Epidemiology of inter-epidemic Rift Valley fever transmission
in the Kilombero Valley, Tanzania

Épidémiologie de la transmission inter-épidémique de la fièvre de la Vallée
du Rift dans la vallée de Kilombero, Tanzanie

Robert David Sumaye
To David, Derrick & Desiree
Acknowledgements

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Rift Valley fever (RVF) epidemics have been associated with periods of unusually high rainfall that lead to sustained flooding over a large area. In Eastern Africa the epidemics have been occurring in cycles of 5-15 years and closely linked to the occurrence of the warm phase of the El Niño/Southern Oscillation (ENSO) phenomenon resulting into periods of heavy rainfall. RVF cause severe disease in young animals and abortion among pregnant animals, and mild to severe disease in people which might lead to haemorrhagic syndrome. However low-level Rift Valley fever phlebovirus (RVFV) transmission occurs during the inter-epidemic periods but most of these remain unrecognized due to inadequate surveillance. The transmission dynamics of RVF both during the epidemics and inter-epidemic periods can be complex and might be uniquely different at fine geographical scales.

Recent and longer-standing infection with and exposure to RVFV in people and livestock were investigated during the inter-epidemic period and the relative importance of the interaction between environment and human behaviour on the RVF exposure risk factors was explored in a seasonal flood plain of the Kilombero river valley in Tanzania, which mimics unusual precipitation increase on annual basis. The findings have demonstrated that indeed RVFV transmission does occur during the inter-epidemic period in the study area. This was possible through detection of antibodies against RVFV in animals that were born after the last RVF disease outbreak of 2006/07 in Tanzania and also detection of IgM antibodies in livestock and people.

In the livestock population an exposure to RVFV of 11.3% was observed, whereas in the human population the prevalence was 11.7%. In both people and livestock populations, seroprevalence was increasing with age. Recent exposure through detection of immunoglobulins M, a short lived class of antibodies (<60 days) upon exposure to RVFV was also evident in both livestock and human populations.

As far as the cattle population was concerned, the presence of four transmission hotspots was demonstrated in the study area with no particular pattern. High animal seropositivity was observed away from the flood plains. Animals that were present during the 2006/07 epidemics had higher seroprevalence compared to younger animals. There was a linear increase in percent seropositivity from 1 year olds to age 5 years, which implies a possible annual challenge by RVFV in the study area.

It was further shown that also people become infected with RVFV during the inter-epidemic
period and that direct infectious mosquito bites contributed to the current observation. Twelve percent of the participants had evidence of past infection and out of those 3% had recent exposure. Various types of contact with livestock were important risk factors including milking the animals and eating raw meat/blood: households keeping livestock had more members with evidence of past infection. Again an increase with age of exposure prevalence was evident.

Lastly, a mathematical model was used to simulate various scenarios of vector-host-environment interactions to elucidate the transmission dynamics of RVFV and associated key determinants during the inter-epidemic period. The mathematical model showed that several factors contributed to the low-level transmission, but invariably included transmission by vector species other than \textit{Aedes mcintoshii}. This species proved nevertheless essential to explain the occurrence of epidemics at regular intervals.

This work adds to the increasing body of knowledge on the transmission dynamics of RVFV during the inter-epidemic period. Further studies in particular those targeting febrile patients in the endemic areas where inter-epidemic transmission is common will provide important insight on the RVFV transmission and generate further information useful for disease control strategies in the event of epidemics. The results also highlight the importance for clinicians in the study area to consider RVF in their differential diagnosis in the case of febrile patients.
Réssumé – Version française

Les épidémies de fièvre de la Vallée du Rift (RVF) ont été associées à des périodes de pluie anormalement élevées qui ont conduit à des inondations prolongées sur une vaste zone. En Afrique de l’Est, les épidémies se sont produites par cycles de 5 à 15 ans et sont étroitement liées à l’apparition de la phase chaude du phénomène El Niño / oscillation australe (ENOA), qui se traduit par des périodes de fortes pluies. Cependant, une transmission de faible fréquence du phlébovirus de la fièvre de la Vallée du Rift (RVFV) se produit pendant les périodes inter-épidémiques, mais la plupart d’entre elles ne sont pas reconnues en raison d’une surveillance inadéquate. La dynamique de transmission de la RVF à la fois pendant les périodes épidémiques et inter-épidémiques peut être complexe et différente à des échelles géographiques précises. La RVF provoque une maladie grave chez les jeunes animaux et un avortement chez les animaux gravides, et une maladie légère à grave chez les personnes pouvant entraîner un syndrome hémorragique.

L’infection/exposition récente et de longue durée avec le RVFV chez les humains et le bétail durant la période inter-épidémique a été invistigée et l’importance relative de l’interaction entre l’environnement et le comportement humain sur les facteurs de risque RVF a été explorée dans une plaine d’inondation saisonnière de la vallée de la rivière Kilombero en Tanzanie, qui imite des précipitations inhabituellement élevées sur une base annuelle. Les résultats ont démontré que la transmission du RVFV se produisait effectivement pendant la période inter-épidémique dans la zone d’étude. Cela a été possible grâce à la détection d’anticorps contre le virus de la RVF chez les animaux nés après la dernière épidémie de RVF de 2006/07 en Tanzanie et également la détection d’anticorps IgM chez le bétail et les humains.

Dans la population animale, une exposition de 11,3% au RVFV a été observée alors que dans la population humaine, la séroprévalence était de 11,7%. Chez les humains comme chez les animaux d’élevage, la séroprévalence augmente avec l’âge. Une exposition récente par la détection d’anticorps IgM, une classe d’anticorps à courte vie (<60 jours) lors de l’exposition à la RVFV est également évidente dans les populations animales et humaines.

La présence de quatre zones à risque de transmission élevée a été démontrée dans les populations de bétail dans la zone d’étude. Une forte séropositivité chez les animaux a été observée loin des plaines inondables. De même, notre travail sur le bétail a montré que les animaux présents lors des épidémies de 2006/07 présentaient une séroprévalence plus élevée que les animaux plus jeunes. Il y a eu une augmentation linéaire du pourcentage de séro-positivité de 1 an à 5 ans,
ce qui implique un risque annuel potentiel de RVFV dans la zone d’étude.

Les résultats de l’enquête menée dans la population humaine ont montré que les personnes sont infectées par le RVFV au cours de la période inter-épidémique, et que les piqûres directes de moustiques infectieuses ont contribué à l’observation actuelle. Douze pour cent des participants avaient des preuves d’infection antérieure et parmi ces derniers 3% ont été récemment exposés à RVFV. Divers types de contact avec le bétail ont été identifiés comme des facteurs à risque importants, y inclus la traite des animaux et la consommation de viande/sang cru, mais aussi les personnes plus âgées avaient plus de chances d’être infectées. Les ménages élevant du bétail avaient plus de membres avec des preuves d’infection passée.

Finalement, un modèle mathématique a été utilisé pour simuler divers scénarios de l’interaction vecteur-hôte-environnement pour élucider la dynamique de transmission de la RVFV et les déterminants clés au cours de la période inter-épidémique. Le modèle mathématique a montré que plusieurs facteurs ont contribué à la transmission de bas niveau, mais, invariablement, l’inclusion d’un vecteur outre 
Ædes mcintoshii était essentielle. La présence de cette dernière espèce était néanmoins nécessaire pour provoquer des épidémies à un interval régulier.

Ce travail enrichit la connaissance sur la dynamique de transmission de la RVFV au cours de la période inter-épidémique. D’autres études, en particulier celles ciblant les patients fébriles dans les zones endémiques où la transmission inter-épidémique est fréquente, fourniront des informations importantes sur la transmission de la RVF et informeront sur les stratégies de lutte contre la maladie en cas d’épidémie. Cela souligne également l’importance pour les cliniciens dans la zone d’étude d’envisager la RVF dans le diagnostic différentiel pour les patients fébriles.

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## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AIC</td>
<td>Akaike Information Criterion</td>
</tr>
<tr>
<td>asl</td>
<td>above sea level</td>
</tr>
<tr>
<td>BSL-3</td>
<td>Biosafety Level 3</td>
</tr>
<tr>
<td>CCHF</td>
<td>Crimean-Congo Haemorrhagic fever</td>
</tr>
<tr>
<td>c-ELISA</td>
<td>Competitive Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>DGDC</td>
<td>Directorate General for Development Cooperation</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ENSO</td>
<td>El Niño / Southern Oscillation</td>
</tr>
<tr>
<td>ESRI</td>
<td>Environmental Systems Research Institute</td>
</tr>
<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Arrestance</td>
</tr>
<tr>
<td>ICTVdB</td>
<td>International Committee on Taxonomy of Viruses database</td>
</tr>
<tr>
<td>IDW</td>
<td>Inverse Distance Weighted</td>
</tr>
<tr>
<td>IEP</td>
<td>Inter-epidemic Period</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IHDSS</td>
<td>Ifakara Health Demographic Surveillance System</td>
</tr>
<tr>
<td>IHI</td>
<td>Ifakara Health Institute</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>ITM</td>
<td>Institute of Tropical Medicine</td>
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<tr>
<td>LAMP</td>
<td>Loop-mediated Isothermal Amplification</td>
</tr>
<tr>
<td>NS</td>
<td>Non-structural</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organisation for Animal Health</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PAF</td>
<td>Population Attributable Fraction</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain reaction</td>
</tr>
<tr>
<td>PI</td>
<td>Percentage Inhibition</td>
</tr>
<tr>
<td>PP</td>
<td>Percentage Positivity</td>
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<tr>
<td>RDT</td>
<td>Rapid Diagnostic Test</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RR</td>
<td>Risk Ratio</td>
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<tr>
<td>RVF</td>
<td>Rift Valley fever</td>
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<tr>
<td>RVFV</td>
<td>Rift Valley fever virus</td>
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<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1

Overview of Rift Valley fever
1.1 Introduction

Rift Valley Fever (RVF) is an arthropod-borne viral disease of animals and humans caused by a RVF virus (RVFV) (Bird et al., 2009; Flick and Bouloy, 2005). It is an acute infection causing severe disease in young animals and inapparent or mild infection in adult sheep, cattle, camels and wild ruminants. It provokes abortion among pregnant ungulates and often is fatal in newborn and young animals (Davies and Martin, 2003; Swanepoel and Coetzer, 2004).

In humans the disease presents as a febrile illness that is characterized by abrupt onset of high fever, severe headache, muscle pain, conjunctivitis and incapacitating prostration of several days duration (Al-Hazmi et al., 2003; Madani et al., 2003; McIntosh et al., 1980b). A small proportion of patients may develop a severe disease in form of haemorrhagic fever or encephalitis, and those who survive either the mild or severe form of the disease may further develop ocular disease, primarily retinal lesions (Al-Hazmi et al., 2005; Kahlon et al., 2010; Siam and Meegan, 1980; WHO, 2008).

RVF epidemics have been associated with periods of unusually high rainfall that lead to sustained flooding over a large area (Davies et al., 1985). In Eastern Africa the outbreaks have been occurring in cycles of 5–15 years closely linked to the occurrence of the warm phase of the El Niño/Southern Oscillation (ENSO) phenomenon resulting into periods of heavy rainfall in the area (Anyamba et al., 2009; Linthicum et al., 1999).

RVF was first described during an investigation of a disease epidemic among sheep in the Rift Valley area of Kenya in the early 1900s (Daubney et al., 1931). Epizootics in animals, sometimes accompanied by epidemics in humans, have been observed in diverse ecologies across Africa (Clements et al., 2007; Eisa et al., 1977; Gear et al., 1955; Hoogstraal et al., 1979; Logan and Linthicum, 1992; McIntosh et al., 1980b; Ringot et al., 2004; Woods et al., 2002; Zeller et al., 1995), Africa’s Indian ocean islands (Morvan et al., 1992; Sissoko et al., 2009) and the Arabian Peninsula (Abdo-Salem et al., 2006; Shoemaker et al., 2002) (Figure 1.1).

Recent serological evidence suggest that RVF is continuing to expand its geographical span to the Maghreb countries and even further north to the Mediterranean region (Di Nardo et al., 2014; Failloux et al., 2017; Gür et al., 2017). RVFV has also been imported into countries outside its historical geographical ranges including crossing the Sahara desert to Egypt in 1977/78 (Meegan, 1979), the Indian ocean to Islands (Sissoko et al., 2009) and crossing the red sea to Arabian Peninsula 2000/01 (Arishi et al., 2000; Nasher et al., 2000), and recently report of a patient diagnosed with RVF in China who acquired the infection in Angola (WHO, 2016).
Figure 1.1: Geographical distribution of Rift Valley fever. The years indicate when the disease was detected in individual countries. Adapted from CDC and https://www.nature.com/articles/emi201381/figures/1, with additional information from Bosworth et al. (2016); Di Nardo et al. (2014); Failloux et al. (2017); Gür et al. (2017).

