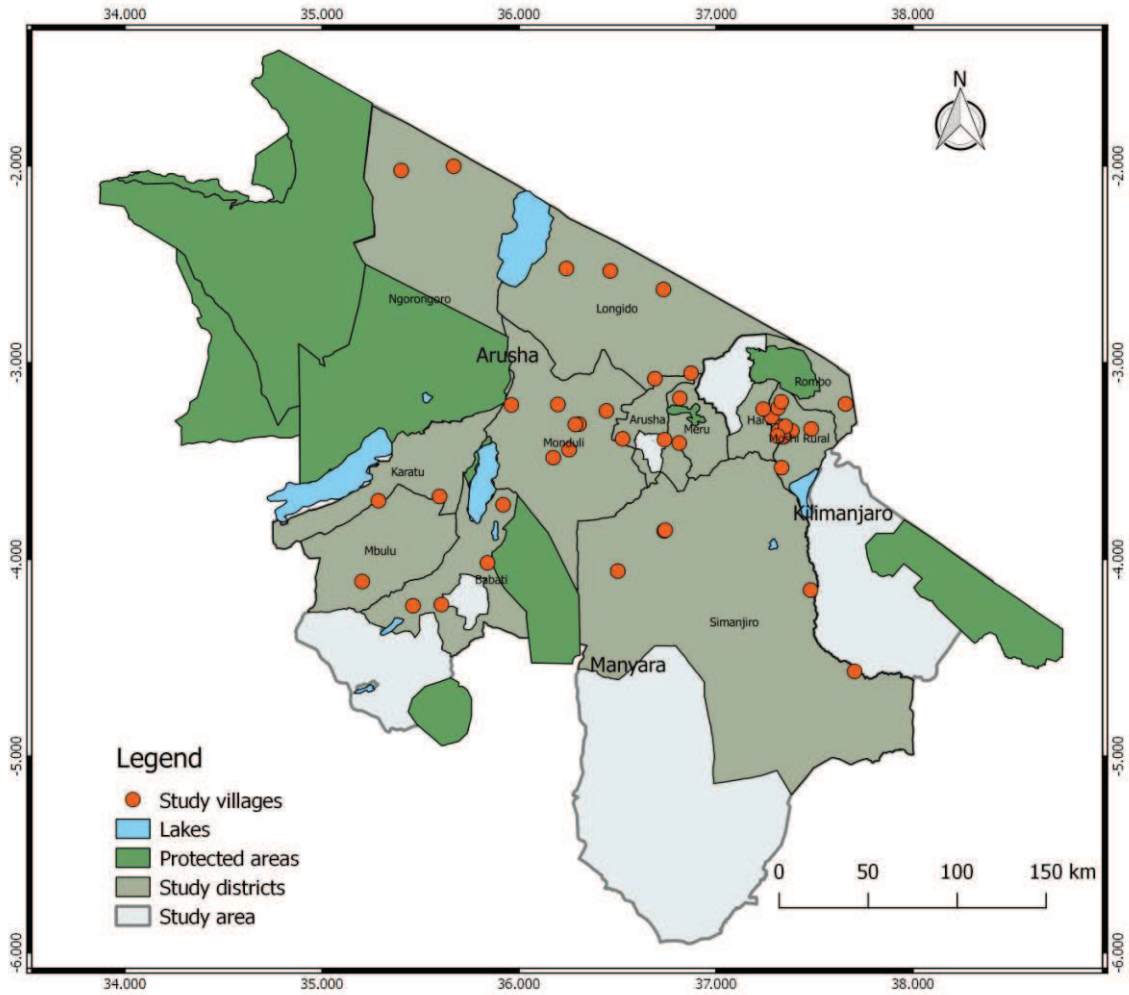

Figure 5.1 Map of northern Tanzania showing regions (Arusha, Kilimanjaro and Manyara), districts and location of the 35 study villages in the study area.

5.2.2 Sample collection

The sampling procedure for the SEEDZ study is described in the methods chapter. Briefly, livestock keepers in each sub-village were encouraged to bring their animals to a central point in the subvillage. Ten households from those who attended were randomly selected for recruitment into the study and a target of 10 cattle, sheep and goats selected in each household for sampling. In cases where a household had less than 10 animals in a herd or flock, all animals would be sampled. A 10ml blood sample was collected by jugular venepuncture and about 2-5 ml of sera was obtained from each blood sample. For the BacZoo study, a list of all livestock keeping households in each village was generated and up to six households randomly selected. Households were visited and, where numbers allowed, 12 of each of cattle, sheep and goats were sampled. Animal blood collection was carried out as described in the SEEDZ study.

A household questionnaire survey was carried out in households in which livestock sampling took place to collect data on household characteristics, demographics, and livestock management practices. In a random selection of SEEDZ study households, 10 ml of whole blood was collected from all assenting and/or consenting household members who had been occupant for the past 12 months and who were 5 years or older. An individual-level questionnaire focusing on risk factors for zoonotic disease was also conducted. Human blood samples and individual level questionnaire data were collected from all BacZoo households. Human blood sampling was performed by a medically qualified practitioner from Kilimanjaro Christian Medical Centre (KCMC) using vacuum extraction method as per World Health Organization guidelines (WHO, 2010). Serum samples obtained from livestock and human subjects were used for serological testing of RVFV exposure.

5.2.3 Laboratory analyses

In this study, livestock sera were analysed for anti-RVFV antibodies using two enzyme linked immuno-sorbent assays (ELISAs). A recombinant nucleocapsid-based in-house ELISA developed at the University of Glasgow was first used as screening test, then results confirmed using a commercial ELISA (ID Screen® Rift Valley fever multi-species ELISA; IDvet Innovative Diagnostics, Grabels, France).

All human sera samples were tested by commercial ID Screen® Rift Valley fever multi-species competitive ELISA. ELISA results were validated by virus neutralization test (VNT) of a subset of randomly selected (30 livestock and 30 human) positive and negative samples. Since the commercial ELISA showed close agreement with results of the VNT, it was used as a method of choice for all livestock and human samples and results presented here are based on the commercial ELISA assay. Details of the assays and how the choices were made are described in Chapters two and four of the thesis, respectively.

5.2.4 Statistical Analysis

Statistical analyses were performed using R version 3.5.3 (R Core Team, 2018). The associations between the response variable and explanatory variables were assessed using binomial generalised linear mixed models (GLMMs), also known as mixed-effects logistic regression. Considering the hierarchical nature of the data, containing clusters of non-independent observational units such as village and households, GLMMs were the method of choice for modelling of random and fixed effects of the explanatory variables (Bolker et al., 2009). GLMMs allows assessment of how the response variable is impacted by variation among individuals and variation among clusters/levels. Random effects allow estimation of variance in the response variable between groups (clusters), and reduces the probability of false positives (Type I errors) and false negatives (Type II errors) (Harrison et al., 2018). In this study, village and households were considered as random effects, RVFV seropositivity in livestock (cattle, goats or sheep) was the response variable, and other variables were considered as fixed effects. These fixed effects included sex, age, farming classification (small holder, agro-pastoral or pastoral), herd/flock size, animal introductions, use of seasonal movements/camps, history of abortions, presence/history of standing water/flooding in the household, history of animal deaths related to disease, animal management (confining cattle with small ruminants) and vector habitat suitability derived from species distribution modelling detailed in chapter three. Variables considered as fixed effects for seropositivity in people included occupation, sleeping in the same house as domestic ruminants, engaging in milking animals, birthing animals, handling placenta, handling aborted materials, handling animal carcasses, slaughtering animals, consuming raw meat or milk and seropositivity in livestock. Model selection was based on purposeful selection as proposed by Hosmer and Lemeshow (Hosmer Jr et al., 2013). The purposeful selection process began with a univariable

analysis of each variable. Any variable that had a Wald test p-value < 0.25 from the mixed-effects logistic regression was selected as a candidate for the multivariable analysis (Bursac et al., 2008). A backward selection procedure was then used to remove variables from the model that were non-significant and which did not act as important confounders of other effects in the model. Significance was evaluated at the 0.05 alpha level and confounding as a change in any remaining parameter estimate greater than 20% when comparing the full model with the model without the confounder (Bursac et al., 2008, Hosmer Jr et al., 2013). At the end of this iterative process of deleting, refitting, and verifying, the model contained all significant covariates and potential confounders.

5.3 Results

5.3.1 Seroprevalence in livestock

This study tested 3582 cattle, 3303 goats and 2586 sheep samples collected from 537 households in northern Tanzania. The overall RVFV seroprevalence based on anti RVFV antibodies in livestock in northern Tanzania was 2.8% (n=9471, 95% CI:2.5-3.2), with higher seroprevalence in cattle 4.4% (n=3582, 95% CI:3.7-5.1), than in sheep 2.6%, (n=2586, 95% CI: 2.0-3.3) and goats 1.4% (n=3303; 95%CI: 1.0-1.8). The odds of seropositivity in goats was about 70% less as compared to cattle (OR = 0.3, CI: 0.2-0.4, p<0.01) and the odds of seropositivity in sheep was 50% less as compared to cattle (OR=0.5, CI: 0.4-0.7, p<0.001) In addition, anti RVFV IgM was detected in 7.5% (n=255, 95% CI: 4.7-11.6) of goats and 12.8% (n=180, 95% CI: 8.4-18.7) sheep samples tested.

Overall seropositivity varied across age groups. The odds of seropositivity increased 1.3 times with increase in age by one year (OR=1.3, CI: 1.2-1.4, p<0.001). In this study seropositivity was detected in young animals (1-2 years) and increased with age in all three species (Figure 5.2 and Figure 5.3).

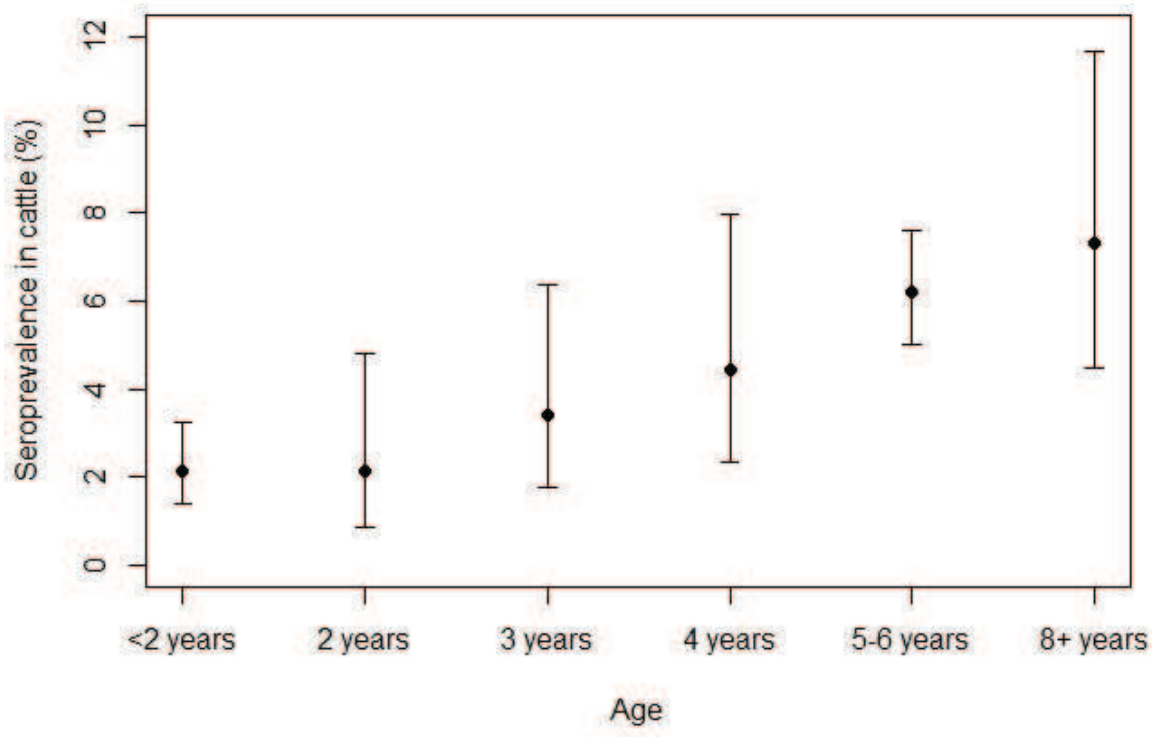

Figure 5.2 A plot of RVFV seroprevalence in different age groups of cattle in northern Tanzania