1.2 The aetiological agent

The Rift Valley fever phlebovirus (RVFV) is an RNA virus which belongs to the order Bunyavirales, the family Phenuiviridae, genus Phlebovirus. Other members of the order include the family Peribunyaviridae with the genus Orthobunyavirus, the family Hantaviridae with the genus Orthohantavirus, the family Nairoviridae with the genus Orthonairovirus, causing a range of diseases in people, animals and plants including Crimean-Congo haemorrhagic fever (CCHF) and Nairobi sheep disease (https://talk.ictvonline.org/taxonomy/). RVFV is an
enveloped virus and the virion particle appears hexagonal in shape and measures about 90 – 110 nm in diameter. The envelope is made of lipid layers and the virion has about 350 – 375 surface spikes (Ellis et al., 1988; Freiberg et al., 2008). The RVFV genome consists of a single-stranded tripartite RNA, among which the large (L) and medium (M) segments have negative polarity, whereas the small (S) segment has ambisense polarity. The S genomic segment encodes NSs proteins, a major virulence factor that mainly functions by counteracting the host’s antiviral interferon system (Ikegami et al., 2009).

Several strains of RVFV have been isolated during different epidemics and inter-epidemic periods from a variety of animal species, mosquito vectors and human populations. Molecular studies of the RVFV isolates indicate presence of closely related lineages of the virus that have been circulating in the animal, human and vector populations (Bird et al., 2007b; Grobbelaar et al., 2011; Sall et al., 1999). These strains have shown differential pathogenicity from mild infections to severe disease in the original hosts as well as in experimental infection to susceptible hosts. The virulence of the RVFV to mammalian host has been shown to be modulated by route of infection and Aedes mosquito saliva (Le Coupance et al., 2013; Schneider and Higgs, 2008).

Survival of RVFV in body fluids depends on the pH (the virus survives better in pH ranges 7–9), and in animal carcasses the virus is inactivated by the low pH associated with rigor mortis in meat (Williams, 2003). Recent detection of genomic RVFV RNA in semen from a patient in France (ex Mali) (Haneche et al., 2016) presents a new paradigm of possible sexual transmission of the virus, a phenomenon, which has also been documented for Ebola and Zika virus (D’Ortenzio et al., 2016; McCarthy, 2016; Thorson et al., 2016). The RVFV survives on air as infectious aerosols for up to one hour depending on relative humidity such that the higher the humidity the lower the viral aerosol stability (Brown et al., 1982). Aerosol survival has an implication for virus transmission in working environments such as abattoirs and laboratories where infectious aerosols are likely to be created (Abu-Elyazeed et al., 1996; Smithburn et al., 1949).

### 1.3 Vectors of RVFV and RVF transmission

A schematic overview of RVFV transmission dynamics and different host/virus/vector interactions is given in Figure 1.2.

Transmission of RVFV to vertebrate hosts is primarily through infectious mosquito bites. Human infections is also of zoonotic nature through contact with infected animals or their
products in the course of executing occupational activities e.g. during treating or butchering RVF infected animals, as well as by inhalation of infectious aerosols released during animal slaughter or in the laboratory environment (Abu-Elyazeed et al., 1996; Archer et al., 2011; Hoogstraal et al., 1979; Morita, 1988; Smithburn et al., 1949; Turell et al., 1996). Close contact with infected animals through common shelter or through consumption of raw animal products such as milk, meat and blood from infected animals are important risk factors for past exposure to the disease (LaBeaud et al., 2008; Woods et al., 2002). Vertical transmission in humans has also been suggested to occur through demonstration of IgM antibodies against RVFV in both newborn and the mother (Adam and Karsany, 2008; Arishi et al., 2006). The vertical acquisition of RVF presents a new paradigm that warrants further exploration in understanding transmission dynamics in people.

Figure 1.2: Rift Valley fever: schematic representation of transmission dynamics
(1) Principal cycle within domestic animal populations; (2) Epidemic and/or annual emergence of dormant, infected vectors, including vertical transmission; (3) Zoonotic and vector-borne infection of humans; (4) Wildlife reservoir
Blue arrow: vectorial transmission; Green arrow: direct contact; Red arrow: vertical transmission
Illustrations used under the GNU Free Documentation License

Potential mosquito vectors include more than 30 species from which RVF virus has been detected either through isolation from field collections or in virus transmission experiments (Table
1). Floodwater *Aedes* species are regarded as the main vectors of RVF that maintain the virus between epidemics, and have been incriminated in several epidemics, however other mosquito species including *Anopheles*, *Culex*, *Eratmopodites*, *Mansonia*, *Mansonoides* and *Coquillettidiae* may also play an important role in either inter-epidemic RVFV maintenance or perpetuation of the epidemics (Gad et al., 1987; Hanafi et al., 2011; Seufi and Galal, 2010). Other arthropods which are vectors of a range of other diseases including *Glossina*, *Stomoxys*, *Phlebotomes*, and *Culicoides species* may transmit the disease mechanically, usually through contaminated mouth parts as a result of interrupted feeding or through regurgitated blood from the crop during next blood meal (Hoch et al., 1985; Turell et al., 1990, 2010). This phenomena of multi-vector involvement can dramatically increase the number of potential vectors during epidemics.

The RVFV can survive long periods of dryness in *Aedes* eggs and can be transmitted vertically (trans-ovarian transmission) in floodwater *Aedes species* (Linthicum et al., 1985). *Aedes* eggs are resistant to desiccation and can survive through a temporary dry period and have the ability to lay dormant in the dry soil for years. The floodwater *Aedes species* lay their eggs on dampened soil or on vegetation at edges of seasonally waterlogged, predominantly grass covered, depressions (referred as dambos in Southern and Eastern Africa) (Gargan et al., 1988; Linthicum et al., 1988). When the dambos are filled up during subsequent rain seasons, the dormant *Aedes* eggs hatch upon being submerged into water (Becker et al., 2010).

Experimental and field studies suggest that certain floodwater breeding *Aedes species*, which emerge from temporary ground pools following flooding, are important epidemic vectors as they maintain the virus in their unhatched infected eggs during inter-epidemic period (Linthicum et al., 1985). Once the outbreak starts, multiple mosquito vectors which emerge in succession upon flooding (Linthicum et al., 1983) play a role in spreading/perpetuating the infection further by transmitting the virus from amplifying hosts e.g. cattle, sheep and goats to other susceptible hosts.

Table 1.1: Selected mosquitoes of medical and/or veterinary importance implicated as biological vectors of RVFV

<table>
<thead>
<tr>
<th>Species</th>
<th>Country</th>
<th>Y/N</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aedes aegypti</em></td>
<td>South Africa, Sudan</td>
<td>Yes</td>
<td>McIntosh et al. (1980a); Seufi and Galal (2010)</td>
</tr>
<tr>
<td><em>Aedes caspius</em></td>
<td>Egypt</td>
<td></td>
<td>Turell et al. (1996)</td>
</tr>
<tr>
<td><em>Aedes cumminsii</em></td>
<td>Burkina-Faso</td>
<td></td>
<td>Fontenille et al. (1988)</td>
</tr>
<tr>
<td><em>Aedes juppi</em></td>
<td>South Africa</td>
<td></td>
<td>Davies and Highton (1980); Linthicum et al. (1983);</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>McIntosh et al. (1980a)</td>
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RVFV transmission by arthropods is further modified by vector factors which dictate their vectorial capacity. The arbovirus pathway within mosquito vectors includes entry and replication within midgut epithelial cells and dissemination into other tissues including salivary glands,
before being secreted together with saliva during blood feeding. This forms the basis of transmission by vectors, however not all mosquitoes that are susceptible to RVFV infection can transmit the virus, due to either midgut or a salivary gland barriers (Romoser et al., 2005). Other mosquito-borne parasites when in co-infection (parasite + virus) within the same vectors may enhance the transmission of arboviruses by helping the virus to bypass these barriers. In endemic areas with mosquito-borne parasites (e.g. malaria or lymphatic filariasis), such co-infection of vectors could make incompetent vector species become competent and thus a higher number of vectors perpetuating the epidemics (Vaughan and Turell, 1996).

1.4 Pathological and clinical features important for diagnosis

1.4.1 Pathology

1.4.1.1 General

For a virus to enter and establish itself in the vertebrate hosts, it has to evade the host’s immune system including interferons, the frontline host protection from virus attack (Finlay and McFadden, 2006). RVFV does so by means of the non-structural protein NSs, which inhibits the secretion of tumor necrosis factor (TNF)-α, interferon (IFN)-β, and IFN-α2 (Terasaki and Makino, 2015). The main entry routes include directly into the circulation (infectious mosquito bites to animals and people), mucous membranes (inhalation & ingestion, humans), or broken skin (humans). The transmission of RVFV by arthropod mosquito vectors may be enhanced by the vector factors in particular proteins in the saliva which help the virus to overcome the primary host defence (Schneider and Higgs, 2008), but also the RVFV may evade host defence by interfering with the local production of interferon, thus allowing replication and local establishment before dissemination into the target organs of the mammalian hosts (Devasthanam, 2014). RVFV is cleared from its mammalian host, but it has been shown to persist for periods up to 21 days in infected animals (particularly in the spleen) (Swanepoel and Coetzer, 2004).

1.4.1.2 Pathology and pathogenesis in people

In people the RVF pathogenesis and pathology is poorly documented due to short disease course in people and relatively few characteristic human cases during epidemics. In humans the main entry routes include directly into the circulation (infectious mosquito bites), mucous membranes (inhalation and ingestion), or broken skin. Although RVFV affects a variety of organs, the initial and main target organ is the liver in which its replication causes hepatocyte
damage and eventual cell death. The subsequent pathological and clinical manifestations are mainly impaired liver functions e.g. elevated liver enzymes (alanine transaminase and aspartate transaminase), increased bilirubin levels and decreased blood albumin levels (Madani et al., 2003; Smith et al., 2010). The main pathological feature of diagnostic importance is hepatic lesions (confined primary foci of necrosis) which are detectable upon gross and microscopic examination (Mohamed et al., 2010; Shieh et al., 2010). The viral antigen can be detected in infected individuals within a few days post infection, and by this time it can be picked by competent vectors and further get transmitted to susceptible hosts (Gargan et al., 1988). This stage of infection is transitory and of short duration as host immune responses would normally clear the infection (Dodd et al., 2013; Nfon et al., 2012).

However in a small fraction of cases/patients RVF may present with severe syndrome including haemorrhagic syndrome (vomiting blood, a purpuric rash or ecchymoses, bleeding from the nose or gums), ocular disease (retinal lesion leading to visual impairment) or meningoencephalitis (prostration). The pathological processes that lead to these severe syndromes in humans that occur late in the course of disease, for example the mechanisms that trigger haemorrhagic fever or the viral entry to the brain are not yet defined. Animal models however suggest that delayed onset of neurological signs in humans may be due to infection with a mutant strain of the RVF virus and/or host genetic factors (Bird et al., 2007a; Peters and Slone, 1982).

1.4.1.3 Pathology and pathogenesis in animals

In animals (livestock and animal models) similar to people the target organ for RVF virus replication is the liver and the main entry point is through infectious bites of mosquitoes. Also the pathological feature of diagnostic importance is primary foci of necrosis (Figure 1.3(a), Figure 1.3(b)), which are detectable upon gross and microscopic examination (Mims, 1957; Shieh et al., 2010; Smith et al., 2010). The necrosis is most severe in liver of aborted sheep foetuses and neonatal lambs. Although the lesions look similar among species, there are significant differences among different age groups. Younger animals have massive hepatic necrosis, which partly explains high mortality in young stock. Haemorrhages in the fore-stomach of ruminants lead to the presence of free blood in the intestines (Figure 1.3(c)). The spleen tends to be slightly enlarged with haemorrhages occurring in the capsule (Davies and Martin, 2003). The other key pathological feature in animals in deaths of new-born animals (kids, lambs, calves) as well as abortion in pregnant animals irrespective of gestation period (Figure 1.3(d)).
1.4.2 Immune responses

Following infection by RVFV, non-human mammalian hosts mount an immune response which involves both the innate and adaptive immunity (Weber and Elliot, 2009). The immune response is key for the initial clearing of the infectious agent and for preventing further progression of the disease as well as future disease development among exposed individuals upon re-infection. This occurs both in natural infection with the RVFV and in case of immunization in which the recovered and immunized individuals remain protected against subsequent future challenges. The protection has been conferred even in situations where no detectable antibodies have been demonstrated in exposed animals (Barnard, 1979). In experimental infections and animal models, innate immunity has been shown to play an important role in protecting individuals from species that are known to be naturally resistant to the disease (Morrill et al., 1990; Nfon et al., 2012; do Valle et al., 2010). The detectable immune responses in infected
individuals develop within 3-15 days post infection in the form of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies (Bird et al., 2009).

The IgM antibodies appear in blood circulation first and have been detected for up to 2 months in natural infection, whereas IgG antibodies are produced almost at the same time but may remain detectable for long period and possibly for life in animals (Elfadil and Shaheen, 2007; Morvan et al., 1992; Paveska et al., 2003; Thonnon et al., 1999). The transitory nature of IgM antibodies in vertebrates has been utilized for serological diagnostics and disease surveillance in populations to detect recent exposure and is thus indicative of recent circulation of RVFV in the population (Paveska et al., 2003, 2005a,b). Since the RVFV antigen is quickly cleared from the body, antibody detection remains a key biological marker of past exposure and/or recent infection.

Very little information is available for the RVFV specific immune response in humans. A, very vague, reference traced was CDC (2019): IgM antibodies appear early as a transient response and IgG antibodies persist for years. It is further stated that both classes of antibodies are specific for RVF. An earlier reference (Findlay and Howard, 1951) reports presence of ‘virucidal bodies’ up to 18 years after contact.

1.4.3 Clinical features

In livestock, RVF is characterized by abortions in susceptible pregnant animals in a herd, which occur irrespective of gestation period. Abortions observed in pregnant animals are accompanied by high mortality in neonates (lambs, kids and calves). Other signs include depression, high fever and abdominal pain in young stock. In adult animals high fever is accompanied by excessive salivation, anorexia, weakness, nasal discharge and a drop in milk production in lactating animals (WHO, 2008). The disease in susceptible newborn lambs and kids can be per-acute with an incubation period of 36 hours whereas in adult animals the incubation period ranges from 3-5 days.

Two main disease courses can be expected when humans contract RVF (Madani et al., 2003); one is a mild form of the disease characterized by influenza-like symptoms with mild fever, nausea, vomiting, headaches and muscle and joint pains. The other is severe form which is characterized by one or more of the three distinct syndromes namely, ocular, meningoencephalitis and haemorrhagic fever syndromes (Siam et al., 1980). The severe form occurs in smaller percentage of patients (WHO, 2008). Based on clinical symptoms, RVF is not easily distinguished from other febrile tropical illnesses in endemic regions such as malaria, typhoid
fever, bacterial gastro-enteritis and rickettsial diseases.

1.5 Economic, public health and social consequences of RVF outbreak

The socio-economic and public health consequences of RVF epidemics include direct and indirect disease burden to the affected populations. Infections in livestock herds result into reduced meat and milk production, and loss of their market value, whereas disease in people results into reduced manpower to perform normal duties. The re-allocation of scarce resources (both personal/family and government) directed towards curbing the spread of epidemics also contribute significantly to the registered economic losses.

Abortions in pregnant animals and mortalities in new-born and young stock during epidemics contribute to a significant economic losses as this lead to loss of future replacement stock thus resulting into poor livelihood of the livestock keepers. A retrospective study in three regions in Tanzania indicated that during the 2006/07 RVF epidemics mortalities in livestock were 0.1%, 0.18% and 0.31% in cattle, goats and sheep respectively whereas loss due to abortions were 0.09%, 0.16% and 0.28% respectively in cattle, goats and sheep (Chengula et al., 2013). Animals infected by RVFV are less productive in terms of growth, milk production and draft power leading to further losses to livestock keepers. Also, extra expenditure that is likely to be incurred by livestock keepers for costs of drugs, vaccines and consultancy services in the course of treating sick animals adds up to these losses.

During RVF epidemics, disease control measures that are implemented including restricted livestock and their products movement and closure of livestock markets disrupt the livestock market chains and contribute to poor livelihood of livestock keepers (Rich and Wanyoike, 2010). Also, import bans imposed on live animals and their products by importing countries hinder trade between affected and disease free countries (Holleman, 2003; Soumare et al., 2006). People get scared to consume meat and milk, so even the local market is disrupted. Apart from mortalities and abortions, the other tragedy that the livestock industry suffers is in terms of trade ban as a result of the quarantine imposed on movement of live animals and animal products. This includes movement bans at the local level, e.g. from one affected location to another, and international trade bans imposed on animals and animal products from infected countries (Geering et al., 2002). During high epizootics all imports from affected countries are banned and can only be resumed 3-6 months after the last evidence of infection. The World Organization for Animal Health (OIE) recommends a trade ban on livestock from an affected country which
can be lifted only if animals are vaccinated or free from infection as determined by laboratory tests that in turn increases trading cost and thus acts as barrier to trade (OIE, 2010).

Sick people will require treatment and occasionally prolonged hospitalisation. The cost of consultation, medicine and/or bed space in the hospital plus the cost of transport to the health service points all add up to the burden of the disease to the community. This is more so when affected people require either the company of a healthy individual to attend sick person or transportation from remote areas, where high risk groups such as pastoralists usually reside and where the epicentre of the disease occurs (Russell, 2004; Sachs and Malaney, 2002). Furthermore, sick people cannot work; therefore an outbreak of a disease leads to loss of man hours for the whole period of outbreak (Jeanmaire et al., 2011). Though it affects less than 2% of people, those who either develop the encephalitic or ophthalmic form of the disease may end up with disability like blindness, thus become dependent. Approximately 1% of infected people succumb to the disease, mostly those with the haemorrhagic form of the disease.

Although most of the RVF epidemic consequences may be felt among pastoralist and agro-pastoralist communities in rural and remote areas, people in other segments of population, for example those which rely on livestock such as livestock product processors, intermediaries, animal traders and consumers, normally get affected through the ban of movement of livestock and their products in the event of RVF outbreak. This disrupts market chains leading to temporary loss among processors, middlemen and business people, and a temporary rise in prices of other protein food sources (Rich and Wanyoike, 2010).

1.6 Prevention and control

1.6.1 Quarantine

Imposing a quarantine on the movement of livestock and their products can only help prevent transfer of the disease to a distant geographical location within a country or across international borders. However within a local area, RVF usually affects relatively large areas due to the involvement of vectors whose abundance in a locale is defined by flooded land and their movement beyond flooded areas.

1.6.2 Vaccines and vaccinations

Inactivated and attenuated vaccine products are available for vaccination of livestock against RVF. The live attenuated Smithburn vaccine confers lifelong protection but does cause abortion in pregnant animals and can cause congenital abnormalities in the offspring of pregnant ewes.
(Smithburn, 1949). Its use is thus restricted to young stock of non-bearing age or in situations where the pregnancy status of individual adult females can easily be established. The inactivated vaccines confer a short-term immunity, so they require boosting immunity throughout life, thereby making it an expensive endeavour. There are no commercially available vaccines for human use but there are several candidate vaccines (protein/capsid) that are in various phases of development (Ikegami and Makino, 2009).

1.6.3 Personal protection

Preventive measures for humans include use of insecticide treated bednets, repellents, avoiding contact with infected animals during outbreaks, wearing protective gear, including masks, when attending sick or dead animals in the event of outbreak, sufficient cooking of food of animal origin before consumption and avoidance of the slaughter of sick animals during outbreaks. It is essential that veterinarians and their assistants performing post-mortem examinations or laboratory investigations during suspected RVF outbreaks wear personal protective equipment i.e. boots, gloves, aprons, masks and goggles, and minimize generation of aerosols (ILCA, 1990). The handling of suspected RVF infected samples should be in type II bio-safety cabinets and HEPA filtered respirators or under P-2/P-3 conditions to minimize risk of acquiring the infection in the laboratory (Ponnusamy et al., 2011).

1.6.4 Control

The main RVF control strategies in livestock relay on quarantine and vaccination whereas control in people depends on education and awareness creation.

In the event of an RVF outbreak, emphasis is on a combination of measures to curb the situation and to mitigate the adverse impact on the livestock industry as well as on public health. Given the geographical remoteness, poor infrastructure and flood conditions of most outbreak locations, which lead to poor accessibility, it is often difficult to detect and subsequently confirm the disease by medical and/or veterinary experts early enough which in turn leads to delays in instituting proper control measures in time.

However, utilizing an early warning system could give up to 6 months lead time before an outbreak (Anyamba et al., 2009, 2006; Linthicum et al., 1999), which would allow vaccination of susceptible animals, provided the vaccine is available, to allow for the animals to develop protective immunity before the disease outbreak. The main challenge though remains, the time needed to mobilize adequate resources and materials such as vaccines which have to be
produced and imported from other countries. Such measures may include ring vaccinations and movement ban in situations where the disease and the flooding condition is not wide spread. Public education and awareness raising is also very important component of control measures to provide the right information to people on ways to protect themselves and to reduce unnecessary worries when dealing with the situation (FAO-TZ, 2007).

Because there is no registered human vaccine against RVF in the market (Pittman et al., 1999), people have to rely on personal protection measures that involves protecting themselves from mosquito bites during the outbreak through use of insecticide treated bed nets and use of mosquito repellents. However, since a high risk of infection to people is through contact with infected animals and their products, people handling sick animals such as livestock keepers and veterinarians attending sick animals should take extra precautions by wearing personal protective equipment.

1.7 Modelling Rift Valley fever epidemiology, forecasting and control

Mathematical modelling techniques have been applied to a number of infectious diseases including malaria and have evolved over time (Mandal et al., 2011) with the overall goal being to investigate and describe complex biological processes through simplification into simple rules and equations. In Rift Valley fever modelling goals have ranged from predicting future epidemics (in space and time to allow early warning systems) to mechanisms of RVFv circulation (for understanding the disease transmission dynamics and maintenance in nature) (Métras et al., 2011).

The original modelling contributions to modern-day epidemiology started with the so-called “Ross model” of Plasmodium transmission (Ross, 1915). The first application of compartmental models to Plasmodium transmission was developed by Kermack and McKendrick (1922). At the same time, the Reed-Frost compartment model was being developed by Lowell Reed and Wade Frost of Hopkins University and used by them in their courses, though never formally published.

In the compartmental model approach the population is divided into mutually-exclusive compartments, with the fundamental assumption that individuals in the same compartment have the same epidemiological characteristics as far as pathogen transmission is concerned. The basic compartments in the currently most widely applied model include (Figure 1.4, note that the original Reed-Frost model included only compartments 1 and 3):
1. **Susceptible:** the individuals that can become infected by individuals in compartment 3

2. **Exposed:** the individuals that have been infected, but cannot themselves transmit the pathogen (≈ incubation period)

3. **Infective:** the individuals that are infective to susceptible individuals and can transmit the pathogen upon sufficient contact with them

4. **Removed:** the individuals that have been removed from the pathogen transmission process (≈ recovered with sterile immunity, dead)

![Figure 1.4: Schematic representation of the basic SEIR model](image)

This basic model has been extended repeatedly and considerably to allow (e.g.) vectorial transmission, age structure, emigration, immigration, etc. There are two main approaches to compartment models, namely difference equation models (discrete time units) and differential equation models (continuous time scale).

An alternative approach commonly used in epidemiology are so-called agent-based models. Agents are individual entities with common characteristics that are followed in time and space, interaction with each other as required. Advantages of this approach are flexibility and realism. The main drawbacks are the complexity of the models, especially the coding and the computing power required (Auchincloss and Totaro García, 2015).

In a recent publication Danzetta et al. (2016) summarised how compartmental models have been utilised in the understanding of RVF transmission dynamics and concluded that there existed an increasing interest and use of mathematical models in this area. RVF modelling first of all concentrated on prediction of epidemics in the Horn of Africa, because of the characteristic link to the El Niño phenomenon (Linthicum et al., 1999). More recently, modelling efforts have focused on RVF epidemiology and ecology away from the drier areas of the continent and have become more concerned with understanding transmission dynamics during epidemics and inter-epidemic periods, rather than simply predicting the occurrence of epidemics (Gaff et al., 2007;
RVF modelling thus more and more becomes a research tool, summarising the knowledge base of the disease and, at the same time, highlighting gaps in this base and offering guidance to further research efforts. A remark commonly made in this respect is that mathematical simulation models remain difficult to understand and apply. Recent developments in open-source, easily accessible software has changed this and especially the availability of \textit{R}® and \textit{RStudio}® has allowed the development of so-called open models, available to all and adaptable with minimum effort (Soetaert and Herman, 2009).
CHAPTER 2

Description of study context and study objectives
2.1 Rift Valley fever in East Africa region

Since the first description of enzootic hepatitis disease in sheep that was later known as RVF in early 1900s and the isolation of its aetiological agent in the Rift Valley province of Kenya, there have been reports on investigations and observations of the disease in Kenya. Relatively few reports and investigations exist in neighbouring countries in the region. The disease has been reported in Tanzania almost every time the RVF has been occurring in Kenya but few investigative reports are available (EMPRES, 2001). Somalia and Ethiopia have been affected at some point, at which an import ban was imposed on their animal and animal products to Saudi Arabia. This prompted stakeholders’ investigation on the spread of the disease in the region for the purpose of defining the problem and thus put in place a certification system for exports of live animals and animal products from respective countries.

2.2 Previous RVF disease reports in Tanzania

In Tanzania, Rift Valley Fever has been reported to occur in a number of epidemics and sporadic cases as early as 1930s (Sindato et al., 2014), with most recent RVF outbreak occurring in 2006/07 (WHO, 2007). The RVF epidemics has traditionally been reported in the northern parts of the country, the area bordering Kenya, which has a high livestock density and lower rainfall compared to other parts of Tanzania. In each subsequent epidemic and follow-up surveys, it was shown that RVF has increased its geographical extent towards south-eastern, central and north-western parts of Tanzania (Kifaro et al., 2014; Sindato et al., 2014).

Historical observations of RVF in Tanzania have been mainly in livestock populations with fewer early reports on human involvement. This is possibly due to absence of severe disease in people, with only self-limiting symptoms that did not catch much attention from public health authorities. In Tanzania, RVF in people was first observed as an accidental human infection contracted during a post-mortem examination of dead calves in 1956 (Newlands, 1957). Also during investigation of abortions among cattle and sheep in various farms in 1978 (Iringa, Dar es Salaam, West Kilimanjaro, and Zanzibar) herdsmen had symptoms suggestive of RVF (Kondela et al., 1985). It was during the 2006/07 RVF outbreak when Tanzania had confirmed human cases with typical clinical features and high mortality distributed in several regions in Tanzania (Mohamed et al., 2010), with reports of cases in Arusha (12), Dodoma (164), Iringa (6), Manyara (6), Morogoro (54), Mwanza (5), Pwani (6), Singida (36) and Tanga (1). These included 117 deaths (case fatality rate, 40%) (WHO, 2007). Serological evidence during the inter-epidemic
period in various occupational groups before the 2006/07 RVF disease outbreak indicates variable exposure in different occupational groups and male individuals being at higher risk compared to women. (Heinrich et al., 2012; Swai and Schoonman, 2008).