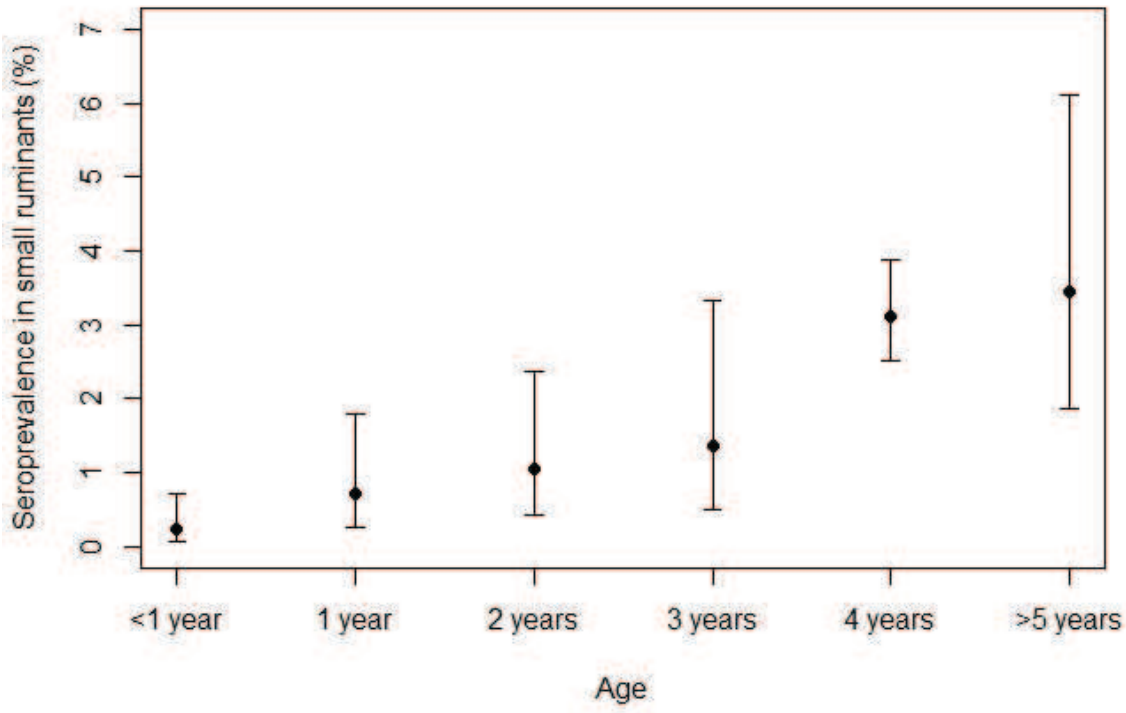

Figure 5.3 A plot of seroprevalence in different age groups of small ruminants (sheep and goats) in northern Tanzania

Seroprevalence varied between villages (Table 5.1), ranging from 0% to 13%. In cattle seroprevalence varied by about 2 times (MOR = 2.12) between villages and almost 3 times (MOR=2.6) between households. In sheep, seroprevalence was notably higher in Ruvu remiti (23%) and generally low in other villages (Table 5.1) varying by around 3 times (MOR=3.2) between villages and 5 times (MOR=5.5) between households. Whereas in goats seroprevalence varied by almost 4 times (MOR=3.6) between villages and about 4 times (MOR=3.6) between households. The distribution of village-level seroprevalence in livestock across northern Tanzania is shown in Figure 5.4.

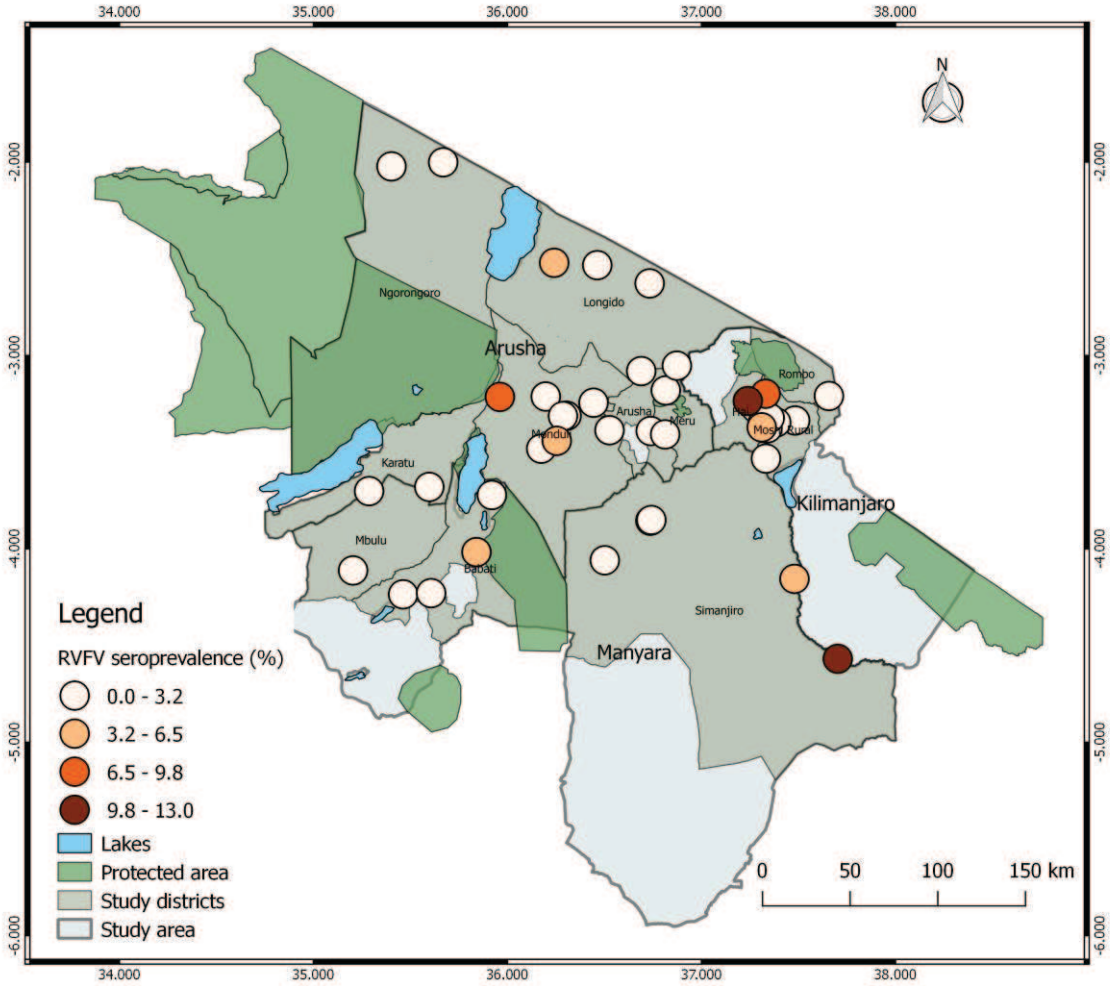

Figure 5.4. Map of northern Tanzania showing study regions, districts and the village-level seroprevalence in livestock

Table 5.1 RVFV seroprevalence in livestock (cattle, goats and sheep) by sampled village in northern Tanzania

District	Village	Cattle		Goats		Sheep		Overall seroprevalence		
		Tested	% positive (n)	Tested	%positive (n)	Tested	%positive (n)	Tested	%positive (n)	95%CI
Arusha	Ilkerin	93	3.2 (3)	117	0.9 (1)	133	1.5 (2)	343	1.7 (6)	0.0 - 3.9
	Sorenyi	4	0.0 (0)	5	0.0 (0)	3	0.0 (0)	12	0.0 (0)	0.0 - 30.1
Babati	Arri	64	3.1 (2)	40	0.0 (0)	22	0.0 (0)	126	1.6 (2)	0.3 - 6.2
	Minjingu	144	6.9 (10)	105	0.0 (0)	99	1.0 (1)	348	3.2 (11)	1.7 - 5.8
	Sarame	113	15.0 (17)	143	0.7 (1)	94	0.0 (0)	350	5.1 (18)	3.2 - 8.2
Hai	Lyamungo sinde	17	17.6 (3)	4	0.0 (0)	2	0.0 (0)	23	13.0 (3)	3.4 - 34.7
Karatu	Kansay	225	1.3 (3)	148	0.0 (0)	84	0.0 (0)	457	0.7 (3)	0.0 - 2.1
	Lositete	135	1.5 (2)	68	1.5 (1)	57	0.0 (0)	260	1.2 (3)	0.3 - 3.6
Longido	Engikareti	197	1.5 (3)	162	1.9 (3)	175	1.1 (2)	534	1.5 (8)	0.0 - 3.1
	Engoshowash	7	0.0 (0)	14	0.0 (0)	6	0.0 (0)	27	0.0 (0)	0.0 - 15.5
	Kimokowa	176	3.4 (6)	158	0.0 (0)	143	0.7 (1)	477	1.5 (7)	0.6 - 3.1
	Mairowa	75	2.7 (2)	71	0.0 (0)	75	0.0 (0)	221	0.9 (2)	0.2 - 3.6
	Ngereyani	63	3.2 (2)	70	0.0 (0)	67	0.0 (0)	200	1.0 (2)	0.2 - 3.9
	Orkejuloongishu	74	2.7 (2)	74	8.1 (6)	75	4.0 (3)	223	4.9 (11)	2.6 - 8.9

District	Village	Cattle		Goats		Sheep		Overall seroprevalence		
		Tested	% positive (n)	Tested	%positive (n)	Tested	%positive (n)	Tested	%positive (n)	95%CI
Mbulu	Endanyawish	102	2.0 (2)	60	0.0 (0)	49	0.0 (0)	211	0.9 (2)	0.0 - 3.7
	Long	175	0.6 (1)	49	0.0 (0)	37	0.0 (0)	261	0.4 (1)	0.0 - 2.5
	Maheri	240	2.1 (5)	167	1.2 (2)	103	1.0 (1)	510	1.6 (8)	0.7 - 3.2
Meru	Kisimiri	100	0.0 (0)	63	0.0 (0)	65	0.0 (0)	228	0.0 (0)	0.0 - 2.1
	Nambala	136	2.2 (3)	113	2.7 (3)	103	0.0 (0)	352	1.7 (6)	0.7 - 3.9
Monduli	Alkaria	36	0.0 (0)	45	0.0 (0)	38	0.0 (0)	119	0.0 (0)	0.0 - 3.9
	Lengoolwa	66	15.2 (10)	75	0.0 (0)	63	1.6 (1)	204	5.4 (11)	2.9 - 9.7
	Meserani	45	6.7 (3)	44	0.0 (0)	46	0.0 (0)	135	2.2 (3)	0.6 - 6.8
	Naiti	197	4.1 (8)	261	3.4 (9)	136	1.5 (2)	594	3.2 (19)	1.9 - 5.0
	Selela	33	6.1 (2)	40	15.0 (6)	42	4.8 (2)	115	8.7 (10)	4.5 - 15.8
Moshi	Bonite	4	0.0 (0)	27	0.0 (0)	7	0.0 (0)	38	0.0 (0)	0.0 - 11.4
	Kaloleni	7	0.0 (0)	85	0.0 (0)	3	0.0 (0)	95	0.0 (0)	0.0 - 4.8
	Karanga	3	0.0 (0)	5	0.0 (0)	0	0.0 (0)	8	0.0 (0)	0.0 - 40.2
	Kariwa chini	8	0.0 (0)	18	0.0 (0)	2	0.0 (0)	28	0.0 (0)	0.0 - 15.0
	Magereza	7	0.0 (0)	7	0.0 (0)	3	0.0 (0)	17	0.0 (0)	0.0 - 22.9

District	Village	Cattle		Goats		Sheep		Overall seroprevalence		
		Tested	% positive (n)	Tested	%positive (n)	Tested	%positive (n)	Tested	%positive (n)	95%CI
	Majengo	7	0.0 (0)	39	0.0 (0)	0	0.0 (0)	46	0.0 (0)	0.0 - 9.6
	Sabasaba	5	0.0 (0)	32	0.0 (0)	19	0.0 (0)	56	0.0 (0)	0.0 - 7.9
	Shirimatunda	8	0.0 (0)	8	12.5 (1)	5	0.0 (0)	21	4.8 (1)	0.2 - 25.9
Ngorongoro	Embarway	30	3.3 (1)	30	0.0 (0)	30	0.0 (0)	90	1.1 (1)	0.0 - 6.9
	Enguserosambu	151	4.0 (6)	88	3.4 (3)	100	1.0 (1)	339	2.9 (10)	0.0 - 5.5
	Oloipiri	143	2.1 (3)	118	0.8 (1)	135	0.0 (0)	396	1.0 (4)	0.3 - 2.7
Rombo	Shimbi mashariki	8	0.0 (0)	43	0.0 (0)	5	20.0 (1)	56	1.8 (1)	0.0 - 10.8
Rural Moshi	Kileuo	12	0.0 (0)	17	0.0 (0)	9	0.0 (0)	38	0.0 (0)	0.0 - 11.4
	Kindi	5	0.0 (0)	7	0.0 (0)	6	0.0 (0)	18	0.0 (0)	0.0 - 21.9
	Kiria	10	10.0 (1)	20	5.0 (1)	0	0.0 (0)	30	6.7 (2)	1.2 - 23.5
	Lombeta	10	0.0 (0)	20	0.0 (0)	0	0.0 (0)	30	0.0 (0)	0.0 - 14.1
	Maua	11	0.0 (0)	3	0.0 (0)	7	0.0 (0)	21	0.0 (0)	0.0 - 19.2
	Utamaduni	16	12.5 (2)	57	0.0 (0)	12	0.0 (0)	85	2.4 (2)	0.4 - 9.0
Simanjiro	Komolo	126	2.4 (3)	157	0.0 (0)	121	1.7 (2)	404	1.2 (5)	0.5 - 3.0
	Ngage	170	12.4 (21)	88	0.0 (0)	93	0.0 (0)	351	6.0 (21)	3.8 - 9.1

District	Village	Cattle		Goats		Sheep		Overall seroprevalence		
		Tested	% positive (n)	Tested	%positive (n)	Tested	%positive (n)	Tested	%positive (n)	95%CI
	Ruvu remiti	164	9.8 (16)	177	1.1 (2)	178	23.0 (41)	519	11.4 (59)	8.8 - 14.5
	Sukuro	159	3.8 (6)	138	0.0 (0)	134	3.0 (4)	431	2.3 (10)	1.2 - 4.4

5.3.2 Risk factors for RVFV Seropositivity in livestock

5.3.2.1 Univariable logistic regression analysis of risk factors for RVFV seropositivity in livestock

Univariable analysis identified potential risk factors considered for inclusion in the multivariable analysis. Some of the variables were statistically significant ($p<0.05$) in the univariable analyses. These included species, sex, age, history of livestock abortions, use of seasonal camps, confining cattle with small ruminants, and mosquito vector suitability. Table 5.2, Table 5.3 and Table 5.4 summarizes the potential risk factors for cattle, goats and sheep that were examined to be considered for inclusion in the multivariable logistic regression analysis.