2.3 Study area

This study was conducted in the Kilombero river valley, which spans Kilombero and Ulanga districts in Morogoro region. The Kilombero valley is a seasonally inundated flood plain up to 52 km wide, at high water, between the densely forested escarpment of the Udzungwa mountains, which rise to 2576 m above sea level (asl) on the north-western side and the grass covered Mahenge mountains, which rise to 1516 m asl on the south-eastern side. The valley receives an average annual rainfall of 1200–1800mm and temperatures range between 25°C and 32°C. The valley has a diverse ecology and demography with villages consisting largely of numerous distinct groups of houses located on the margins of the flood plain where rice cultivation is the predominant economic activity. Other land use types include hunting, fishing, forestry, pastoral livestock rearing and other crops cultivation. There has been intense malaria transmission in the valley with the main vectors being *Anopheles gambiae* complex and *Anopheles funestus* (Killeen et al., 2007). Other mosquito species inhabiting the valley, some of which are vectors of RVF virus, include *Culex spp*, *Aedes spp* and *Mansonia spp* (Ogoma et al., 2010).

2.4 Hypothesis

Low-level endemic transmission of RVF occurs regularly throughout much of the African continent apart from the epidemics that results from unusual high precipitation, but most of this low-level transmission remains unrecognised due to inadequate surveillance. Endemicity is traditionally associated with areas of high annual rainfall, causing annual flooding of dambos (waterlogged, seasonally wet depressions) and floodplains, and/or tropical rainforest ecological features.

2.5 Study goal and objectives

The main goal of this study was quantify recent and longer standing infections with RVF in both humans and livestock and to better understand the interaction between the spatial living environment, human behaviour and migration on the risk for endemic and epidemic transmission of RVF. The approach was to investigate the disease in both humans and livestock in a seasonal flood plain of the Kilombero river valley in Tanzania, which mimics unusual precipitation increase
on annual basis, by examining the presence of circulating antibodies against RVF in serum samples from livestock and people.

Specifically this study aimed to address the following objectives:

1. To describe RVF exposure status in livestock and humans in the former epizootic areas of the Kilombero valley, distinguishing between recent (post epidemic/epizootic) transmission and longer-standing exposure.

2. To assess temporal and spatial risk factors for recent and longstanding RVF sero-positivity in livestock.

3. To assess demographic, behavioural, temporal, occupational and spatial risk factors for recent and longstanding RVF sero-positivity in the human population.

4. To establish the relative importance of risk factors related to mosquito-borne transmission and transmission through direct contact with infected animal fluids.
Inter-epidemic transmission of Rift Valley fever in livestock in the Kilombero river valley, Tanzania: A cross-sectional survey

This chapter has been published in the journal *PLoS Neglected Tropical Diseases*. The unit of the hotspot analysis in Figure 1 is the within household seroprevalence.
Inter-epidemic Transmission of Rift Valley Fever in Livestock in the Kilombero River Valley, Tanzania: A Cross-Sectional Survey

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Abstract

Background: In recent years, evidence of Rift Valley fever (RVF) transmission during inter-epidemic periods in parts of Africa has increasingly been reported. The inter-epidemic transmissions generally pass undetected where there is no surveillance in the livestock or human populations. We studied the presence of and the determinants for inter-epidemic RVF transmission in an area experiencing annual flooding in southern Tanzania.

Methodology: A cross-sectional sero-survey was conducted in randomly selected cattle, sheep and goats in the Kilombero river valley from May to August 2011, approximately four years after the 2006/07 RVF outbreak in Tanzania. The exposure status to RVF virus (RVFV) was determined using two commercial ELISA kits, detecting IgM and IgG antibodies in serum. Information about determinants was obtained through structured interviews with herd owners.

Findings: An overall seroprevalence of 11.3% (n = 1680) was recorded; 5.5% in animals born after the 2006/07 RVF outbreak and 22.7% in animals present during the outbreak. There was a linear increase in prevalence in the post-epidemic annual cohorts. Nine inhibition-ELISA positive samples were also positive for RVFV IgM antibodies indicating a recent infection. The spatial distribution of seroprevalence exhibited a few hotspots. The sex difference in seroprevalence in animals born after the previous epidemic was not significant (6.1% vs. 4.6% for females and males respectively, p = 0.158) whereas it was significant in animals present during the outbreak (26.0% vs. 7.8% for females and males respectively, p<0.001). Animals living >15 km from the flood plain were more likely to have antibodies than those living <5 km (OR 1.92; 95% CI 1.04–3.56). Species, breed, herd composition, grazing practices and altitude were not associated with seropositivity.

Conclusion: These findings indicate post-epidemic transmission of RVFV in the study area. The linear increase in seroprevalence in the post-epidemic annual cohorts implies a constant exposure and presence of active foci transmission preceding the survey.

Introduction

Rift Valley fever (RVF) is known to occur in outbreaks in cycles of 3–15 years in the Eastern Africa region and the Horn of Africa, following unusual high precipitations that lead to sustained flooding [1,2]. In recent years, evidence of RVF transmission during the inter-epidemic periods in some parts of the African continent has increasingly been reported [3–5]. The inter-epidemic transmissions generally pass undetected clinically, but can be revealed where active serological surveillance is regularly done in either livestock or human populations [4,6,7].

Rift Valley fever is a mosquito borne viral zoonosis that affects both livestock and wild ruminants [4,5,8]. It is caused by Rift Valley fever virus (RVFV) belonging to the genus Phlebovirus of the family Bunyaviridae and in susceptible animals is manifested clinically by high fever, and causes abortion in susceptible pregnant animals irrespective of the gestation period and high mortality in newborn animals [9]. In humans, RVF can be asymptomatic, but can also cause mild illness (associated with headache, fever, muscle and joint pains) or severe illness (associated with hemorrhagic fever, encephalitis or ocular disease) [10–12]. The disease was first described in the early 1910s and the aetiological agent was isolated in the 1930s in Kenya [13]. The disease pattern in the Eastern Africa region and the horn of Africa is driven by climatic conditions linked to the El Niño/Southern Oscillation (ENSO) phenomenon, which leads to unusual high rainfall and floods alternated by long dry spells [2]. In other parts of Africa, RVF emerged in relation to the construction of
Rift Valley fever (RVF) is an arthropod-borne viral disease that affects people, livestock and wild animals. It occurs mostly in Africa, and epidemics have been reported in the Arabian Peninsula. RVF is transmitted to humans and animals by mosquitoes, but people can also get the infection through direct contact with blood or tissues of infected animals. The disease occurs in epidemic form in a cycle of 5–15 years, but some reports also indicate occurrences of the disease during non-epidemic periods. We report here inter-epidemic period transmission of RVF in livestock population, evidenced by demonstration of RVFV antibodies in animals that were born after the 2006/07 RVF outbreak in Tanzania and demonstration of immunoglobulin M (IgM), a short lived class of antibodies, following infection by RVF virus in 9 samples. We have also identified hotspots of transmission in the study area, with exposure being higher away from the main flood plain. There was a linear increase in percent seropositivity from 1 year olds to age 5 years, implying a possible annual challenge.

Design, sampling and data collection

The sero-survey was done from May to August 2011, approximately four years after the end of the 2006/07 RVF outbreak in Tanzania. Blood samples were collected from three livestock populations, namely cattle, goats and sheep from 44 villages in both districts. These villages were selected based on their inclusion in a demographic surveillance system and the proximity to the Kilombero river flood plains. In each village four households that kept at least one of the three species, were randomly selected from the list of livestock keepers in that particular village. For each participating household a maximum of 18 samples were collected (i.e. maximum 10 cattle, maximum 4 goats and maximum 4 sheep, the actual numbers sampled depending on the number of a particular species present). The sampling strategy was based on selecting animals of all age groups in order to be able to characterize epidemic and inter-epidemic transmission. The blood samples were collected in 6 ml vacutainer tubes with clot activator, labeled and stored in a cooler box with ice packs while in the field. After coagulation, serum was eluted from the whole blood into a 1.8 ml cryovial tube, labeled and stored in a car fridge until transfer to the laboratory for laboratory analysis. Characteristics of individual animals together with the herd history were obtained through a structured interview with the herd owner.

Age determination

Individual animal age was estimated using history taking, review of available records on date of birth and dentition. Records were available only from households keeping exotic dairy cattle. Dentition was used in determining age of cattle between 24 to 34 months only. When the above two methods yielded no useful results, age was estimated by probing the head of the household, herd boys and other members of the household for the animal’s mouth/season and year of birth and for female animals also by taking into account number of births and average birth rate for the particular species in the valley.

Serological assays

To determine the individual animals’ longstanding exposure status to previous Rift Valley fever virus (RVFV), a commercial, inhibition enzyme-linked immunosorbent assay (c-ELISA) for the
detection of antibodies against RVFV in humans, domestic and wildlife ruminants was used (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [38]. Recent infection was determined using a commercial IgM ELISA kit (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [39]. The IgM ELISA test was employed for c-ELISA positive samples only since the c-ELISA detects both IgG and IgM antibodies against RVFV [38].

**Data analyses**

The data was analysed in STATA version 12 [Stata Corp., College Station, Texas, USA]. To examine the determinants for RVF seropositivity, first a univariable analysis of individual factors was performed by fitting a logistic regression model with wards and villages as random effects to account for clustering. Variables with p-value $<0.25$ were selected as potential covariables in the multivariable analysis, where a p-value $<0.05$ was considered statistically significant. Forward model-building was done with subsequent models evaluated against sparser models by means of the Akaike information criterion (AIC). Two-way interactions between variables included in the model were also tested. Lastly, all factors that were dropped in the process of model building were later tested for any confounding effect. Factors were considered a confounder if they led to a change of $\pm 25\%$ in the coefficient estimates of other determinants.

The spatial analysis of the seropositivity was performed using ArcGIS software version 10 [ESRI, Redlands, USA] using hot spot analysis and inverse distance weighted (IDW) tools. The Getis-Ord G* statistic for each feature (household) was computed with the resultant Z-score values indicating where households with either high (hot spot), median (random) or low (cold spot) values cluster spatially. The IDW is a deterministic interpolation model that assigns values to locations where no measurements have been taken to produce a surface pattern, based on how far those locations are to the sentinel locations where measurements have been taken.

**Ethics statement**

The blood collection procedure from livestock was performed by a qualified veterinarian following proper physical restraint of animals that ensured both personnel and animal safety. Livestock owners were explained the study purpose and procedures and upon agreeing to participate they provided a written consent prior to study procedures and blood collection from their animals. Ethical approval for this study protocol was obtained from the Institutional Review Board of the Ifakara Health Institute and Medical Research Coordination Committee of the Tanzania’s National Institute for Medical Research (permit number NIMR/HQ/R.8a/Vol. IX/1101).

**Results**

A total of 1680 livestock serum samples were tested by RVF c-ELISA, of which 1234 samples were from Kilombero district and 446 samples were from Ulanga district. Out of the samples tested 970, 455 and 255 were from cattle, goats and sheep respectively. Several potential animal-level risk factors were investigated; table 1 shows the univariable logistic regression model output of the risk factors. The proportion of seropositive animals by c-ELISA was 11.3%. A seroprevalence of 5.5% was recorded among animals that were born after the 2006/07 RVF outbreak (less than 4 years of age), compared to 22.7% in those that were born before and thus present during the outbreak. There was a linear increase in
Table 1. Potential animal level risk factors associated with RVF sero-positivity for cattle, goats and sheep in the Kilombero Valley, Tanzania based on a univariable random effect logistic regression model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>%Positive (n)</th>
<th>OR</th>
<th>95% CI</th>
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<td><strong>Species</strong></td>
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<td></td>
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<tr>
<td>Cattle</td>
<td>11.03 (970)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>11.86 (455)</td>
<td>1.06</td>
<td>0.74–1.52</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>11.37 (255)</td>
<td>1.07</td>
<td>0.68–1.69</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14.16 (1144)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5.22 (536)</td>
<td>0.30</td>
<td>0.19–0.46</td>
<td></td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exotic</td>
<td>9.54 (220)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>11.61 (1420)</td>
<td>1.23</td>
<td>0.70–2.15</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>10.00 (40)</td>
<td>0.95</td>
<td>0.28–3.14</td>
<td></td>
</tr>
<tr>
<td><strong>District</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kilombero</td>
<td>10.69 (1234)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulanga</td>
<td>13.00 (446)</td>
<td>1.17</td>
<td>0.68–2.03</td>
<td></td>
</tr>
<tr>
<td><strong>Present during RVF outbreak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5.55 (1116)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22.69 (564)</td>
<td>5.29</td>
<td>3.79–7.38</td>
<td></td>
</tr>
<tr>
<td><strong>Herd composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One species</td>
<td>10.27 (360)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two species</td>
<td>10.30 (359)</td>
<td>1.08</td>
<td>0.64–1.83</td>
<td></td>
</tr>
<tr>
<td>Three species</td>
<td>12.07 (961)</td>
<td>1.24</td>
<td>0.80–1.92</td>
<td></td>
</tr>
<tr>
<td><strong>Distance to the edge of floods (km)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 km</td>
<td>10.74 (1117)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–10 km</td>
<td>14.01 (157)</td>
<td>1.27</td>
<td>0.72–2.21</td>
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<tr>
<td>10–15 km</td>
<td>7.94 (214)</td>
<td>0.75</td>
<td>0.41–1.37</td>
<td></td>
</tr>
<tr>
<td>&gt;15 km</td>
<td>16.14 (192)</td>
<td>1.62</td>
<td>0.91–2.86</td>
<td></td>
</tr>
<tr>
<td><strong>Feeding practices</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>4.76 (21)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing</td>
<td>11.30 (1592)</td>
<td>2.29</td>
<td>0.29–18.18</td>
<td></td>
</tr>
<tr>
<td>Cut &amp; carry</td>
<td>13.43 (67)</td>
<td>2.80</td>
<td>0.31–25.06</td>
<td></td>
</tr>
<tr>
<td><strong>Altitude (m)</strong></td>
<td>Continuous</td>
<td>n/a</td>
<td>1.007</td>
<td>0.99–1.01</td>
</tr>
</tbody>
</table>