Table 5.2 Univariable analysis of risk factors RVFV seropositivity in cattle in northern Tanzania

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Cattle					
Sex					
Female	2409	128 (5.3)	Ref.	Ref.	Ref.
Male	1169	28 (2.4)	0.4	0.3 - 0.7	<0.001
Age					
Age (years)	3582	156 (4.4)	1.2	1.1 - 1.2	<0.001
Farming classification					
Small-holder	146	7 (4.8)	Ref.	Ref.	Ref.
Agro-pastoral	973	35 (3.6)	0.7	0.3 - 1.7	0.479
Pastoral	2463	114 (4.6)	0.9	0.4 - 2.1	0.926
Herd size					
Number of cattle			1.1	1.0 - 1.3	0.0389

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Number of cross breed cattle			0.7	0.4 - 1.3	0.272
Number of exotic breed cattle			0.9	0.8 - 1.2	0.677
Cattle introduced into compound					
No	2433	114 (4.7)	Ref.	Ref.	Ref.
Yes	973	37 (3.8)	0.8	0.6 - 1.2	0.259
Cattle free range					
No	3250	144 (4.4)	Ref.	Ref.	Ref.
Yes	153	7 (4.6)	1.0	0.5 - 2.3	0.932
Cattle herding					
No	298	12 (4.0)	Ref.	Ref.	Ref.
Yes	3109	139 (4.5)	1.1	0.6 - 2.0	0.757
Cattle zero grazing					
No	3137	144 (4.6)	Ref.	Ref.	Ref.
Yes	266	7 (2.6)	0.5	0.3 - 1.2	0.142
Cattle ronjo (seasonal camps)					
No	1553	52 (3.4)	Ref.	Ref.	Ref.
Yes	1164	57 (4.9)	1.5	1.0 - 2.2	0.043
Graze cattle with small ruminants					
No	2605	118 (4.5)	Ref.	Ref.	Ref.
Yes	801	33 (4.1)	0.9	0.6 - 1.3	0.622
Confine cattle with small ruminants					
No	3178	148 (4.7)	Ref.	Ref.	Ref.
Yes	228	3 (1.3)	0.3	0.1 - 0.8	0.0269
See buffalo in village					

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
No	2365	90 (3.8)	Ref.	Ref.	Ref.
Yes	486	23 (4.7)	1.3	0.8 - 2.0	0.341
Standing water in the compound					
No	3116	140 (4.5)	Ref.	Ref.	Ref.
Yes	285	11 (3.8)	0.8	0.5 - 1.6	0.62
Cattle abortion					
No	2468	95 (4.0)	Ref.	Ref.	Ref.
Yes	936	56 (6.0	1.6	1.1 - 2.2	0.0074
Cattle death by disease					
No	1963	65 (3.3)	Ref.	Ref.	Ref.
Yes	888	48 (5.4)	1.7	1.1 - 2.4	0.00857
Aedes spp suitability			2.6	1.4 - 4.6	0.0018
Culex spp suitability			2.1	1.1 - 3.8	0.0182

Table 5.3 Univariable analysis of risk factors for RVFV seropositivity in goats

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Sex					
Female	2533	40 (1.6)	Ref.	Ref.	Ref.
Male	770	5 (0.7)	0.4	0.2 - 1.0	0.0592
Age			1.6	1.4 - 1.8	<0.001
Farming Classification					
Small holder	424	2 (0.5)	Ref.	Ref.	Ref.
Agro-pastoral	683	7 (1.0)	2.2	0.5 - 10.6	0.3311

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Pastoral	2196	36 (1.6)	3.5	0.8 - 14.7	0.0843
Flock size					
Number of goats			1.1	0.8 - 1.4	0.676
Number of exotic breed goats			1.3	1.1 - 1.5	0.00204
Goats intro into compound					
No	2440	35 (1.4)	Ref.	Ref.	Ref.
Yes	716	9 (1.3)	0.9	0.4 - 1.8	0.722
Goats free range					
No	2964	34 (1.2)	Ref.	Ref.	Ref.
Yes	192	10 (5.2)	4.7	2.3 - 9.7	<0.001
Goats herding					
No	475	12 (2.5)	Ref.	Ref.	Ref.
Yes	2681	32 (1.2)	0.5	0.2 - 0.9	0.0257
Goats ronjo (seasonal camps)					
No	1472	17 (1.2)	Ref.	Ref.	Ref.
Yes	710	10 (1.4)	1.2	0.6 - 2.7	0.616
Graze cattle with small ruminants					
No	2421	30 (1.2)	Ref.	Ref.	Ref.
Yes	721	14 (2.0)	1.6	0.8 - 2.9	0.181
Confine cattle with small ruminants					
No	2945	42 (1.4)	Ref.	Ref.	Ref.
Yes	211	2 (1.0)	0.7	0.2 - 2.8	0.57
See buffalo in village					
No	1932	22 (1.1)	Ref.	Ref.	Ref.

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Yes	373	5 (1.3)	1.2	0.4 - 3.1	0.74
Standing water in the compound					
No	2849	39 (1.4)	Ref.	Ref.	Ref.
Yes	294	5 (1.7)	1.3	0.5 - 3.2	0.645
Goats abortion					
No	1722	15 (1.0)	Ref.	Ref.	Ref.
Yes	1434	29 (2.0)	2.4	1.3 - 4.4	0.00763
Goats death by disease					
No	1095	11 (1.0)	Ref.	Ref.	Ref.
Yes	1210	16 (1.3)	1.3	0.6 - 2.8	0.48
***Aedes spp* suitability**			1.8	1.5 - 2.3	<0.001
***Culex spp* suitability**			2.0	1.3 - 2.6	<0.001

Table 5.4 Univariable analysis of risk factors for RVFV seropositivity in sheep

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Sex					
Female	1971	62 (3.2)	Ref.	Ref.	Ref.
Male	615	5 (1.0)	0.3	0.1 - 0.6	0.00319
Age (years)			1.5	1.3 - 1.7	<0.001
Farming classification					
Small-holder	89	1 (1.1)	Ref.	Ref.	Ref.
Agro-pastoral	458	1 (0.2)	0.2	0.0 - 3.1	0.246
Pastoral	2039	65 (3.2)	2.9	0.4 - 21.1	0.294

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Flock size (continuous)			1.1	0.9 - 1.3	0.0976
Sheep intro into compound					
No	1984	42 (2.1)	Ref.	Ref.	Ref.
Yes	492	19 (4.0)	1.8	1.1 - 3.2	0.0277
Sheep herding					
No	316	5 (1.6)	Ref.	Ref.	Ref.
Yes	2160	56 (2.6)	1.7	0.7 - 4.2	0.284
Sheep ronjo (seasonal camps)					
No	1222	19 (1.6)	Ref.	Ref.	Ref.
Yes	625	32 (5.1)	3.4	1.9 - 6.1	<0.001
Graze cattle with small ruminants					
No	1857	55 (3.0)	Ref.	Ref.	Ref.
Yes	619	6 (1.0)	0.3	0.1 - 0.7	0.00855
Confine cattle with small ruminants					
No	2340	59 (2.5)	Ref.	Ref.	Ref.
Yes	136	2 (1.5)	0.6	0.1 - 2.4	0.448
See buffalo in village					
No	1582	45 (3.0)	Ref.	Ref.	Ref.
Yes	380	7 (2.0)	0.6	0.3 - 1.4	0.278
Standing water in the compound					
No	2219	53 (2.4)	Ref.	Ref.	Ref.
Yes	255	8 (3.1)	1.3	0.6 - 2.8	0.467
Sheep abortion					
No	1449	23 (1.6)	Ref.	Ref.	Ref.

Variable	Tested (n)	Positive (n (%))	OR	95% CI	P- value
Yes	1027	38 (3.7)	2.38	1.4 - 4.0	0.00117
Sheep death by disease					
No	1065	21 (2.0)	Ref.	Ref.	Ref.
Yes	887	31 (3.5)	1.82	1.0 - 3.2	0.0369
***Aedes spp* suitability (continuous)**			1.01	0.8 - 1.3	0.949
***Culex spp* suitability (continuous)**			0.80	0.6 - 1.0	0.0818

5.3.2.2 Multivariable logistic regression analysis of risk factors for seropositivity in livestock

Factors included in the multivariable model for all livestock species combined include age of livestock, species, age, occurrence of abortions, and confining cattle with small ruminants. Two factors (age and abortions) were observed as significant for all three livestock species combined as well as for sheep and goats when species were treated separately. Table 5.5 summarises risk factors as identified by the multivariable models for all species, cattle, goats and sheep respectively.

Table 5.5 Multivariable analysis of risk factors for RVFV seropositivity in all species combined, cattle, goats and sheep in northern Tanzania

Variable	OR	95% CI	P-value
All species combined			
Species			
Cattle	**ref.**	**ref.**	**ref.**
Goats	0.4	0.3 - 0.6	<0.001
Sheep	0.8	0.5 - 1.1	0.100

Variable	OR	95% CI	P-value
Age (years)	1.3	1.2 - 1.3	<0.001
Abortions in past 12 months	1.5	1.1 - 2.1	0.017
Cattle			
Age (years)	1.2	1.1 - 1.3	<0.001
Confine cattle with small ruminants	0.2	0.1 - 0.9	0.035
Goats			
Age (years)	1.6	1.4 - 1.8	<0.001
Abortions in past 12 months	2.5	1.1 - 5.4	0.023
Sheep			
Age (years)	1.7	1.4 - 2.1	<0.001
Abortions in past 12 months	2.7	1.1 - 6.3	0.025

Cattle had higher odds of seropositivity than sheep and goats. The odds of seropositivity increased with age of animals of all species. Seropositivity in cattle increased 1.2 times with each increase in age by one year and herds that confined cattle with small ruminants were about 0. 2 times more likely to be seropositive. In goats and sheep, seropositivity increased about 2 times with increase in age by one year. On the other hand, the odds of seropositivity in goats and sheep from flocks that had abortions were about three times higher than those with no record of abortions.

5.3.3 Seroprevalence in humans

In humans, the overall RVFV seroprevalence was 8.5% (n=565, 95% CI: 6.4 - 11.2). Although the difference in exposure between males and females was not statistically significant, seroprevalence was relatively higher in males (10.4%, n=270, 95% CI: 7.1 - 14.8) than females (6.8%, n=295, 95% CI: 4.3 - 10.4). Seropositivity was also recorded in young people <13 years old. Seropositivity was recorded in 15 out of 37 villages sampled with notably higher

seroprevalence (>20%) recorded in 5 villages across the study area (Figure 5.5). Seroprevalence in humans varied between villages and between households within villages. Seroprevalence varied by around four times (MOR=4.4) between villages and by almost five times (MOR = 4.6) between households.

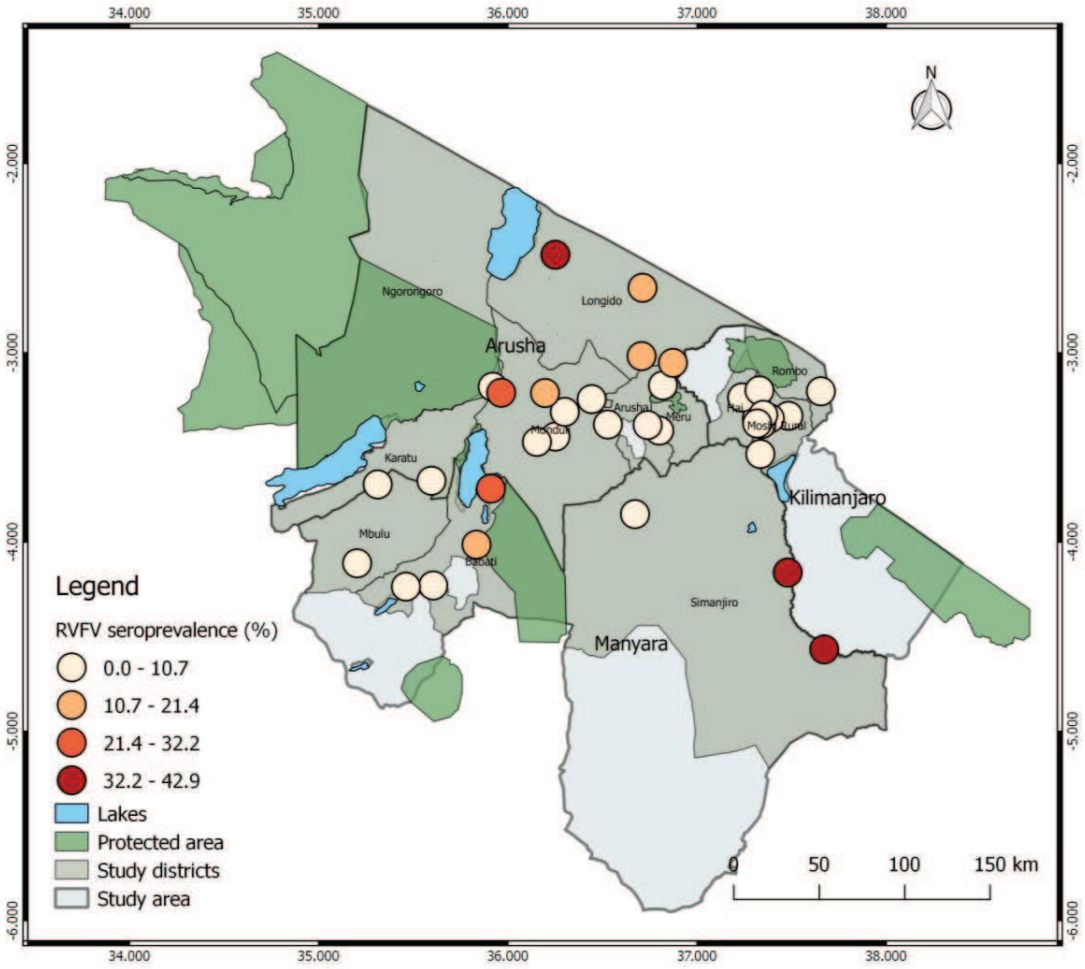

Figure 5.5. Map of northern Tanzania showing study regions, districts and the village-level seroprevalence in humans.