OR = Odds ratio; CI = Confidence interval.

doi:10.1371/journal.pntd.0002356.t001

Figure 2. Seropositivity to Rift Valley fever virus in cattle, sheep and goats pooled by age. The error bars indicate 95% confidence intervals of the percentage.
doi:10.1371/journal.pntd.0002356.g002
Table 2. Animal level risk factors associated with RVF seropositivity for cattle, goats and sheep in the Kilombero valley in multivariable analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Levels</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>0.41</td>
<td>0.26–0.63</td>
</tr>
<tr>
<td>Present during RVF outbreak</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4.67</td>
<td>3.33–6.55</td>
</tr>
<tr>
<td>Distance to the edges of floods (km)</td>
<td>&lt;5 km</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5–10 km</td>
<td>1.28</td>
<td>0.72–2.29</td>
</tr>
<tr>
<td></td>
<td>10–15 km</td>
<td>0.89</td>
<td>0.48–1.65</td>
</tr>
<tr>
<td></td>
<td>&gt;15 km</td>
<td>1.92</td>
<td>1.04–3.56</td>
</tr>
</tbody>
</table>

OR = Odds ratio; CI = Confidence interval.

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Discussion

The findings from this serosurvey indicate post-epidemic and recent transmission of RVFV in livestock populations in the Kilombero valley. The demonstration of RVFV antibodies in animals as young as one year old, and the observed linear increase in the proportion of seroconverted animals from the age of 1 year to 5 years implies a constant exposure to infectious mosquito bites. The IgM antibodies detected in some animals illustrate presence of active foci of recent transmission preceding the serosurvey as the median duration of IgM antibodies to RVFV is two months [39–41]. These observations add to the increasing body of serological [3,4,42,43] and virological [44,45] evidence pertaining to RVFV transmission during the inter-epidemic periods in parts of Africa. Inter-epidemic transmission can be detected where active disease surveillance is in place in the livestock and/or human populations, as most of the inter-epidemic infections either are subclinical or mistaken for other diseases in the absence of public awareness of RVF presence [6,46,47].

The high prevalence observed in animals that were present during the outbreak is not surprising as during epidemics there is high exposure to RVFV with resulting high herd immunity [48,49]. Such increased prevalence with age was also reported in sero-surveys in Madagascar, Nigeria and Senegal [47,48,50]. One of the few other studies reporting sex differences in RVF prevalence in livestock, a study in a slaughter house in Chad also found higher prevalence in female animals [51]. In contrast to our observation, a serosurvey in Madagascar reported higher prevalence in male animals [30]. Our observation might be as a result of female animals staying longer in a herd due to their role in reproduction and consequently most of sampled female animals were relatively older than their male counterparts. The different timing of our survey and the Madagascar survey, i.e. 4 years versus 3 months post-outbreak, might explain the different result on risk associated with sex in livestock populations.

Despite the sero-conversions observed in animals born after the 2006/07 outbreak, there have been no reports of epidemic or clinical disease in the area during the study period. This might be as a result of high herd immunity following the RVF outbreak as demonstrated by high prevalence in animals that were present during the previous epidemic in the study area. In such scenario high proportion of offspring born to immune dams would acquire maternal antibodies thus protected during vulnerable young age whereas old animals of local breeds are naturally less susceptible to clinical disease [52,53]. Another explanation could be a circulation of non-virulent strain of RVFV [54] during inter-epidemic period in the area as it has been hypothesized in other reports of seroconversion with no previous epidemic or clinical disease reports [46]. The sporadic cases of RVF could easily be confused with other livestock diseases which present similar clinical features of fever, including sporadic abortions and thus overlooked and under reported [53].

The contact between infected vectors and naïve mammalian hosts is the main determinant of arboreal disease transmission [55]. Mosquito vector population dynamics are driven by environment and ecology, which provide essential life resources. The trend observed in this study of increased seroprevalence away from the main flood plain and in high altitude is contrary to findings in other studies [6,46]. This could be an indication of other factors playing a role including localized floods unrelated to the main flood area, but also proximity to dense vegetation (forested environments) that could harbor a variety of mosquito vectors [6], as the main forested areas are further away from the flood plain. Another explanation could be movement of previously exposed animals from one locality to another within the area through animal trade among livestock keepers, dowry or establishment of new households. In such circumstances, naïve animals can be transferred to an infected area or infected animals can be introducing the disease into a naïve location. In this way vector populations which are abundant and of diverse species within the valley [34] are exposed and maintenance mechanisms established where conditions are favorable. The observed separate hotspots further point to fine scale factors playing major role in transmission dynamics. On the other hand the only cold spot is located around Ifakara town, a semi urban environment in which livestock keeping is characterized by small herds, sedentary and mixed feeding practices as compared to relatively large herds with extensive system in the villages. The urban environment might be unfavorable to the main vector species but also due to limited daily livestock movements there is little interaction between neighbouring herds thus possibility of infection to limit itself to isolated herds in the event of disease outbreak.

Given the possible active transmission observed in this study within Kilombero valley and the moving out of livestock from the valley as a result of environmental degradation of wetland due to overstocking and overgrazing, there are chances for incubating or sick animals to introduce the disease into new areas. This might be
enhanced by the quick means of transportation employed and possibilities for animals to harbour the RVFV for up to three weeks [52,56]. In view of that, follow up of these moved herds and their new environment through serological and vector population monitoring will help to inform various stakeholders of the currently unidentified consequences, as livestock movement have been implicated to spread RVF in previously free areas [16,37].

The interaction of livestock keepers with their animals is intense and includes milking, taking care of sick animals, grazing, using as draft animals, slaughtering and butchering and even children playing with animals. Future work should establish to what degree this inter-epidemic zoonotic circulation of RVFV leads to human infection as well. If considerable transmission to humans exists, health care providers within the Kilombero valley should consider RVF in their differential diagnosis in all fever cases presented in their facilities as RVF in humans may present with similar clinical signs to malaria, which is thought to be the main cause of fever in the valley [58]. We think this should be of priority in particular when dealing with patients from agro-pastoralist communities, especially when the malaria test is negative.

Conclusion

The findings from this study indicate post-epidemic and recent transmission of RVFV in livestock populations in the Kilombero valley. The linear increase in prevalence of RVFV antibodies in the post-epidemic annual cohorts implies a constant exposure and presence of active foci of recent transmission preceding the survey.

Supporting Information

Table S1 Comparison of RVF prevalence across species, sex and presence during the 2006/07 RVF epidemic. (DOC)

Acknowledgments

We would like to extend our sincere gratitude to district authorities of Kilombero and Ulanga, agriculture/livestock extension officers and village leaders for their support in the course of conducting this study. We are also indebted to livestock keepers from respective villages who willingly agreed to participate in this study and also by providing assistance in many ways thus making the smooth running of activities. We also thank the anonymous reviewers for their important critiques on this manuscript.

Author Contributions

Conceived and designed the experiments: RDS EG DB. Analyzed the data: RDS EM. Wrote the paper: RDS EG DB.

References


Inter-epidemic acquisition of Rift Valley fever virus in humans in Tanzania

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RESEARCH ARTICLE

Inter-epidemic Acquisition of Rift Valley Fever Virus in Humans in Tanzania

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Abstract

Background

In East Africa, epidemics of Rift Valley fever (RVF) occur in cycles of 5–15 years following unusually high rainfall. RVF transmission during inter-epidemic periods (IEP) generally passes undetected in absence of surveillance in mammalian hosts and vectors. We studied IEP transmission of RVF and evaluated the demographic, behavioural, occupational and spatial determinants of past RVF infection.

Methodology

Between March and August 2012 we collected blood samples, and administered a risk factor questionnaire among 606 inhabitants of 6 villages in the seasonally inundated Kilombero Valley, Tanzania. ELISA tests were used to detect RVFV IgM and IgG antibodies in serum samples. Risk factors were examined by mixed effects logistic regression.

Findings

RVF virus IgM antibodies, indicating recent RVFV acquisition, were detected in 16 participants, representing 2.6% overall and in 22.5% of inhibition ELISA positives (n = 71). Four of 16 (25.0%) IgM positives and 11/71 (15.5%) of individuals with inhibition ELISA sero-positivity reported they had had no previous contact with host animals. Sero-positivity on inhibition ELISA was 11.7% (95% CI 9.2–14.5) and risk was elevated with age (odds ratio (OR) 1.03 per year; 95% CI 1.01–1.04), among milkers (OR 2.19; 95% CI 1.23–3.91), and individuals eating raw meat (OR 4.17; 95% CI 1.18–14.66). Households keeping livestock had a higher probability of having members with evidence of past infection (OR = 3.04, 95% CI = 1.42–6.48) than those that do not keep livestock.

Conclusion

There is inter-epidemic acquisition of RVFV in Kilombero Valley inhabitants. In the wake of declining malaria incidence, these findings underscore the need for clinicians to consider RVF in the differential diagnosis for febrile illnesses. Several types of direct contact with livestock are important risk factors for past infection with RVFV in this study’s population.
However, at least part of RVFV transmission appears to have occurred through bites of infected mosquitoes.

Author Summary

Rift Valley fever (RVF) is a disease of animals and people that is caused by the RVF virus. During epidemics, humans get RVF through direct contact with animals or through mosquito bites. In East Africa, epidemics occur every 5–15 years following unusually high rainfall. In between epidemics, the transmission of RVF might occur at low level. In an epidemic-free period, we measured whether people in the Kilombero Valley in Tanzania had evidence of past and recent RVF infection in their blood sample, and studied risk factors. Three per cent of people had been infected recently, and 12% had evidence of past infection, with increased risk with age, among milkers and among people eating raw meat. Some people with past or recent infection reported they had not had contact with animals. Households keeping livestock had more members with evidence of past infection. The findings show that people get infected with RVF in between epidemics, and that various types of contact with livestock are important risk factors. There is also evidence that some people get infected with RVFV by mosquitoes in the epidemic free period. Clinicians in the Kilombero Valley should consider RVF in the differential diagnosis of patients with fever.

Introduction

Rift Valley fever (RVF) is one of the major viral zoonoses in Africa. The disease is caused by the Rift Valley fever virus (RVFV) of the genus *Phlebovirus* in the family *Bunyaviridae* [1], and it is transmitted to animals through infectious mosquito bites and other arthropod vectors [2]. People become infected either from mosquito bites or by direct or indirect contact with infectious material when exposed to blood, body fluids or tissues of viraemic animals when handling sick or dead animals as well as through aerosol transmission, consumption of raw milk, meat or blood [3–5].

The disease was first described in the Rift Valley of Kenya in the early 1900s and the etiological agent demonstrated in the early 1930s [6]. RVF epidemics occur in cycles of 5–15 years in the Eastern Africa region as a result of abnormally high precipitation, for example during the warm phase of the El Niño/Southern Oscillation (ENSO) phenomenon [7]. In other regions the disease has been driven by floods caused by other sources including construction of hydroelectric dams [8]. During the outbreaks the disease causes devastation in livestock populations and economies of livestock keepers as a result of morbidity, mortality in new-borns and abortions (irrespective of gestation period) with direct negative consequences in the next crop of new-borns [9].

Public health consequences during epidemics involve a wide range of clinical manifestation in people including mild illnesses characterized by fever, muscle pain, joint pain, and headache, which can cause RVF to be confused clinically with other febrile illnesses such as malaria. In mild cases, symptoms persist for about a week and subside without specific treatment. A small percentage (0.5–2%) of patients may develop severe forms of the disease characterized by either ocular disease, meningo-encephalitis or haemorrhagic fever which last for 1–4 weeks after onset of symptoms [10, 11]. People most at risk include those in close contact with infected animals and infectious materials [4], but also those unprotected from infectious bites of infected
mosquitoes. Apart from general supportive therapy, there is no established treatment for people, and a commercial vaccine for humans is not available either. The control of RVF therefore relies mainly on vaccination of livestock and preventive measures by humans (including protection from mosquito bites and avoidance of contact with infected animals and infectious material during epidemics). [11].

Inter-epidemic transmission has increasingly been reported in recent years, including in our study area, but its consequences are not fully understood and its incidence not explored enough for future epidemic preparedness [8, 12–16]. Relatively little is known regarding the natural history of RVF as the epidemics occur in remote areas inaccessible during heavy rains; on the other hand, inter-epidemic RVF transmission presents an opportunity for studying its natural history as it normally occurs when affected areas are accessible.

In Tanzania, RVF with human involvement has been reported in the past [17, 18], with few studies demonstrating inter-epidemic transmission in livestock and people [12, 19]. During the 2006/07 RVF epidemic in Tanzania, livestock and people in the Kilombero Valley were affected [20], and a sero-survey in livestock indicated presence of inter-epidemic period transmission of RVF [12]. The Kilombero Valley is a seasonally inundated floodplain between the densely forested escarpment of the Udzungwa mountains to the northwest and the grass covered Mahenge mountains to the southeast. The annual floods in the valley mimic flooding that may occur elsewhere during ENSO years. In the Kilombero Valley, there has been intense malaria transmission due to abundance of the *Anopheles gambiae* complex, but other mosquito species including vectors of RVF virus (e.g. *Culex* spp., *Aedes* spp. and *Mansonia* spp.) are present [21]. The current study therefore aimed to 1) determine whether people do acquire RVF during the inter-epidemic period in the Kilombero Valley and 2) evaluate the demographic, behavioural, occupational, and spatial determinants of recent and longstanding RVF sero-positivity in people.

**Methodology**

**Study population and area**

We conducted the study in rural areas of the Kilombero River Valley, located in the Kilombero and Ulanga districts in south-eastern Tanzania [22]. The Kilombero Valley is characterized by seasonal flooding which supports reproduction of large numbers of mosquitoes including arbovirus vectors such as *Aedes* spp [21]. The inhabitants of the two districts engage mainly in smallholder farming, fishing, and livestock keeping. A serological survey was carried out from March to August 2012 in six villages, three from each study district, with a total population of 14,517 in 3716 households. About a quarter of households keep livestock [23]. We selected the villages from hotspots of RVF transmission in the livestock populations in the Kilombero Valley [12]. This aimed at maximizing the probability of detecting inter-epidemic virus activity in the human population, since the hotspots indicated presence of ecological features that promote RVF transmission.

**Data and sampling**

The sample size calculation took into account the fact that sampling was done in households (clusters), with an average cluster size of 5 individuals per household considered appropriate for the valley [22] so a design effect of 3 was applied. The design effect adjusted sample size was further adjusted for the expected number of covariates we hoped to evaluate, which overall gave a sample size of 726 in 145 clusters. To ensure equal representation, we selected livestock keepers’ and farmers’ households independently as sampling units, because the two sub-populations are exposed in different ways to RVF risk factors [24]. In the four villages that were within the health and demographic surveillance system (IHDSS) of the Ifakara Health Institute, we
randomly selected farmers’ households from the master list of IHDSS [23]. For farmers’ households in the other two villages and for livestock keepers’ households in all villages, we obtained the lists of households from the village office and manually picked every nth household from the list.

We took blood samples from all members of the household who provided written consent to participate in the study. For children under 18 years the written consent was provided by parents or guardians. We collected blood samples into vacutainer tubes containing clot activator and after clotting, eluted the sera into cryovial tubes and kept these in a car fridge until transferred to the laboratory. We collected demographic characteristics and individuals’ exposure to risk factors to RVF through a structured questionnaire.

Serological analyses

We analysed the serum samples for presence of RVFV antibodies by two commercial enzyme-linked immunosorbent assay (ELISA) kits, an inhibition ELISA and a capture ELISA. The inhibition ELISA simultaneously detects immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against RVFV in humans, domestic and wildlife ruminants (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [25]. We converted the net optical density (OD) reading for each sample to a percentage inhibition (PI) value using the equation: \[(100 – \frac{\text{net OD of test sample}}{\text{mean net OD of negative control}} \times 100)\]. Test results producing PI values ≥38.6 are considered positive (following the manufacturer’s recommendations) whereas below that threshold is negative, with sensitivity and specificity being 99.5% and 99.7% respectively [25]. To determine recent infection, we then tested the positive samples for the presence of IgM using the capture IgM ELISA (Biological Diagnostic Supplies Limited, Dreghorn, United Kingdom) [26, 27]. For this test, we used the two intermediate net OD values of positive controls (C+) for the calculation of the net mean OD value of C+. We then used this value in subsequent calculations of percentage positivity (PP) of C+, C- and test sera as follows: \[\text{PP} = \frac{\text{net OD serum}}{\text{net mean OD C+}} \times 100\]. The cut off for positive samples’ PP values was ≥7.1, with sensitivity and specificity being 96.4% and 99.6% respectively [27].

Data analyses

We analysed the data in STATA version 13 (Stata Corp., College Station, Texas, USA). Samples that were positive by inhibition ELISA were considered to give evidence of past infection in the individual, as IgG antibodies last long in persons infected in the past [26]. Samples that were positive by IgM ELISA were considered to indicate recent infection in the individual, as IgM antibodies are short lived following infection by RVF virus [26, 28]. To examine risk factors of RVF virus infection and help identify households at higher risk for targeted public health interventions, we developed three separate mixed effect logistic models. We built two models for individual level risk factors for recent and past infection as outcome variables respectively and treated households as a random effect variable. We built a third model for household sero-positivity as outcome variable and villages as random effect variable. For each model, we first determined the univariable association of individual factors with the outcome by fitting a logistic regression model. Variables with p-value <0.25 were selected as potential covariates in the multivariable analysis, where a p-value ≤0.05 was considered statistically significant. We performed manual forward model-building with subsequent models evaluated against sparser models by means of the Akaike Information Criterion (AIC). We also tested two-way interactions between variables included in the model. Lastly, all factors that were dropped in the process of model building were later tested for any confounding effect. We considered factors to be a confounder if they led to a change of ≥25% in the coefficient.
estimates. We calculated the population attributable fraction (PAF), a fraction of all cases in
the study population due to exposure to a certain risk factor, as follows: PAF = \( \frac{Px \times (RR-1)}{1+(Px \times (RR-1))} \), where \( Px \) = estimated population exposure and \( RR \) = risk ratio.

Ethics statement
We obtained ethical approval from both the Institutional Review Board of the Ifakara Health
Institute (IHI-IRB) and Medical Research Coordination Committee of the Tanzania’s National
Institute for Medical Research for this study, permit number NIMR/HQ/R.8a/Vol.IX/1101. Prior to study procedures, participants were explained the study purpose and procedures and
upon agreeing to participate, individual adult participants provided a written informed consent whereas parents or guardians provided written consent for the under-age participants.

Results
The analyses were based on data from 606 participants in 141 households with complete ques-
tionnaire and laboratory results. We could not attain the a priori calculated sample size because of consenting issues among household members and because family size was smaller than expected. We do not anticipate this has introduced underrepresentation of participants with cer-
tain characteristics given the number of clusters involved. Out of 606 participants, 55.6% were
females with age ranging between 2 and 90 years. Fifty four per cent and 46% of the partici-
pants originated from Kilombero and Ulanga districts respectively.

The inhibition ELISA results indicated an overall RVF sero-prevalence of 11.7% (95% CI = 9.2–14.5). There was a linear increase in sero-prevalence in the 10 year cohorts (Fig. 1). Evi-
dence of recent infection by RVFV was found in 16 participants representing 2.6% overall
(\( n = 606 \)) and 22.5% of inhibition ELISA positive individuals (\( n = 71 \)). Four of 16 (25.0%) IgM
positives and 11/71 (15.5%) of individuals with inhibition ELISA sero-positivity reported they had had no previous animal contact, suggesting that at least part of the transmission in the area occurred through infected mosquito bites.

In the univariable analyses, factors associated with past RVF infection were history of partici-
pating in slaughter of animals (odds ratio [OR] 1.85; 95% CI 1.01–3.42), assisting birthing

![Fig 1. Prevalence of Rift Valley fever by age groups. The trend line indicates gradual increase of sero-
positivity with age.](doi:10.1371/journal.pntd.0003536.g001)
animals (OR 2.02; 95% CI 1.12–3.63), milking animals (OR 2.45; 95% CI 1.35–4.45), eating raw meat/blood (OR 6.01; 95% CI 1.86–19.39), disposing aborted foetus (OR 2.04; 95% CI 1.13–3.67) and being older (OR 1.03 per year; 95% CI 1.02–1.04) (Table 1). In the multivariable model, age (OR 1.03; 95% CI 1.01–1.04), milking animals (OR 2.19; 95% CI 1.23–3.91) and eating raw meat/blood (OR 4.17; 95% CI 1.18–14.66) remained significantly associated with past infection (Table 2). The PAFs of milking animals and eating raw meat in the past were 29% and 6% respectively. None of the risk factors studied were associated with recent infection (results not shown).

Though keeping livestock was not associated with individuals' sero-positivity, households keeping livestock had a higher chance of having at least one member with past infection (OR = 3.04, 95% CI = 1.42–6.48) than households that do not keep livestock (table 3). Participant’s gender, eating meat from dead animals, drinking raw milk, bed net use, proximity to the main flood area, elevation and district were not associated with inhibition ELISA sero-positivity.

Discussion

We report here presence of IgM antibodies against RVFV among inhabitants of Kilombero Valley. This confirms recent infection and thus transmission of RVF which is not linked to the previous epidemic which happened five years prior in the study area [20]. This finding affirms our previous report, which highlighted IEP transmission of RVF in livestock [12]. Inter-epidemic sero-positivity to RVF in people has also been previously documented in other parts of Tanzania and Africa [13, 14, 16, 29], with IgM antibodies detected in Nigeria and Chad [14, 29]. The observed sero-prevalence by inhibition ELISA (11.7%) in this study is high compared to studies from other parts of Tanzania during inter-epidemic period with sero-prevalence of 5.2% and 4% in Mbeya and Tanga regions respectively [13, 19], possibly as a result of our sampling of participants from hotspots of RVF circulation in animals. In Gabon, a country with no RVF epidemic history, a sero-prevalence of 3.3% has been reported [30], in Kenya, an epidemic prone country, a mixed picture for inter-epidemic sero-positivity has been recorded in people in different geographical locality and time [16, 31].

In this study, participants who milked animals were more likely to have evidence of past RVF infection. This points to a potential public health consequence of RVFV shedding in milk which occurs during the viraemic phase of the disease. The traditional milking practices create a lot of aerosols, and if one is milking a viraemic animal the RVFV containing milk particles could result into infection to milkers through inhalation of the infectious aerosols [32]. Also skin abrasions on hands of milkers could form an easy route of infection when people have broken skin. However, drinking raw milk was not associated with longstanding sero-positivity. Although raw milk consumption is considered an important risk factor during epidemics [18, 33], the infection through oral route comes across barriers including acidic environment in the stomach [34]. The findings in our study might also be explained by the practice of consuming fermented milk by the livestock keepers in which case the virus would die when exposed to acidic environment of sour milk [34].

People who ate raw meat (including blood and internal organs such as kidneys and liver) were more likely to have evidence of past RVF infection. The animal products (meat and blood) from infected animals could have a high concentration of RVFV which has the ability to persist at neutral pH in carcasses. When meat is consumed raw before the pH drops with rigor mortis this could lead to infection in people. Eating meat from animals that died before slaughter was not associated with sero-positivity which might be because individuals who reported
Table 1. Prevalence of RVF inhibition ELISA sero-positivity and association of individual-level variables with sero-positivity.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Factor</th>
<th>Level</th>
<th>%Positive (n)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>District</td>
<td>Kilombero</td>
<td>11.6 (327)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Village</td>
<td>Ulanga</td>
<td>11.8 (279)</td>
<td>1.01</td>
<td>0.53–1.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Iragua</td>
<td>12.6 (119)</td>
<td>1.01</td>
<td>0.53–2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lungongole</td>
<td>10.2 (137)</td>
<td>0.75</td>
<td>0.31–1.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lupiro</td>
<td>12.0 (75)</td>
<td>0.91</td>
<td>0.33–2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mofu</td>
<td>15.8 (101)</td>
<td>1.33</td>
<td>0.55–3.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nakafuru</td>
<td>10.5 (85)</td>
<td>0.84</td>
<td>0.31–2.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sagamaganga</td>
<td>8.9 (89)</td>
<td>0.70</td>
<td>0.25–1.93</td>
</tr>
<tr>
<td>3</td>
<td>Sex</td>
<td>Female</td>
<td>10.6 (337)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>13.0 (269)</td>
<td>1.26</td>
<td>0.75–2.12</td>
</tr>
<tr>
<td>4</td>
<td>** Age (year categories)</td>
<td>0–10</td>
<td>1.9 (105)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11–20</td>
<td>6.5 (168)</td>
<td>3.59</td>
<td>0.76–16.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21–30</td>
<td>13.8 (108)</td>
<td>9.02</td>
<td>1.94–41.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31–40</td>
<td>14.7 (68)</td>
<td>8.95</td>
<td>1.85–43.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41–50</td>
<td>23.3 (77)</td>
<td>16.97</td>
<td>3.67–78.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51–60</td>
<td>17.0 (47)</td>
<td>10.87</td>
<td>2.12–55.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61–70</td>
<td>14.2 (14)</td>
<td>10.24</td>
<td>1.21–86.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71–80</td>
<td>25.0 (12)</td>
<td>19.71</td>
<td>2.65–146.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt; 80</td>
<td>28.5 (7)</td>
<td>21.43</td>
<td>2.22–206.86</td>
</tr>
<tr>
<td>5</td>
<td>Occupation</td>
<td>Farmer</td>
<td>11.9 (242)</td>
<td>1.09</td>
<td>0.08–0.167</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Livestock keeper</td>
<td>11.7 (356)</td>
<td>0.117</td>
<td>0.086–0.156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
<td>0.0 (8)</td>
<td>0.000</td>
<td>0.000–0.369</td>
</tr>
<tr>
<td>6</td>
<td>Bed net ownership</td>
<td>Yes</td>
<td>11.4 (577)</td>
<td>0.56</td>
<td>0.18–1.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>19.2 (26)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Bed net use</td>
<td>Yes</td>
<td>11.0 (532)</td>
<td>0.75</td>
<td>0.34–1.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>16.4 (73)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Keeping livestock</td>
<td>Yes</td>
<td>12.0 (365)</td>
<td>1.10</td>
<td>0.61–1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>11.2 (241)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>** Slaughter animal in the past</td>
<td>Yes</td>
<td>17.2 (110)</td>
<td>1.85</td>
<td>1.01–3.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>10.5 (493)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Slaughter a sick animal in the past</td>
<td>Yes</td>
<td>16.6 (30)</td>
<td>1.47</td>
<td>0.56–3.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>11.5 (562)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>** Eat raw meat</td>
<td>Yes</td>
<td>42.8 (14)</td>
<td>6.01</td>
<td>1.86–19.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>10.9 (583)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>* Eat meat from dead animal</td>
<td>Yes</td>
<td>14.0 (249)</td>
<td>1.59</td>
<td>0.89–2.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>9.8 (314)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Don’t know</td>
<td>14.2 (35)</td>
<td>1.50</td>
<td>0.51–4.46</td>
</tr>
<tr>
<td>13</td>
<td>** Milking</td>
<td>Yes</td>
<td>16.5 (254)</td>
<td>2.45</td>
<td>1.35–4.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>8.2 (350)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Drink raw milk</td>
<td>Yes</td>
<td>12.2 (450)</td>
<td>1.28</td>
<td>0.67–2.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>9.8 (152)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>** Help with birthing animal</td>
<td>Yes</td>
<td>17.3 (127)</td>
<td>2.02</td>
<td>1.12–3.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>9.6 (458)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>** Dispose of aborted foetus</td>
<td>Yes</td>
<td>18.5 (113)</td>
<td>2.04</td>
<td>1.13–3.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>10.1 (464)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Significance levels at univariable mixed effect logistic regression model,
** > 0.05; * > 0.05 but < 0.25
doi:10.1371/journal.pntd.0003536.t001
eating meat from dead animals also reported cooking the meat before consumption, which would have destroyed the virus.