5.3.4 Risk factors for seropositivity in humans

5.3.4.1 Univariable logistic regression analysis of risk factors for RVFV seropositivity in humans

Table 5.6 summarizes the potential risk factors that were considered for inclusion in a multivariable logistic regression analysis.

Table 5.6 Univariable analysis of risk factors for RVFV seropositivity in human

Variable	Tested	Prevalence (% (n))	OR	95% CI	P-value
Sex					
Female	295	6.8 (20)	ref	ref	ref
Male	270	10.4 (28)	1.8	0.8 - 3.7	0.12
Age (years)			1.0	0.9 - 1.0	0.52
Farming classification					
Small holder	143	1.4 (2)	ref	ref	
Agro-pastoral	231	2.6 (6)	3.2	0.5 - 21.8	0.229
Pastoral	235	14 (33)	11.5	2.1 - 64.3	0.005
Education					
No formal education	208	12 (25)	ref	ref	ref
Primary education	342	4.1 (14)	0.6	0.3 - 1.4	0.257
Secondary/High school	44	16.7 (2)	1.5	0.2 - 9.7	0.696
University education	11	10 (1)	1.6	0.1 - 22.9	0.746
Occupation					
Agric/crop production	128	2.3 (3)	ref	ref	ref
Livestock/rancher	358	10.1 (36)	5.1	1.1 - 23.6	0.04
Other	121	2.5 (3)	1.3	0.2 - 8.1	0.76
Milked animals					
No	375	6.9 (26)	ref	ref	ref

Variable	Tested	Prevalence (% (n))	OR	95% CI	P-value
Yes	234	6.8 (16)	0.7	0.3 - 1.5	0.392
Sleep in the same house as animals					
No	512	6.8 (35)	ref	ref	ref
Yes	96	7.2 (7)	1.3	0.5 - 3.8	0.618
Handled animal waste					
No	328	8.5 (28)	ref	ref	ref
Yes	281	5 (14)	0.4	0.2 - 0.9	0.0428
Birthing animals					
No	385	3.4 (13)	ref	ref	ref
Yes	222	13.1 (29)	2.7	1.2 - 6.1	0.0137
Handled placenta					
No	431	6 (26)	ref	ref	ref
Yes	178	9 (16)	0.97	0.4 - 2.2	0.933
Handled aborted product					
No	548	5.7 (31)	ref	ref	ref
Yes	61	18 (11)	3.5	1.4 - 9.0	0.008
Slaughtered animals					
No	351	3.7 (13)	ref	ref	ref
Yes	258	11.2 (29)	2.4	1.1 - 5.3	0.024
Handled carcass					
No	453	5.3 (24)	ref	ref	ref
Yes	156	11.5 (18)	1.8	0.8 - 4.2	0.129
Consumed raw milk					
No	512	4.1 (21)	ref	ref	ref

Variable	Tested	Prevalence (% (n))	OR	95% CI	P-value
Yes	94	22.3 (21)	3.6	1.5 - 9.1	0.005
Consumed raw yoghurt					
No	379	4.7 (18)	ref	ref	ref
Yes	229	10.5 (24)	1.4	0.6 - 3.1	0.397
Seropositive livestock					
No	334	5.1 (17)	ref	ref	ref
Yes	175	14.3 (25)	3	0.9 - 3.7	0.065

5.3.4.2 Multivariable logistic regression analysis of risk factors for seropositivity in humans

Factors that were significant in the multivariable model included handling of aborted material, and consumption of raw milk (Table 5.7). People who had handled aborted material and those who consumed raw milk were four times more likely to be RVFV seropositive than those who did not.

Table 5.7 Multivariable analysis of risk factors for RVFV seropositivity in humans

Variable	OR	95% CI	P-value
Handled aborted material	4.3	1.7 -10.8	0.002
Consumed raw milk	4.1	1.8 - 9.3	0.001

5.4 Discussion

The sero-epidemiological study presented here reports detection of RVFV antibodies (IgG and IgM) in apparently healthy domestic ruminants and also evaluates the risk of exposure to the virus in livestock and human populations of northern Tanzania. Although recent outbreaks

have been reported in neighbouring countries, Uganda , Kenya and Rwanda (Anyamba et al., 2018), no RVF outbreak has been reported in Tanzania since the last epidemic of 2006–2007. This study reports evidence for RVFV exposure in domestic ruminants and humans in northern Tanzania ten years after the last outbreak. Inter-epidemic seropositivity in livestock has been reported by similar studies in the region including 10% (n=654) seroprevalence in small ruminants in central Mozambique (Fafetine et al., 2013), 16.8% (n=595) in cattle in Rwanda (Umuhoza et al., 2017) and 9.8% (n=1470) in small ruminants in Uganda (Magona et al., 2013). Inter-epidemic seropositivity in humans has also been reported (LaBeaud et al., 2008, Lichoti et al., 2014a, Mbotha et al., 2018) including 13% (n=248) in north-eastern Kenya (LaBeaud et al., 2008) and 12% (n=655) in Kabale district, Uganda (Nyakarahuka et al., 2018).

In the current study, seroprevalence was significantly higher in cattle than sheep or goats. Difference in seroprevalence between ruminant species has been reported in other parts of Tanzania (Sumaye et al., 2013, Sindato et al., 2015), Kenya (Lichoti et al., 2014a) and Uganda (Nyakarahuka et al., 2018) suggesting different levels of exposure between host species. The effect of species remained significant even when controlling for age, indicating that the species effect cannot be explained only by the greater longevity of cattle in comparison with sheep and goats. The difference in seroprevalence could also be due to mosquito feeding behaviour, as mosquitoes tend to select large (Takken and Verhulst, 2013) and coloured host species. In periods of drought, pastoralists would prefer to take cattle than small ruminants for seasonal camps (ronjo) searching for pasture and water in areas which stay wet most of the year, usually lowlands experiencing floods or with permanent water bodies. This would also expose cattle to potential mosquito bites and infection as many herds gather together at these camps.

In this study, RVFV seropositivity was detected across all age groups of ruminant species tested, with an increase with age consistent with endemic circulation of the virus. Similar observations were reported in serosurveys in Kenya (Anyamba et al., 2009, LaBeaud et al., 2008) and Mozambique (Fafetine et al., 2007) which were conducted one and two years from the 2006-2007 outbreak in East Africa. In this study we report seropositivity in animals as young as 1-2 years and the detection of IgM antibodies may suggest recent infections of the

virus in the study area about ten years from the previous outbreak. These findings suggest undetected circulation of the virus in the area outside of an epidemic period. The detection of IgM antibodies illustrates recent infection preceding the serosurvey as IgM antibodies to RVFV can only be detected up to two months after infection (Morvan et al., 1991, Paweska et al., 2003a). The circulation of RVFV during the inter-epidemic period suggests that clinical RVF cases may have occurred but have been mistaken for other diseases or not reported in the absence of public awareness.

In addition to age of an animal, occurrence of abortions in the herd/flock was associated with inter-epidemic seropositivity. Although abortions can be linked to a number of other infections, the association of RVFV seroposivity with abortions may be attributed to missed or unreported RVF cases in the study area. This could be due to a lack of adequate surveillance. Abortions could also represent an RVFV transmission route within herds/flocks through contact with aborted materials. This also poses a risk for RVFV transmission to humans which is supported by findings from the current study and other studies (LaBeaud et al., 2008, Magona et al., 2013) which have found a strong association between seropositivity in humans and handling aborted materials.

Since the last RVF outbreak in Tanzania, seropositivity in human populations has been reported in other parts of the country (Heinrich et al., 2012b, Sumaye et al., 2015, Ahmed et al., 2018). In the current study 8.5% seroprevalence is reported in northern Tanzania, including in young people less than 13 years old. Although a higher seroprevalence in humans was observed in pastoral than agro-pastoral and smallholder settings, the difference was not statistically significant. RVF is typically reported as outbreaks in pastoral areas of Tanzania, but the finding of human seropositivity across settings suggests that RVF cases may be occurring in other agro-ecological systems and surveillance efforts should not be directed exclusively to pastoral settings. This conclusion is further supported by results from Chapter Six.

The current study reports a significant association between RVFV seropositivity in people and consumption of raw milk. The consumption of raw milk has been considered an important risk factor for human exposure during epidemics (Woods et al., 2002, LaBeaud et al., 2008,

LaBeaud et al., 2011) and other studies have reported similar observations (Anyangu et al., 2010, Nicholas et al., 2014). Small amounts of RVFV have been found in the milk of experimentally infected cattle, (Alexander, 1951), and saliva and nasal discharges of infected sheep and cattle (Swanepoel and Paweska, 2011a), suggesting that consuming infected raw milk could represent another pathway for the virus to be introduced into the body (Nicholas et al., 2014). This question needs to be further explored as there is not enough evidence supporting RVFV transmission through milk. However, unpasteurised milk is known to be associated with a number of other diseases including tuberculosis and brucellosis. It is important therefore, to ensure people are aware of the risks of raw milk consumption especially in communities where this is a traditional and widespread practice.

People who handled aborted materials were more likely to be seropositive than those who did not. Similar observations have been reported in other studies in Tanzania (Sumaye et al., 2015), Kenya (Anyangu et al., 2010), and Uganda (Nyakarahuka et al., 2018). This could be possible because RVFV is highly infectious via the aerosol route, which is evident by the number of laboratory workers who have become infected (Alexander, 1951) and the potential for infection of veterinarians and abattoir workers who handle infected animals (Hoogstraal et al., 1979, Ross et al., 2012). Infection might also be possible through broken skin if handled infected material without appropriate protective equipment.

5.5 Conclusion

Results reported here shows RVFV seropositivity in cattle, goats and sheep of all age groups and human populations during an inter-epidemic period in northern Tanzania and identifies risk factors for exposure. The association of seropositivity with abortions may suggest missed or unreported cases due to lack of active surveillance. Therefore, enhanced surveillance of RVF and other abortigenic pathogens is recommended. This should involve specific training to livestock field officers and community healthcare workers to be able to quickly identify the initial RVF cases for immediate response and reporting which would improve preparedness, response and control strategies. In addition, continuous farmer education and awareness or sensitization on zoonoses such as RVF and risks for exposure, is essential. The education of

farmers should be in a simple language and translated to local languages for specific communities. The education materials should include messages that emphasize animal disease signs and symptoms, reporting of suspect RVF cases to livestock field officers or veterinarians, general hygiene such as washing hands after handling aborted materials or other animal materials of suspect case, cooking meat and boiling milk thoroughly.

Chapter Six

6 Molecular survey of Rift Valley fever virus in mosquitoes and diagnostic samples and milk from aborting livestock in northern Tanzania

6.1 Introduction

Rift Valley fever virus (RVFV) is a zoonotic *Phlebovirus* that can be transmitted to ruminants and humans by mosquitoes or through direct contact with contaminated bodily fluids and tissues (Davies et al., 1985, Gerdes, 2004). In East Africa, major Rift Valley fever (RVF) outbreaks have occurred in intervals of 5 to 15 years (Mohamed et al., 2010, Nderitu et al., 2010) usually associated with unusually heavy rains. The most recent outbreak in East Africa was reported in Kenya, Uganda and Rwanda between June and August 2018, but, despite an RVF alert being issued by the Ministry of Livestock and Fisheries in Tanzania, no outbreak was reported at the time. Although RVFV has been studied for about eight decades since it was first reported in East Africa, many aspects of its maintenance and ecology in periods between the epidemics ("inter-epidemic periods") are not fully understood. Evidence for RVFV circulation in the region has been supported by studies that are based primarily on serological evidence and limited molecular epidemiological studies, mostly following outbreaks. Epidemiological studies following the 2007 outbreak in Kenya detected RVFV using molecular techniques performed on samples from cattle, sheep and goats (Munyua et al., 2010) and mosquitoes (Lutomiah et al., 2014, Sang et al., 2010). In Tanzania, similar studies involving humans (Mohamed et al., 2010) and livestock (Chengula et al., 2014) were conducted using molecular tests using samples collected following the outbreak of 2006/2007. However, there is still limited molecular evidence for the inter-epidemic circulation of RVFV in Tanzania and the region. Molecular diagnostic tools provide confirmation of the presence of RVFV RNA for improved confidence in clinical diagnostic results and confirmation of RVF cases (Njenga et al., 2009). Molecular techniques such as reverse-transcriptase polymerase chain reaction (RT-PCR) allow for a rapid and accurate detection of RVFV (Escadafal et al., 2013, Njenga et al.,

2009) and is recommended as an efficient diagnostic tool for the investigation of endemic circulation of the RVFV. It allows the detection of low viral RNA loads adapted for the investigations of reservoirs or specific epidemiological situations such as inter-epidemic periods (Maquart et al., 2014).

Human exposures to RVFV have been attributed to a number of infection routes as observed in previous outbreaks. These routes include direct contact with infected animal tissues, blood, or other body fluids by handling aborted materials, helping with parturition, and slaughter, inhalation of aerosolized infected fluids, and transmission through bites of infected mosquito vectors (Gerdes, 2004, Pepin et al., 2010, Anyangu et al., 2010). Ingestion of raw and unpasteurized milk has also been epidemiologically associated with RVF exposure in humans in previous outbreaks (LaBeaud et al., 2011, Mohamed et al., 2010, Woods et al., 2002). However, the link between consumption of milk from infected animals and human exposure has not been demonstrated conclusively, and there is only limited evidence from experimentally infected animals (Alexander, 1951) and no reports of RVFV detection in milk from naturally infected animals. Findings from our serological study (see Chapter four) and as analysed previously (Grossi-Soyster et al., 2019) suggests an association of consumption of raw milk with human exposure to RVFV.

The main aim of this study was to detect RVFV genomic RNA in mosquitoes, aborted materials and milk samples collected during the inter-epidemic period in study areas of northern Tanzania during a time period (2017-2019) when there were no outbreaks reported in study areas of northern Tanzania. This study used RT-PCR to investigate RVFV circulation during the inter-epidemic period through testing of (a) mosquito vectors and (b) diagnostic material collected from abortion cases reported in cattle, sheep and goats. Data generated in this study will lead to a better understanding of RVFV maintenance in the inter-epidemic periods and risk factors for human exposure. This will also inform surveillance strategies and national and regional emergency preparedness and response plans, and identify avenues for future research.

6.2 Methods

6.2.1 Study area

The study involved analysis of mosquitoes, aborted materials and milk samples collected from Arusha, Kilimanjaro and Manyara Regions of northern Tanzania (Figure 6.1) between March 2016 and August 2019. The area include districts where RVF cases were reported in previous outbreaks in 1977, 1998 and 2006/2007 (Mohamed et al., 2010, Fyumagwa et al., 2011, Chengula et al., 2013). Samples used in this study were collected as part of the two epidemiological studies carried out in the area, namely 'Social, Economic and Environmental drivers of Zoonoses' (SEEDZ) and 'Supporting Evidence Based Interventions to Achieve Agricultural Development Goals in Tanzania' (SEBI-TZ) described in Chapter Two.

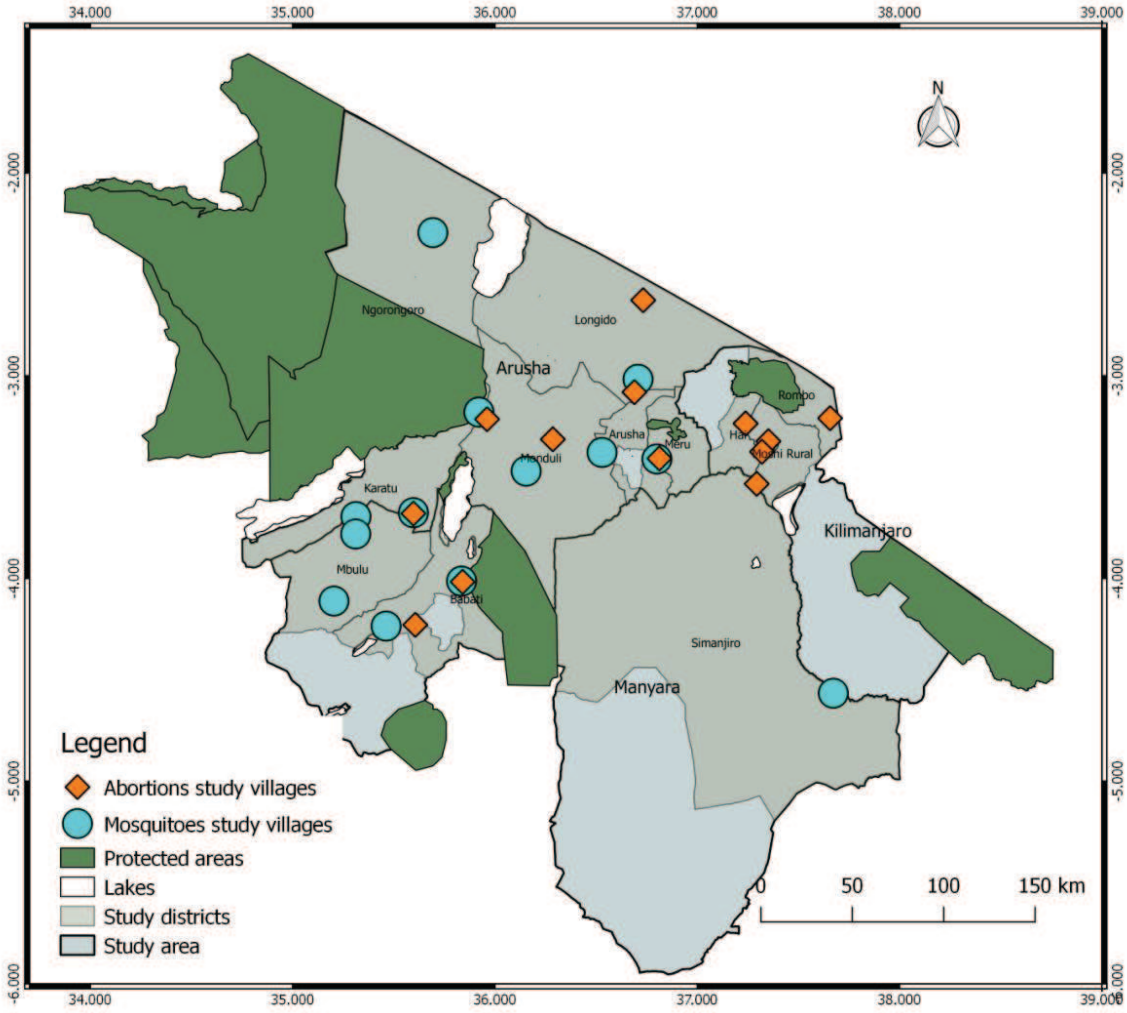

Figure 6.1 Map of northern Tanzania (Arusha, Kilimanjaro and Manyara regions) showing districts and location of SEEDZ villages where mosquito sampling was carried out and villages where abortion events were reported and from which samples were collected through the SEBI-TZ study project.

6.2.2 Sample collections

Details of sample collection and processing are described in Chapter two. Briefly, mosquitoes were collected in 12 villages randomly selected from the 20 SEEDZ study villages between

2015 and 2017. Collected mosquitoes were identified to genera or species level using morphological identification keys (Gillies and Coetzee, 1987, Huang, 2001, Jupp, 1996), separated according to genera, sex, trap type, site and date of collection, into pools of 1-25 mosquitoes and preserved in labelled 2-ml cryovials containing TRIzol reagent (Thermo Fisher Scientific, Loughborough, UK) and stored in -80 ^{0}C freezer until further RNA extraction and PCR.

Livestock abortion samples and milk, were collected in response to reported abortion events from the SEBI study villages. Following the report of an abortion or peri-natal mortality event, recruited Livestock Field Officers (LFOs) or members of the study field team attended the cases to collect samples within 72 hours of the abortion/still birth event. In addition to basic farm level data, the following samples were collected: (i) blood, milk and vaginal swab samples from the aborting cow/ewe/doe; (ii) tissue from the placental inter-cotyledonary space; (iii) placental cotyledon; (iv) foetal organs (liver, lung and kidney, thymus); and (iv) foetal stomach contents. Only four sample types were used in the current study, namely vaginal swabs and milk samples from the aborted dam, swabs from aborted foetus, placenta cotyledon tissue samples. The abortion cases reported here are those which were recorded, followed by sample collection as part of the SEBI-TZ study, between October, 2017 when the study began and July, 2019.

6.2.3 RNA preparation

The RNA extraction process is described in detail in Chapter two. RNA extraction from mosquito samples was based on Direct-zol™ (Zymo Research, CA, U.S.A) RNA preparation protocol as per manufacturers' instructions. Whereas RNA preparation from swabs, milk and placental tissue was based on the Qiagen RNeasy Mini kit (QIAGEN, Manchester, UK) according to manufacturer's instructions with slight modification.

6.2.4 Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Detection of RVFV by one-step quantitative reverse-transcription real-time PCR (RT-qPCR) using RNA samples was carried out as previously described (Drosten et al., 2002). One-step

assay combine reverse transcription and PCR in a single tube, using a reverse transcriptase along with a DNA polymerase and utilizing sequence-specific for RVFV Gc gene primers RVS (AAAGGAACAATGGACTCTGGTCA[349-371]), RVAs (CACTTCTTACTACCATGTCCTCCAAT [443-417]), and dual-labelled probe RVP (FAM-AAAGCTTTGATATCTCTCAGTGCCCCAA-BHQ1 [388-416]).

6.2.5 Data management

Data from sample collection records and laboratory results were managed in Excel 2007. Data were cleaned and analysed in R version 3.5.3 (R Core Team, 2019). In this study, samples (mosquitoes and/or abortion materials) with complete information (ID, date, village, species, sample type) and RT-PCR results for RVFV were considered for analyses. Maps were produced in QGIS v 2.14.0-Essen. Sample collection villages were geocoded using GPS coordinates recorded during sample collections.

6.3 Results

Three hundred and twelve pools of mosquitoes, 190 vaginal swabs from dams, 61 foetal swabs, 42 aborted placenta cotyledon tissue samples, and 159 milk samples from abortion events involving cattle, goats and sheep were tested for RVFV by real time RT-qPCR. About 190 abortion events were investigated.

6.3.1 RVFV detection in mosquito vector species

A total of 312 mosquito pools, including 17 *Aedes spp*, 137 *Culex spp*, 20 *Mansonia spp*, 133 *Anopheles spp*, and 5 *Coquillettidia spp*, were screened for Rift Valley fever virus by RT-qPCR. None of the mosquito pools tested positive for RVFV infection (Figure 6.2).

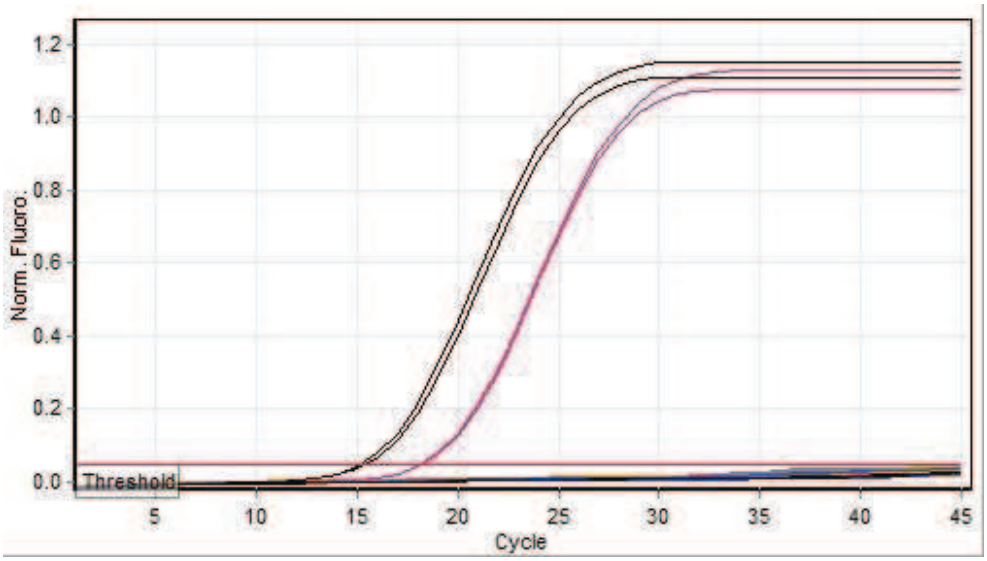

Figure 6.2 Amplification curves for RT-qPCR of RVFV, showing normalized fluorescence intensity over time (PCR cycles) for mosquito samples, positive controls and negative controls. The black and red curves above the amplification threshhold in duplicates are positive controls (two different dilutions) and the lines below the threshold line are negative controls and test samples which did not amplify above the threshold.