The high PAF values for milking and for eating raw meat as risk factors present important educational intervention targets for risk reduction even during epidemic free periods. The increased sero-prevalence in older individuals suggests stable rates of on-going transmission in the population. The increased sero-prevalence was also evident when participants were categorized into ten-year cohorts, with drops in the 51–60 and 61–70 groups. Older individuals might have either been infected in one or more previous epidemics or through clinically undetected low-level virus circulation in the study area.

Although there was no significant risk difference between individual livestock keepers and farmers, households keeping livestock had a higher probability of having at least one member with past RVF infection. This might imply presence of either higher risk through animal contact as compared to mosquito bites or higher exposure to infectious mosquito bites among livestock keepers, as mosquitoes living in close proximity to livestock can pick up infection from amplifying infected hosts and transmit to livestock keepers even in circumstances of low-level virus circulation in the general vector populations.

Helping with birthing animals and disposal of aborted foetuses are high risk activities when dealing with infected animals or infectious materials especially when not wearing proper protective attire. In this study both were not statistically significant in the final model. People who reported participating in slaughtering animals in the past (including skinning and butchering)

Table 2. Multivariable analysis of correlates of RVF antibody sero-positivity.

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor</th>
<th>Level</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Help with birthing animal</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>0.83</td>
<td>0.36–1.90</td>
</tr>
<tr>
<td>2</td>
<td>Age (years)</td>
<td>n/a*</td>
<td>1.03</td>
<td>1.01–1.04</td>
</tr>
<tr>
<td>3</td>
<td>Milking</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>2.19</td>
<td>1.23–3.91</td>
</tr>
<tr>
<td>4</td>
<td>Eat raw meat</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>4.17</td>
<td>1.18–14.66</td>
</tr>
<tr>
<td>5</td>
<td>Dispose of aborted foetus</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>1.35</td>
<td>0.59–3.09</td>
</tr>
</tbody>
</table>

* Age was included as a continuous variable, OR = odds ratio, CI = confidence interval

doi:10.1371/journal.pntd.0003536.t002

Table 3. Household-level factors for RVF sero-positivity.

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor</th>
<th>Level</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Keep livestock</td>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>3.04</td>
<td>1.42–6.48</td>
</tr>
<tr>
<td>2</td>
<td>Elevation (meters)</td>
<td>n/a*</td>
<td>0.98</td>
<td>0.97–1.00</td>
</tr>
</tbody>
</table>

* Elevation was included as a continuous variable, OR = odds ratio, CI = confidence interval

doi:10.1371/journal.pntd.0003536.t003
were more likely to be sero-positive but the sero-positivity was not associated with slaughtering sick animals suffering from other unknown conditions. Slaughtering animals sick from RVF exposes individuals through direct contact with infectious materials such as aerosols from oozing blood and other organs during skinning and butchering [3].

Although sero-prevalence in male individuals was slightly higher, sex was not associated with sero-positivity. The sex difference in RVF prevalence has been reported in some studies [16, 30] but was not apparent in others [13, 19, 35] and where it existed, it has been mostly attributed to gender-biased distribution of animal handling in affected populations. The lack of association between gender and sero-positivity in this study indicates that either the specific risk-increasing animal handling activities are equally distributed between genders or that direct mosquito bites as source of infection to people in the valley was equally important. The latter possibility is supported in our study area because men were more involved with animal handling duties.

Bed net ownership and use were not associated with sero-positivity. This is possibly because in the study area there is high bed net coverage [36], but also because the main RVF vector *Aedes* mosquitoes are day biting mosquitoes.

**Conclusion**

These findings, coupled with our previous report in livestock [12], indicate persistent IEP transmission of RVFV in both livestock and human populations in the Kilombero Valley. The animal contact risk factors, especially milking and eating raw meat are important and present educational intervention targets for risk reduction. In the wake of declining malaria incidence [37] these findings underscore the need for clinicians to consider RVF in the differential diagnosis for febrile illnesses among Kilombero Valley inhabitants. This is relevant regardless of the person’s occupation, because part of the transmission likely happens through infectious mosquito bites. The findings also suggest the opportunity and need to further investigate the circulating RVFV strain as well as the main vectors responsible for IEP transmission.

**Supporting Information**

S1 Checklist. STROBE Checklist. (DOC)

**Acknowledgments**

We thank people of Kilombero and Ulanga for their kind consideration and for agreeing to participate in this study. We would like to extend our gratitude to village leaders for their support in the course of conducting this study. We thank Athuman Mtandanguo and Fidelis Mbena for their immense assistance during data and sample collection. We thank Maarten Hoek for his helpful discussion and comments on the initial draft of this manuscript. We also thank the three anonymous reviewers for their important critiques on this manuscript.

**Author Contributions**

Conceived and designed the experiments: RDS DB EG. Performed the experiments: RDS MA EG. Analyzed the data: RDS ENA ET DB. Wrote the paper: RDS ENA ET DB EG.

**References**


Modelling Rift Valley fever transmission dynamics at fine-scale ecological level

This chapter has been published in the journal *PLoS ONE*. Supporting material is presented in appendix A (the user manual on how to install, set up and run the model), appendix B (R code for the user interface and differential equations) and appendix C (C++ code for additional computations).
Rift Valley fever: An open-source transmission dynamics simulation model

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Abstract

Rift Valley fever (RVF) is one of the major viral zoonoses in Africa, affecting humans and several domestic animal species. The epidemics in eastern Africa occur in a 5-15 year cycle coinciding with abnormally high rainfall generally associated to the warm phase of the El Niño event. However, recently, evidence has been gathered of inter-epidemic transmission. An open-source, easily applicable, accessible and modifiable model was built to simulate the transmission dynamics of RVF. The model was calibrated using data collected in the Kilombero Valley in Tanzania with people and cattle as host species and *Aedes mcintoshi, A.egypti* and two *Culex* species as vectors. Simulations were run over a period of 27 years using standard parameter values derived from two previous studies in this region. Our model predicts low-level transmission of RVF, which is in line with epidemiological studies in this area. Emphasis in our simulation was put on both the dynamics and composition of vector populations in three ecological zones, in order to elucidate the respective roles played by different vector species: the model output did indicate the necessity of *Culex* involvement and also indicated that vertical transmission in *Aedes mcintoshi* may be underestimated.

This model, being built with open-source software and with an easy-to-use interface, can be adapted by researchers and control program managers to their specific needs by plugging in new parameters relevant to their situation and locality.

Introduction

Rift Valley fever (RVF) is caused by the Rift Valley fever virus (RVFv), which belongs to the genus *Phlebovirus* in the family Bunyaviridae. RVF is one of the major viral zoonoses in Africa, affecting man and several domestic animal species [1, 2].

A syndrome compatible with RVF was first described in the Rift Valley of Kenya in the early 1900s and the virus was isolated in the 1930s [3]. The known range of RVFv is shown in Fig 1. RVF was confined to eastern and southern Africa until about 1975. Since then it has expanded its range first to Egypt (1977), then to western Africa (ca. 1980) and finally to the
Arabian peninsula in 2000 [4]. It has so far not been officially confirmed from the Maghreb countries, although there is at least serological evidence of import into south-western Algeria [5], evidence of human exposure in Tunisia [6], mention of viral presence in Morocco, Algeria and Libya [7] and mention of exposure of camels, gazelle and water buffalo in Turkey [8].

Currently, an epidemic is being experienced in East Africa (Kenya, Rwanda, Tanzania and Uganda reporting cases in humans and animals, ProMED-mail, several postings http://www.promedmail.org). RVFv has been imported into countries outside the normal range, the most recent report being that of a patient, being diagnosed in China and having acquired the infection in Angola [9].

The epidemics in eastern Africa and the Horn of Africa involve a 5–15 year cycle marked by abnormally high rainfall, e.g. during the warm phase of the El Niño/Southern Oscillation phenomenon (ENSO) [10, 11]. In other regions of Africa, the occurrence of the disease is...
linked to other sources of flooding, e.g. the construction of a hydroelectric dam along the Sene-
gal river [12, 13].

In the past, the above was the traditional view of the epidemiology of RVF, but recently there is more and more evidence of so-called inter-epidemic transmission: previously unnoticed low-level viral transmission in all species involved [12, 14–18]. In Tanzania, human involvement in RVF inter-epidemic transmission has been reported in the past [19, 20]. During the 2006/07 RVF epidemic in eastern Africa, livestock and people in the Kilombero valley in Tanzania were affected [21]. Two serological surveys in this region since this last epidemic, one in livestock and one in people, effectively showed the presence of inter-epidemic transmission in the area [17, 22].

RVF is transmitted to humans and other mammalian hosts, both livestock and wild ruminants (e.g. cattle, buffalo, sheep, goats and camels) through mosquito (e.g. *Culex* spp., *Aedes* spp. and *Mansonia* spp.) and other arthropod vector bites [1, 2, 16, 23]. *Aedes* mosquitoes are capable of transovarial (= vertical) transmission of RVFV to the eggs, which can survive long droughts (several years) and hatch when new water arrives during e.g. the ENSO phenomenon, resulting in infected larvae and adult mosquitoes [2]. The highest risk for humans to become infected is through direct and indirect contact with infectious animal materials (blood, body fluids or tissues of viraemic animals). Aerosol formation during e.g. milking or consumption of raw milk, meat or blood form another risk for transmission [13, 24–28]. An established treatment method or a vaccine for humans currently does not exist. Control of the disease needs to be done through vaccination of livestock and preventive measures by humans [29, 30].

Clinical manifestation in humans can go from only mild illness, including fever, muscle pain, joint pain and headache to severe forms with ocular disease, meningo-encephalitis or haemorrhagic fever [29, 31]. The disease manifests itself in livestock through morbidity and mortality in newborns and abortions during all stages of the pregnancy. This has devastating effects on livestock populations and has severe economic repercussions for livestock keepers [2, 26, 32, 33].

Quantitative analysis and simulation modelling of RVFv dynamics have been undertaken on several occasions. Note that the list that follows cites only typical examples and that many more publications exist dealing with RVF modelling. The analytical models use environmental characteristics and range from post-hoc predictions of where outbreaks were to be expected during the 2006-2007 epidemic in East Africa [10] over statistical modelling in order to identify landscape features related to RVFv transmission [34] to the identification of ranges of potential vectors [35]. Simulation models include temporal models using differential equations [36] with extensions to spatial components [37]. Risk analysis of introduction into new territory (*in casu* The Netherlands) [38] has also been carried out. An overview of compartmental models, applied to the simulation of RVF dynamics, is provided by Danzetta and colleagues [39].