6.3.2 RVFV detection in vaginal and foetal swabs, placenta tissue and milk

RVFV was detected in 14 (7.4%) out of 190 abortion cases tested by RT-qPCR. RVFV was detected in 11 (5.8%) of the 190 dam vaginal swabs, 9 (14.8%) of the 61 foetal swabs, 6 (14.3%) of the 42 aborted placenta cotyledon tissue samples, and 3 (1.9%) of the 159 milk samples tested. All positive swabs, placenta tissue and milk samples were from cattle, no RVFV was detected in samples from goats and sheep. The prevalence of RVFV based on RT-qPCR by species and sample type is shown in Table 6.1.

Table 6.1 RT-qPCR results of samples collected from abortion cases clustered by species and sample type showing number of each sample type tested and those tested positive for RVFV

Species	Sample type	Tested (n)	Positive (n)	Prevalence (%)
Cattle	Dam-swab	64	11	17.2
	Foetus-swab	35	9	25.7

Species	Sample type	Tested (n)	Positive (n)	Prevalence (%)
	Milk	63	3	4.8
	Placenta	34	6	17.7
Goats	Dam-swab	85	0	0
	Foetus-swab	21	0	0
	Milk	76	0	0
	Placenta	7	0	0
Sheep	Dam-swab	41	0	0
	Foetus-swab	5	0	0
	Milk	20	0	0
	Placenta	1	0	0
Total		452	29	6.42

In this study four sample types (dam swab, foetus swab, milk, and placenta tissue) collected from abortion cases were used. Serological (ELISA) testing was also carried out for each case and results for the same are included in Table 6.2. It is worth noting that of the 14 abortion cases which were RVFV positive, 11 tested positive for more than one sample type. Two of the three positive milk samples corresponded with the positive swabs from the dam and foetus, whereas one corresponded with the positive placenta tissue. Table 6.2 shows RT-qPCR results of different sample types per abortion case clustered by study village.

Table 6.2 RT-qPCR results of samples collected from abortion cases clustered by site abortion case and sample type showing RVFV status as positive (+) or negative (-) and unavailable sample (na)

Village	Sample/ case ID	Serum serology	Dam swab	Foetus swab	Milk	Placenta
Arusha chini	SEBI-051	+	+	+	-	na
	SEBI-058	+	+	+	+	na
	SEBI-064	+	+	+	+	na
	SEBI-075	+	+	+	-	na
	SEBI-076	+	+	+	-	+

Village	Sample/ case ID	Serum serology	Dam swab	Foetus swab	Milk	Placenta
	SEBI-079	+	-	-	+	+
	SEBI-084	+	-	+	-	+
	SEBI-089	+	+	na	-	na
	SEBI-095	+	+	na	-	na
	SEBI-106	+	+	+	-	na
Rau	SEBI-094	+	+	+	-	na
Machame mashariki	SEBI-099	+	-	na	-	+
	SEBI-110	+	+	+	-	+
Kindi	SEBI-115	+	+	na	-	+

RVFV was detected in samples from abortion events that occurred between May and August, 2018 (Figure 6.3). Other abortion cases were either found negative for all pathogens under study or were associated with other abortigenic pathogens the results of which are not described in this thesis.

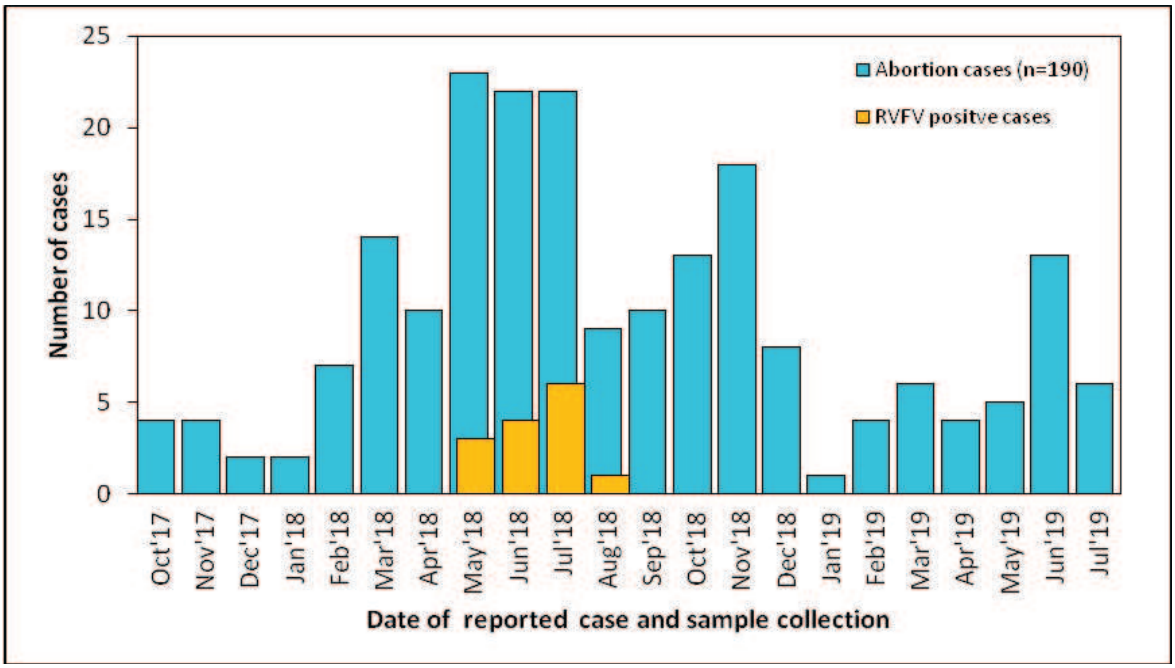

Figure 6.3 Outbreak curve showing all abortion cases (blue bars) included in the study and the number which were RVFV positive (orange bars).

6.3.3 Distribution of the RVFV positive cases

All of the RVFV positive samples were collected from abortion events from four villages in Kilimanjaro Region (Table 6.2) with the majority from Arusha chini. Figure 6.4 shows the location of the four villages.

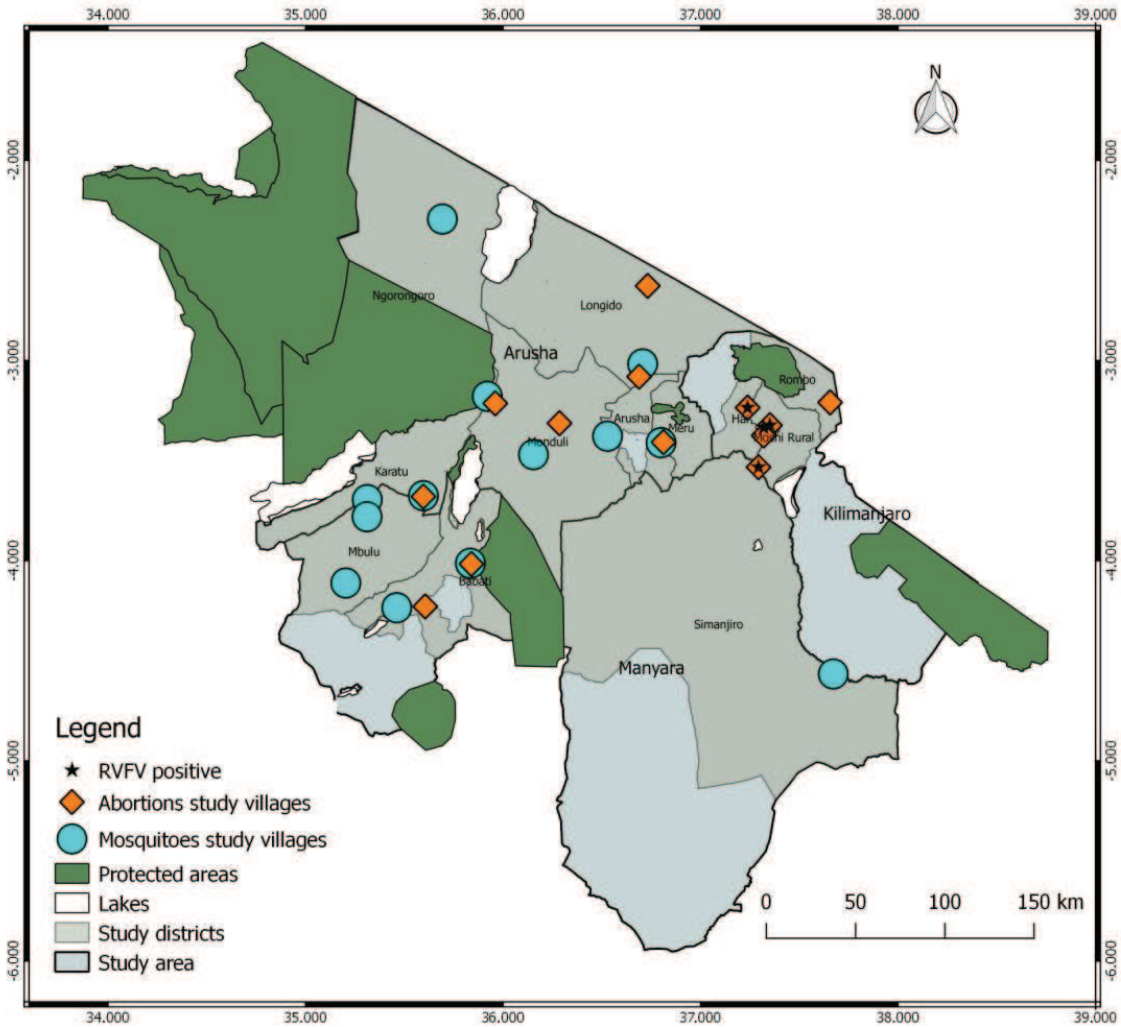

Figure 6.4 Map of northern Tanzania - Arusha, Kilimanjaro and Manyara regions, showing districts and mosquitoes and abortions study sites and locations of the RVFV positive abortion cases based on RT-qPCR

6.4 Discussion

This study reports detection of RVFV RNA in aborted materials and milk from cattle but not goats or sheep in northern Tanzania outside a reported outbreak period. In addition, this study reports no RVFV was detected in potential mosquito vectors collected from the study area. The inability to detect RVFV in mosquitoes can be explained by low numbers of mosquitoes collected and timing of collection (mostly the dry season) outside the outbreak period. The detection of RVFV in aborted materials and milk provides strong evidence for a previously

unreported outbreak of RVF occurring in Moshi and Hai Districts below the detection threshold of current surveillance systems in northern Tanzania, despite the existence of an RVF alert that had been issued by the Ministry of Livestock and Fisheries.

Although a number of other pathogens can cause abortions in livestock in the study area, some of these abortions are linked to RVFV infection as evidenced by data from the current study where 14 out of 190 abortion cases tested were RVFV-positive. To our knowledge, this is the first report of the detection of RVFV in milk samples from naturally infected livestock using RT-PCR. A number of epidemiologic studies have suggested that consumption of unpasteurized milk is associated with RVFV infection in humans (Woods et al., 2002, Anyangu et al., 2010, LaBeaud et al., 2011). This is supported by results of the serological study detailed in Chapter five suggesting a strong association between RVFV exposure in humans and consumption of raw milk. Other recent studies following the 2006/2007 RVF outbreak in Kenya suggested that individuals who milked and also consumed raw milk had greater odds of RVFV exposure than individuals whose only contact to raw milk was through milking (Grossi-Soyster et al., 2019). However, there has been little direct evidence that raw milk may be a high-risk driver of human RVFV transmission. There is limited data on studies that have attempted to examine the potential infectivity and risk of raw milk and milk products obtained from infected animals. The study that confirmed shedding of live infectious virus in the milk of experimentally infected lactating cows was conducted in 1951 (Alexander, 1951), suggesting that virus could be found in milk from acutely infected cattle for 3-5 days (Alexander, 1951). Although the current study reports detection of RVFV in cattle milk, it is clear that there is still a requirement for additional data to address this question. Further studies are required to explore and accurately communicate the potential food-borne risk of RVFV in raw milk. However, it is essential to emphasise the importance of pasteurising or boiling milk in preventing human exposure to RVFV and other potential pathogens in raw milk.

The detection of RVFV in aborted materials (foetus and placenta) from livestock poses risks of animal-to-animal and animal-to-human infection risks especially in farming communities where unprotected handling and disposal of aborted materials is a common practice. In approximately 70-90% of affected livestock animals, RVFV virus easily crosses placental

barriers and can cause a variety of foetal malformations or death (Bird et al., 2009, Bird and McElroy, 2016, Ali et al., 2012). Typically, aborted foetuses and fluids contain exceptionally high viral titres, often exceeding 1.0 x 10^7 plaque forming units (PFU)/g and pose a great risk for human infection (Swanepoel and Coetzer, 2004, Ali et al., 2012). Therefore, it is of the utmost importance that emphasis is given to safe handling of animals during helping with birthing and handling of aborted materials. It is also essential to determine other causes of abortion in livestock as this study demonstrates that not all the abortion cases recorded were associated with RVFV. This suggest that the identification of all possible causes of abortions and implementation of integrated control measures will reduce devastating socio-economic losses owing to abortions.

In the current study, RVFV was detected in samples collected between May and August 2018 which was towards the end of the long rainy season in Kilimanjaro Region. This is the season when there is high potential vector (*Aedes spp*) activity in the region (Hertz et al., 2016), which could play a role in virus circulation in the area. All of the RVFV-positive samples were from smallholder farms in the lower lands around Moshi, suggesting a rather small, localized outbreak that was not detected by standard surveillance. This coincided with events of heavy rains in parts of East Africa which led to floods and RVF outbreak in Kenya, Uganda and Rwanda in mid-2018. Despite the fact that there were unusual rains, floods and increased mosquito activity, including reported outbreaks of dengue fever in different parts of Tanzania, there were no reports of RVF cases.

Enhanced surveillance that integrate RVF and other emerging zoonotic diseases would help generate real time, and accurate data enabling better preparedness and response plans and inform the development of control strategies. This is demonstrated by RVF enhanced surveillance applied in a pilot study in Kenya (Oyas et al., 2018) which enabled collection of useful data on RVF-associated syndromes, particularly abortions and hemorrhagic disease (Oyas et al., 2018). In Uganda, RVF enhanced surveillance under the Viral Haemorrhagic Fever (VHF) surveillance program, enabled detection and recording of 10 RVF sporadic outbreaks between 2016-2018 (Maurice, 2016, Nyakarahuka et al., 2019).

Although cattle are said to be less susceptible to RVFV infection than sheep (Gerdes, 2004, Swanepoel and Coetzer, 2004, Magona et al., 2013), in the current study all of the RVFV-positive samples were from the 14 cattle abortion cases and none from sheep and goats. Swanepoel and Coetzer (2004) suggest that cattle are less susceptible to lethal RVFV infections than sheep, with estimates of mortality ratios range from 10% to 70% in calves (Swanepoel and Coetzer, 2004) and around 40–100% of pregnant cows reportedly abort at any gestational stage, particularly imported breeds (Gerdes, 2002, Coetzer, 1982). Sheep are highly susceptible to RVFV infection (Bird et al., 2009, Gerdes, 2002, Gerdes, 2004), with mortality rates typically reaching approximately 90% to 100% of lambs and approximately 10% to 30% among affected adults (Bird et al., 2009). Abortion rates can be high (90% to 100%), which gives rise to the characteristic abortion storms (Bird et al., 2009, Easterday et al., 1962). Although goats are also susceptible to infection, they appear to be more refractory to severe or lethal disease than sheep (Easterday et al., 1962). The absence of RVFV detection in sheep and goats in the current study could be explained by the small number of sheep and goats kept by smallholder farmers in villages where cattle abortion cases were positive for RVFV. Farmers from around Moshi, for example, keep mostly dairy breeds or cross breeds of cattle for milk. Sheep and goat abortion cases from other villages particularly agro-pastoral or pastoral communities could be associated with other abortogenic pathogens that are not the reported in the current study.

Results from the current study suggest that prompt reporting of suspect cases by mobile phones, timely sample collection and transportation, and existence of a laboratory facility with RVF diagnostic capacity can make a big difference on the amount and quality of data on the status of circulation of RVFV and other zoonotic pathogens. In order to enhance RVF and other emerging diseases surveillance in both animal and human populations, it is important to strengthen laboratory facilities in terms of trained personnel, equipment and reagents in the country and the East African region. Although the available vaccines have limitations (Dungu et al., 2010, Botros et al., 2006), vaccination would not only protect animals and reduce socio-economic losses, potentially, it would also prevent human RVF cases. Additionally, public awareness creation involving mass media such as radio and television programmes among others has also demonstrated success in getting the message to the public, for instance

the use of mosquito nets as part of malaria control programmes (Bowen, 2013, Ankomah et al., 2014). Making use of the available media platforms to send would be useful in creating public awareness on RVF and risks for exposure.

6.5 Conclusion

Strong surveillance in both animal and human populations is critical for early detection of RVF and timely response. This will minimize the extent and impact of potential outbreaks and widespread transmission of the virus. It is also critical that the mass media be engaged for the dissemination of factual information regarding the health risks of RVFV infection and to highlight measures the public can take to reduce potential exposures through unsafe handling of abortions and consumption of raw milk consumption.

Chapter Seven

7 Discussion

7.1 Ecology of RVF virus vectors in northern Tanzania

Rift Valley fever virus (RVFV) infects a wide range of host species including livestock, wild animals and humans, and a number of mosquito species are also known to be involved in the transmission of the virus. However, many gaps still exist in our understanding of the complex ecology and maintenance of RVFV, particularly in the inter-epidemic period. Although the current study did not find evidence of RVFV in mosquitoes collected, the entomological study described in chapter three reports varied abundance and distribution of the potential RVFV vectors in the study area. *Culex spp* and *Anopheles spp* were the most abundant in all 12 sampled villages, *Aedes spp* recorded in seven villages, *Mansonia spp* recorded in four villages, and *Coquillettidia spp* recorded in two villages only. All these mosquito genera are known to be able to transmit RVFV to livestock and wild ruminants (Swanepoel, 1976, McIntosh et al., 1980, Logan et al., 1991) hence the occurrence of these vectors suggest their potential role in RVFV circulation in the area. The relatively high abundance of *Anopheles* and their potential to act as vectors may warrant further investigation as to the role of this mosquito genus in RVF epidemiology.

Species distribution modelling by MaxEnt, indicate wide and different extents of overlapping distribution of suitable habitat for *Aedes, Culex* and *Anopheles* mosquitoes including areas within and around the Serengeti ecosystem. Suitable habitats identified in the current study include districts in northern Tanzania which were not sampled before and the distribution of RVF vector species was previously not known. This suggests that potential RVFV vectors could be widely distributed across the country in areas with similar environments that are conducive to vector survival. Although the association of mosquito habitat suitability with seropositivity in livestock or humans was not statistically significant, it was noted that some of the highly suitable parts of the study area include areas that have reported repeated RVF outbreaks in the past (Sindato et al., 2014), and include districts within which high RVFV seroprevalence

(chapter five) was recorded as well as where RVFV was detected in aborted materials (chapter six). This suggest that the potential role of local occurrence and abundance of vectors in local RVFV circulation. However, further entomological surveillance is needed to detect and/or isolate the virus in mosquitoes in the inter-epidemic period to confirm this role.

While some risk maps for RVF in the region have identified parts of northern as risk areas (Anyamba et al., 2010, Anyamba, 2015, Mweya et al., 2013, Njenga and Bett, 2019), the current study validates previous predictions and provides evidence and details of local occurrence of the potential vectors in the area. This is based on collection of potential vector mosquitoes across 12 districts in northern Tanzania.

Results of the species distribution modelling shows that the distribution of *Aedes*, *Anopheles* and *Culex* genera of mosquitoes was influenced by varied ecological requirements and overlapping habitats related to soil characteristics, amount of rainfall and vegetation, confirming findings of other studies (Minakawa et al., 1999, Mahande et al., 2007, Sattler et al., 2005, Arum et al., 2016). The distribution of *Aedes spp* and *Culex spp* was positively correlated with precipitation of the wettest month, temperature seasonality, and enhanced vegetation index (EVI) respectively and negatively correlated with elevation. Similar to findings of other studies in East Africa (Anyamba et al., 2009, Sang et al., 2010, Sang et al., 2017), the distribution of *Anopheles spp* was predicted by temperature and rainfall. Integrating climate and other environmental factors, vector distribution and records of inter-epidemic RVF cases would help to more precisely identify risk areas. The need for greater spatial resolution is reinforced by findings from the serological analyses, which demonstrate high variability in RVF exposure patterns across villages in northern Tanzania (chapter five).

7.2 RVFV infection in livestock in northern Tanzania

Since RVF outbreaks are periodic in nature, a number of questions remain regarding the maintenance of the RVFV during inter-epidemic periods (IEP). In line with other studies from East Africa (Njenga and Bett, 2019), this thesis provides evidence of RVFV exposure in people and domestic ruminant species, as well as evidence of variable, but widespread infection in villages of Northern Tanzania. Serology results demonstrate increase in seropositivity with

animal age, consistent with a pattern of endemic circulation of RVFV. The detection of seropositivity in animals as young as 1-2 years also provides evidence of inter-epidemic transmission of RVFV, given the previous large outbreak was reported in 2006/7.

Data on risk factors for seropositivity in livestock (Chapter five) demonstrate that age of an animal and occurrence of abortion cases in the herd/flock were associated with inter-epidemic seropositivity. Although abortions can be linked to a number of other infections, the association of RVFV seropositivity with abortions suggests that abortions caused by RVF are being missed or mis-diagnosed during inter-epidemic periods, and/or that RVF abortions may be a source of infection for other livestock in the herd/flock.

The finding of RVFV as a cause of livestock abortions during a previously unreported outbreak (chapter six) is consistent with the hypothesis that cases of RVF are being missed and are occurring below current surveillance detection and reporting thresholds. Enhanced surveillance of livestock abortion events would clearly be valuable for detection of RVF cases and would improve capability for early detection and response to cases that could potentially prevent large outbreaks.

7.3 Detection of Rift Valley fever virus RNA in milk

This study provides valuable evidence in relation to RVFV infection in milk which has important public health implications. Very little has previously been reported on shedding of RVFV in milk with evidence of RVFV in milk limited to one experimental study (Alexander, 1951), reported about six decades ago. The current study presents two key findings that provide evidence of the potential risks of RVFV transmission from consumption of milk. First, consistent with previous studies (Woods et al., 2002, LaBeaud et al., 2008, LaBeaud et al., 2011, Anyangu et al., 2010, Nicholas et al., 2014), analysis of human serological data showed that consumption of unboiled milk was a highly significant risk factor for seropositivity (chapter five). Second, the detection of viral RNA in milk from aborting cattle confirmed that the virus can be shed in milk (chapter six). These findings indicate that public health information given during RVF outbreaks needs to include advice as to the risks of consuming unboiled milk, particularly from aborting animals.

Strains of RVFV has been shown to be inactivated by heat treatment of 56°C for 80 minutes (Daouam et al., 2014, Szemiel and Willett, 2019) and Szemiel and Willett personal communication, but some viral activity can be detected following heat treatment (at 56°C) for up to 70 minutes (Daouam et al., 2014), raising concerns about the thermal stability of the virus. OIE regulations stipulate the need for pasteurization of imported milk and milk products from infected areas in order to prevent introduction and spread of infection into countries that are not free of RVF (OIE, 2017). However, little, if any, published data exist on the effectiveness of pasteurization, heat-treatment and fermentation in inactivating RVFV in milk and milk products, and further work is clearly needed.

7.4 RVF as an emerging disease threat in urban communities

The RVF cases detected in this study affected improved breed dairy cattle in smallholder farms and raises questions about the potential for RVF to emerge as a disease threat to peri-urban and urban communities. A particular concern in relation to the detection of RVF in milk from peri-urban dairy cattle is that most of the dairy products consumed within the municipality of Moshi (predominantly milk and fermented milk, *mtindi*) originate from milk produced by smallholder farmers in areas surrounding the town (Ladbury, 2018). While the peri-urban dairy cattle sector in Tanzania is targeted for rapid expansion (Katjiuongua and Nelgen, 2014). Awareness is also needed about potential risks during RVF outbreaks, with the possibility of infected milk products being consumed by a very large urban population. Particular attention needs to be given to risks that may arise from the common practice of bulking milk that has been sourced from multiple different farms, and the widespread consumption of *mtindi*, which is often made from unboiled leftover milk and may be highly contaminated (Ladbury, 2018).

While this study provides a significant contribution to our understanding of the public health risks of unboiled milk consumption, it also raises questions as to the potential role of milk as a source of infection to suckling livestock. However, the finding of a very low seroprevalence in young animals suggests that transmission in milk is unlikely to be an important element of transmission dynamics in livestock and RVFV transmission via vectors or through direct contact

with aborted materials or infected carcasses remains a more likely explanation for livestock-to-livestock transmission both within and between large outbreaks.

7.5 RVFV infection among humans in northern Tanzania

While there is no documentation of human-to-human transmission of RVF, human cases and exposure have been widely reported and attributed to handling infected animals or animal material coupled with behaviours related to occupational tasks, homestead responsibilities, or consumption of animal products such as meat and milk. In the current study (chapter five) 8.5% of RVFV seroprevalence was reported in northern Tanzania including in young people less than 13 years old (i.e. people born since the previous large outbreaks in 2006/7). As for livestock a higher seroprevalence was recorded in pastoral than agro-pastoral and small-holder settings, but with evidence of widespread infection and high variability between villages. In addition to the findings on consumption of raw milk, the risk factor analysis demonstrated a significant association between RVFV seropositivity in people with handling of aborted materials. This finding further suggests that livestock abortion cases caused by RVF are being missed during inter-epidemic periods. Public health recommendations should also continue to highlight the risks associated with handling of aborted materials, including periods outside the large reported outbreaks.

7.6 Serological and molecular detection of RVFV infection

This study demonstrated the value of serological and molecular data for RVFV epidemiological studies, but also identified several challenges that had to be overcome in generating reliable data for analyses. The in-house serological test which was initially trialled as a more cost-effective approach to screening large numbers of serum samples was shown to be highly non-specific. The commercial assay performed well, but the relatively high costs are likely to preclude widespread application for large-scale studies in low-resource settings.

The real-time PCR assay (Drosten et al., 2002) performed well in detecting RVFV cases in aborted tissues as well as in vaginal swabs. However, challenges were encountered in extracting sufficient quantities of RNA during the initial stages of the study. This study also

demonstrates that vaginal swabs as low-cost, safe, and practical samples for surveillance which can be collected up to 72 hours after abortion, hence should be considered for RVF surveillance

7.7 Future prospects for RVF research, surveillance and control in Tanzania

To further understand the inter-epidemic RVFV circulation in livestock and human populations, we should consider the following:

1) Further entomological surveillance is needed to detect and/or isolate the virus in mosquitoes in the inter-epidemic period and typing of RVFV strains circulating in the area.

2) The use of rapid field diagnostic capabilities for infections in livestock and humans, vector identification, virus isolation.

3) Enhanced surveillance of livestock abortion events to detection of RVF cases.

These will help improve our capability for early detection, preparedness and response to cases that could potentially prevent large outbreaks.

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Appendices

Appendix I: Mosquito survey consent form (English version)

"Zoonoses and Emerging Livestock Systems (ZELS) Research Project

RVF Mosquito Vector study

Household Participant Consent (English)

Introduction

You are being asked to take part in a research study to find out if mosquitoes in this area are carrying diseases that can also cause illness in people. We hope that the results from this research will lead to recommendations for control of these diseases in animals, and particularly ways to stop people catching these diseases. This research is being conducted by experts from the Kilimanjaro Christian Medical Centre, Kilimanjaro Clinical Research Institute and Nelson Mandela African Institution of Science and Technology in Tanzania, and the University of Glasgow in the UK. The work is funded by research councils and the Department of International Development in the UK. Please read or have read to you the information in this sheet which explains what is involved and any benefits or dangers for your household. We encourage you to ask the study representative to explain parts of the study that are not clear or if you have further questions. Take as much time as you need to make a decision about whether you would like to be involved.

Why has my household been chosen?

Your village and household were chosen at random. Your household is one of several in this village that have also been randomly selected to be involved.

What is involved in the study?

We will do mosquito trapping in your compound and ask you some questions about the structure of your household, the belongings that you own, animal ownership, the way that you look after your animals, and illnesses of people and animals. Some basic information about you, such as your education, will also be collected.

Are there dangers of being involved?

The equipment used for mosquito trapping are not harmful to humans. However, the discussion of illness or personal details could cause feelings of discomfort, sadness or anxiety. You do not have to answer any questions that make you uncomfortable, and you can stop the discussion at any time.

What are the costs and compensation?

There will be no additional costs to you other than your time and the information you provide as a result of being in this study. No compensation will be provided for your participation.

What will happen to my information?

We will keep personal identifiers (such as your name) in locked storage in Tanzania and, unless required by law, we will not give this information to anyone outside the study. The mosquitoes trapped and the answers you give during the questionnaire will be sent to the University of Glasgow, where scientists will look for reasons why disease in people and animals is more likely to occur in some households than others. Again, your name and other personal identifiers will not be included in this information.

What are the benefits of being involved?

You may not receive any direct benefit from participating. We hope that in the future the information learned from this study will benefit people in this area and help to prevent human and animal disease.

What do I do if I decide I no longer want to be involved?

You can withdraw from the study at any time and you do not need to give a reason. Withdrawal will not incur any sort of penalty. If you make this decision whilst project staff are still in your household, please talk to the study coordinator. If you decide to withdraw, no new information will be collected, and you can also ask us to not use any information we have already collected about your household and animals. If you would like to withdraw after we have left your household, please call Mr. James Nyarobi on +25578459998. Alternatively, you can write to: Prof. Sarah Cleaveland, c/o Prof. Blandina Mmbaga, KCRI-KCMC, PO Box 2236, Moshi, Tanzania.

Whom do I call if I have questions or problems?

For questions about the study or if you have complaints, concerns or suggestions about the research, please contact Mr. James Nyarobi on +255784459998. For questions about your rights as a research participant, or to discuss problems, concerns or suggestions related to the research, or to obtain information or offer input about the research, contact Prof. Blandina Mmbaga on +255-27-275-4201 or the National Health Research Ethics committee (NatHREC) on +255-22-2121400.

On behalf of the whole project team, thank you for your time.

……

Statement of consent

"The purpose of this study, and the study procedures, risks and benefits have been explained to me. I have been allowed to ask questions, and my questions have been answered to my satisfaction. I have been told that I may contact the National Health Research Ethics committee (NatHREC) on +255-22-2121400 if I have questions about my rights as a research subject, to discuss problems, concerns, or suggestions related to the research, or to obtain information or offer input about the research. I confirm that I have had time to read the information in this document, or that it has been read to me. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. I agree to take part in this study."

_____	_____	_____
Name of subject	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Witness (if applicable)	Date	Signature

Appendix II: Mosquito survey consent form (Swahili version)

“Zoonoses and Livestock Systems Research” (ZELS) Project

Utafiti wa Mbu waenezao ugonjwa wa Homa ya Bonde la Ufa

Fomu ya maelezo kwa mshiriki na ridhaa ya kushiriki katika utafiti

Unakaribishwa kushiriki kwenye utafiti kuangalia kama mbu katika eneo hili wanabeba magonjwa yanayoweza kusababisha maradhi kwa wanyama na binadamu. Tunatumaini kuwa matokeo ya utafiti yatatuwezesha kupendekeza kuzuia magonjwa haya kwa wanyama, na njia za kuzuia watu wasipate haya magonjwa. Utafiti huu unaendeshwa na wataalam kutoka Hospitali ya rufaa ya KCMC, Tasisi ya Utafiti wa Kitabibu KCRI na Chuo kikuu cha sayansi na Teknolojia cha Mandela cha Tanzania, na Chuo Kikuu cha Glasgow cha Uingereza. Kazi imefadhiliwa na wahisani na kitengo cha maendeleo ya kimataifa cha Uingereza. Tafadhali soma au utasomewa taarifa kwenye fomu hii ambayo inaelezea nini kinachohusika na faida yeyote au madhara kwa kaya yako. Tunakuhamasisha umuulize mwakilishi wa utafiti akueleze vipengele vya utafiti ambavyo hujaelewa vizuri au kama una maswali ya ziada. Chukua muda wa kutosha kufanya maamuzi kama utataka kushiriki.

NI KWANINI KAYA YAKO IMECHAGULIWA

Kijiji chako na kaya yako vimechaguliwa kwa bahati nasibu. Kaya yako ni miongoni mwa kaya katika kijiji iliyo chaguliwa kwa bahati nasibu kushiriki.

UTAFITI HUU UNAHUSU NINI?

Tutatega mbu katika boma/kaya yako kwa kutumia aina tofauti za mitego ya mbu, ndani ya nyumba na nje ya nyumba. Pia tutakuuliza baadhi ya maswali kuhusu muundo wa kaya yako vitu unavyomiliki, umiliki wa mifugo, jinsi unayotunza wanyama wako, na magonjwa ya wanyama na binadamu. Taarifa muhimu kuhusu wewe, kama vile elimu yako pia vitakusanywa.

KUNA MADHARA YEYOTE KAMA NITASHIRIKI?

Vifaa tunavyotumia kutegea mbu havina madhara yoyote kwa binadamu.

KUTAKUWA NA MALIPO AU GHARAMA?

Hakutakuwepo na gharama ziada ya muda wako na taarifa utakazotupatia kama sehemu ya kushiriki utafiti. Hakuna malipo yeyote kwa ushiriki wako

NINI KITATOKEA KWA TAARIFA ZANGU?

Tutaweka taarifa za mtu binafsi (kama jina lako) sehemu iliyofungwa hapa Tanzania na labda kama zitahitajika kisheria, hatutatoa taarifa hizi kwa mtu yeyote nje ya mradi. Matokeo ya utafiti wa mbu pamoja na majibu utakayotoa wakati wa dodoso yatatumwa Chuo kikuu cha Glasgow, ambapo wanasayansi wataangalia kwanini magonjwa ya binadamu na wanyama yanatokea sana kwa baadhi ya kaya kuliko nyingine na uhusiano wa magonjwa hayo na kuwepo kwa mbu. Kwa mara nyingine jina na taarifa zako na utambulisho binafsi havitajumuishwa kwenye taarifa hii. Sampuli za mbu na taarifa nyingine zilizokusanywa katika utafiti, vitahifadhiwa kwa miaka 10 au zaidi.

MATOKEO YA UTAFITI YATATUMIKAJE?

Matokeo ya utafiti huu yanachangia sehemu ya Shahada ya Uzamivu ya James Nyarobi katika Chuo Kikuu cha Glasgow cha Uingereza. Taarifa za matokeo ya utafiti huu pia zitakabidhiwa katika Hospitali ya rufaa ya KCMC, Taasis ya Utafiti wa Kitabibu KCRI, Taasis ya Taifa ya Utafiti wa Kitabibu NIMR, Tume ya Sayansi na Teknolojia COSTECH na Wizara ya Kilimo, Mifugo and Uvuvi. Jina lako na vitu vingine vinavyokutambulisha wewe havitakuwa kwenye taarifa hizi.

NINI FAIDA YA KUSHIRIKI?

Unaweza usipate faida ya moja kwa moja kwa kushiriki. Tunaamini kuwa kwa baadae taarifa tuliojifunza katika utafiti huu itawafaidisha watu katika eneo hili na kusaidia kuzuia magonjwa ya binadamu na wanyama

NITAFANYA NINI KAMA NITAAMUA KUTOKUENDELEA KUSHIRIKI?

Unaweza kujitoa ushiri wako wakati wowote na huhitaji kutoa sababu. Kujitoa kwako hakutasababisha adhabu yeyote. Kama utaamuwa kujitoa wakati mwakilishi wa utafiti bado yuko kwenye kaya yako tafadhali ongea na mratibu wa utafiti. Kama utaamuwa kujitoa hakuna taarifa mpya itakayochukuliwa juu yako, na unaweza pia kutuambia tusitumie taarifa ambayo tumeshakusanya kuhusu kaya na wanyama wako. Kama unataka kujitoa wakati tumeshaondoka kwenye kaya yako tafadhali mpigie Bwana James Nyarobi kwa +255784459998. Badala

yake unaweza kumwandikia Prof. Sarah Cleaveland, kupitia kwa Prof. Blandina Mmbaga, KCRI-KCMC, PO Box 2236, Moshi, Tanzania.

NITA WASILIANA NA NANI KAMA NINA MASWALI AU MATATIZO?

Kwa maswali kuhusiana na utafiti au kama unamalalamiko, wazo au ushauri kuhusu utafiti , tafadhali wasiliana na Bwana James Nyarobi kwa +255784459998. Kwa maswali kuhusina na haki zako kama mshiriki wa utafiti au kujadili matatizo, mawazo au ushauri kuhusiana na utafiti au kupata taarifa au kutoa mapendekezo kuhusiana na utafiti wasiliana na Prof. Blandina Mmbaga kwa +255-27-275-4201 au kamati ya maadili ya NIMR (National Health Research Ethics committee (NatHREC)) kwa +255-22-2121400.

Kwa niaba ya wawakilishi wote wa utafiti, asante kwa muda wako

……..

NENO LA IDHINI YA RIDHAA

“Lengo la utafiti huu na taratibu za utafiti, madhara na faida imeelezwa kwangu. Nimeruhusiwa kuuliza maswali na maswali yangu yamejibiwa nikaridhika. Nimeelezwa kwamba naweza kuwasiliana na kamati ya maadili ya NIMR (National Health Research Ethics committee (NatHREC)) kwa +255-22-2121400, kama nina maswali kuhusiana na haki zangu kama mshiriki wa utafiti na kujadili matatizo, mawazo, au ushauri kuhusiana na utafiti au kupata taarifa au kutoa mapendekezo kuhusu utafiti. Nathibitisha kwamba nilipata muda kusoma taarifa katika nakala hii au nilisomwewa. Naelewa kwamba ushiriki wangu ni wa hiari na niko huru kijitoa wakati wowote bila kutoa sababu na bila kunyimwa haki zangu za msingi. Nakubali kushiriki kwenye utafiti huu.”

_____	_____	_____
Jina la mshiriki	Tarehe	Saini
_____	_____	_____
Jina la mtu anaepewa idhini	Tarehe	Saini
_____	_____	_____
Shahidi (kama anahitajika)	Tarehe	Saini

Appendix III: Mosquito field data record sheet

Project Name: Epidemiology of Rift Valley Fever in northern Tanzania

Entomological Study of RVF vector species

Field trip	Date	Village name	Sub-village	Household ID	GPS Northing's	GPS Easting's	Trap ID	Trap location	Taxon (Tx)	Taxon Description	Total Male	Fed	Unfed	Gravid/semi gravid	Sample label code	Number of individuals	Number in pool	Number of pools	Notes/observations
							LT1		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
									6										
									7										
									8										
									9										
									10										
							LT2		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							LT3		1	*Aedes spp*									
									2	*Culex spp*									

									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							LT4		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB1		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB2		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									

							RB3		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB4		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB5		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB6		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									

							RB7		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB8		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB9		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							RB10		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							MM1		1	*Aedes spp*									

									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							MM2		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							BG1		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									
							BG2		1	*Aedes spp*									
									2	*Culex spp*									
									3	*Anopheles spp*									
									4	*Coquillettidia spp*									
									5	*Mansonia spp*									

BG-BG Sentinel trap
LT - Light trap
MM- Mosquito Magnet
RB - Resting bucket

Household - a compound consisting of the members of a family or relatives who live together which may consist of more than one dwelling or house
House - a building or dwelling in household compound in which a family unit lives