The existing models all suffer from being closed, inaccessible and specialised. The combination of R/RSStudio® with the libraries shiny and deSolve offers the possibility to develop open-source, easily applicable, accessible and modifiable models that can, on the one hand, be adapted to a specific situation with minimal programming effort and, on the other hand, be perused by the epidemiological researcher to study different scenarios and/or the effects of different parameter settings. The model presented here has been developed for the specific situation in East Africa, but as explained above, it can easily be adapted to other areas/situations, mostly by switching on or off certain parameters or parameter groups or by the inclusion of extensions with minimal new coding. The model presented in this paper is thus to be considered a research tool, allowing the user to study the effect(s) of different scenarios in order to better understand RVFv transmission dynamics and the mammalian hosts and arthropod
vectors involved, and ultimately to assist in the formulation of new research questions. The model is not a predictive tool, as too much uncertainty still exists with regards to the actual dynamics of inter-epidemic transmission of the virus.

**Model—General description**

The model describes the RVFv transmission dynamics in six species (human population, domestic animal population and four vectors) in three different areas. The model attempts to offer maximal flexibility, whilst remaining manageable. The model allows for migration of the various species between the different areas. The different compartments in the model are presented in Table 1 and a simplified schematic representation of the model is shown in Fig 2.

Each human and animal population consists of a susceptible $S$, exposed $E$, infected $I$ and removed $R$ (= recovered/immune) compartment. There is a flow back from the removed to the susceptible compartment in both populations, i.e. immunity is not lifelong. All individuals are born susceptible and a proportion of the pregnant infected animals abort. Vectors $A$ and $B$ allow for vertical transmission: infected females ($I$ compartment) transmit infection to their eggs ($Q$ compartment), where the virus survives until the larva hatch and the resulting adults are infective. Vector $A$ furthermore has the possibility of long-term dormancy in the egg stage (both infected and non-infected).

A challenge lies in the correct modelling of the vector dynamics. More specifically, a point of attention is the distribution of feeding individuals over the different host populations (both species-wise and zone-wise). Vectors can feed on the two modelled host species (human and domestic animal), but they can also use alternative hosts (especially so in the forest zone). The latter means there is no increased mortality in case the two main hosts are not available, but this of course also influences infection prevalence in the vector population. The vector populations are furthermore limited by a density-dependent oviposition rate. The approach currently taken uses the following basic parameters (see Vector feeding and infection rates for details):

- $\epsilon$: proportion of vector $\Xi$ feeding on host $\Lambda$ in zone $i$; it is the user’s responsibility to ensure that the sum of the various $\epsilon$ per species per zone does not exceed one
- $\eta$: (maximum) number of successful bites per time unit of vector $\Xi$ on host $\Lambda$
- $\pi_{uv}$: probability to transmit infection from species $u$ to species $v$ ($v \neq u$) upon a successful bite
- $\Omega_{alt}$: number of alternative hosts

<p>| Table 1. Different compartments in the model. |</p>
<table>
<thead>
<tr>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_1$</td>
<td>$H_1^a$</td>
<td>$H_1'$</td>
</tr>
<tr>
<td>$M_1$</td>
<td>$M_1^a$</td>
<td>$M_1'$</td>
</tr>
<tr>
<td>$A_1$</td>
<td>$A_1^a$</td>
<td>$A_1'$</td>
</tr>
<tr>
<td>$B_1$</td>
<td>$B_1^a$</td>
<td>$B_1'$</td>
</tr>
<tr>
<td>$C_1$</td>
<td>$C_1^a$</td>
<td>$C_1'$</td>
</tr>
<tr>
<td>$D_1$</td>
<td>$D_1^a$</td>
<td>$D_1'$</td>
</tr>
</tbody>
</table>

$H = \text{People}; M = \text{Domestic animals}; A = \text{Vector } A; B = \text{Vector } B; C = \text{Vector } C; D = \text{Vector } D; \\
\subset S = \text{susceptible}; \subset X = \text{exposed}; \subset I = \text{infected}; \subset R = \text{removed}; \\
\subset Q = \text{infected eggs}; \subset P = \text{non-infected eggs}; \\
\subset Z = \text{Zone } 1; \subset Z' = \text{Zone } 2; \subset Z'' = \text{Zone } 3$

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El Niño events are currently modelled to occur every ten years. Additionally, the user is given the opportunity to include annual overall climate variability through the choice of a random series of ‘dry’ or ‘wet’ years and a seasonal within-year variation in egg eclosion to model seasonal effects on vector population size. Finally, there is the possibility of including a ‘fixed’ annual domestic animal movements between zones 1 and 2, simulating seasonal transhumance of (e.g.) cattle between the plateau and the floodplain. Details are to be found in Seasonality and El Niño effect.

\[ \kappa_j \] maximum number of vector \( \Xi \) individuals in zone \( j \) (‘carrying capacity’)

Fig 2. Diagrammatic representation of the model. Note: for the sake of clarity, inter-zone movement is indicated only for the susceptible animal compartment (\( M_S \)); it is identical for all other compartments. Also for the sake of clarity, compartments are only shown for human population (\( H \)), domestic animal population (\( M \)) and one vector species (\( A \)); see Table 1 for a list of all compartments.

https://doi.org/10.1371/journal.pone.0209929.g002
It is understood that the necessary calculations for these density-dependent oviposition-feeding, climatic variability and transhumance processes slow down the model considerably. It was therefore decided to rewrite part of the code, doing the preparatory computations before calling the deSolve routines (using the classical Runge-Kutta 4\textsuperscript{th} order method), in C++ (making use of the RCCP library). This speeds up execution by a factor of about sixty, but of course means a lower accessibility of the code. Therefore, a slower version, entirely written in R is also offered. Full details on how to install and run the model are given in the accompanying user’s manual \textit{S1 Appendix}. The R and C++ code is provided in \textit{S2 Appendix}.

**Model—Differential equations**

For every zone \(i(i = 1, 2, 3)\), we compute the differential equations of each compartment of the human, the animal and the vector populations.

### Human population

\[
\frac{dH_i}{dt} = \gamma_i N_{ih} + \sum_{j \neq i}^3 \lambda_{ij}^H H_j^i + \rho_{ih} H_r^i - (\mu_H + \beta_H + \sum_{j \neq i}^3 \lambda_{ij}^H) H_s^i; \quad (i = 1, \ldots, 3)
\]

Eq 1 describes the rate of change in the susceptible human compartment in Zone \(i\). \(\gamma_i N_{ih}\) refers to the newborn individuals, \(\sum_{j \neq i}^3 \lambda_{ij}^H H_j^i + \rho_{ih} H_r^i\) refers to the immigration into Zone \(i\) from the other two zones and individuals losing their immunity while \((\mu_H + \beta_H + \sum_{j \neq i}^3 \lambda_{ij}^H) H_s^i\) refers to the losses through natural mortality, people becoming infected and emigration out of Zone \(i\).

Eq 2 describes the rate of change in the human exposed (incubating) compartment in Zone \(i\). \(\beta_H H_s^i\) refers to the individuals having become infected, \(\sum_{j \neq i}^3 \lambda_{ij}^H H_j^i\) refers to the immigration into Zone \(i\) and \(\mu_H + \delta_H + \sum_{j \neq i}^3 \lambda_{ij}^H\) refers to the losses through natural mortality, changing from incubation to the infective stage and emigration out of Zone \(i\).

Eq 3 describes the rate of change in the infective human compartment: \(\delta_H H_i^i\) refers to the individuals having become infective, \(\sum_{j \neq i}^3 \lambda_{ij}^H H_j^i\) refers to the immigration into Zone \(i\) and \((\mu_H + \delta_H + \sum_{j \neq i}^3 \lambda_{ij}^H) H_i^i\) refers to the losses through natural mortality, changing from infective to the infective stage and emigration out of Zone \(i\).
refers to the losses through natural mortality, disease-specific mortality, recovery and emigration from Zone \(i\). Eq 4 describes the rate of change in the recovered (immune) human compartment: \(\dot{r}_H^i\) refers to individuals having recovered (gained immunity), \(\sum_{j=1}^{3} \lambda_{ji}^H M_j^i\) refers to immigration into Zone \(i\) and \((\mu_M + \rho_M + \sum_{j=1}^{3} \lambda_{ji}^M) H_i^i\) refers to losses through natural mortality, loss of immunity and emigration from Zone \(i\).

**Animal population**

\[
\frac{dM_i^a}{dt} = \left(\gamma_M N_M^i + \gamma_M M_i^i\right) \left(1 - \frac{N_M^i}{k_M}\right) + \sum_{j=1}^{3} \lambda_{ji}^a M_j^i + \rho_M M_i^i - (\mu_M + \beta_M + \sum_{j=1}^{3} \lambda_{ji}^a) M_i^i; \quad (i = 1, \ldots, 3) \tag{5}
\]

\[
\frac{dM_i^e}{dt} = \beta_M M_i^e + \sum_{j=1}^{3} \lambda_{ji}^e M_j^i - (\mu_M + \xi_M + \sum_{j=1}^{3} \lambda_{ji}^e) M_i^e; \quad (i = 1, \ldots, 3) \tag{6}
\]

\[
\frac{dM_i^o}{dt} = \xi_M M_i^o + \sum_{j=1}^{3} \lambda_{ji}^o M_j^i - (\mu_M + \rho_M + \sum_{j=1}^{3} \lambda_{ji}^o) M_i^o; \quad (i = 1, \ldots, 3) \tag{7}
\]

Eq 5 describes the rate of change in the susceptible animal host compartment:

\[
\left(\gamma_M N_M^i + \gamma_M M_i^i\right) \left(1 - \frac{N_M^i}{k_M}\right) \text{ refers to the newborn individuals, respectively born from uninfected and infected individuals and corrected for population density to simulate removal (sales) in function of herd size.} \sum_{j=1}^{3} \lambda_{ji}^a M_j^i + \rho_M M_i^i \text{ refers to immigration into Zone } i \text{ from the other two zones and individuals losing their immunity and } (\mu_M + \beta_M + \sum_{j=1}^{3} \lambda_{ji}^a) M_i^i \text{ refers to losses through natural mortality, animals becoming infected and emigration out of Zone } i. \quad \text{Eq 6 describes the rate of change in the animal host exposed (incubating) compartment in Zone } i:\n\beta_M M_i^e \text{ refers to the animals becoming infected, } \sum_{j=1}^{3} \lambda_{ji}^e M_j^i \text{ refers to immigration into Zone } i \text{ and } (\mu_M + \xi_M + \sum_{j=1}^{3} \lambda_{ji}^e) M_i^e \text{ refers to losses through natural mortality, changing from incubation to the infective stage and emigration from Zone } i. \quad \text{Eq 7 describes the rate of change in the animal infective compartment in Zone } i:\n\xi_M M_i^o \text{ refers to the individuals becoming infective, } \sum_{j=1}^{3} \lambda_{ji}^o M_j^i \text{ refers to the immigration into Zone } i \text{ and } (\mu_M + \delta_M + \xi_M + \sum_{j=1}^{3} \lambda_{ji}^o) M_i^o \text{ refers to losses through natural mortality, emigration and recovery from Zone } i. \quad \text{Eq 8 describes the rate of change in the animal recovered (immune) compartment in Zone } i:\n\xi_M M_i^r \text{ refers to the individuals having recovered (gained immunity), } \sum_{j=1}^{3} \lambda_{ji}^r M_j^i \text{ refers to immigration into Zone } i \text{ and } (\mu_M + \rho_M + \sum_{j=1}^{3} \lambda_{ji}^r) M_i^r \text{ refers to losses through natural mortality, disease-specific mortality, recovery and emigration from Zone } i.\]

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to the losses through natural mortality, disease-specific mortality, recovery and emigration from Zone $i$. Eq 8 describes the rate of change in the recovered (immune) animal compartment in Zone $i$: $z_{0M}^i M_i^r$ refers to the animals having recovered (gained immunity), $\sum_{j \neq i}^{3} \lambda_{ij}^s M_j^r$ refers to immigration into Zone $i$ and $(\mu_{M} + \rho_{M} + \sum_{j \neq i}^{3} \lambda_{ij}^s) M_i^r$ refers to losses through natural mortality, loss of immunity and emigration from Zone $i$.

**Vector A**

$$\frac{dA_i^o}{dt} = \omega_{o,i}^A_1 \left(1 - \frac{N_i^o}{K_i^o}\right) \zeta_A^i A_i^o - (\mu_{A}^o + \tau_A^o) A_i^o; (i = 1, \ldots, 3)$$  \hspace{1cm} (9)

$$\frac{dA_i^p}{dt} = \gamma_A^i \left(1 - \frac{N_i^p}{K_i^p}\right) \left[\omega_A^p (1 - \zeta_A^i) A_i^p + (\omega_A^p + \omega_A^i) A_i^o\right] - \left(\mu_{A}^p + \tau_A^p\right) A_i^p; (i = 1, \ldots, 3)$$  \hspace{1cm} (10)

$$\frac{dA_i^s}{dt} = \tau_A^s A_i^p + \sum_{j \neq i}^{3} \lambda_{ij}^s A_j^p - (\mu_{A} + \omega_A^s + \sum_{j \neq i}^{3} \lambda_{ij}^s) A_i^s; (i = 1, \ldots, 3)$$  \hspace{1cm} (11)

$$\frac{dA_i^r}{dt} = \tau_A^r A_i^q + \omega_A^r A_i^o + \sum_{j \neq i}^{3} \lambda_{ij}^r A_j^o - (\mu_{A} + \sum_{j \neq i}^{3} \lambda_{ij}^r) A_i^r; (i = 1, \ldots, 3)$$  \hspace{1cm} (12)

Eq 9 describes the rate of change in the infected-egg compartment of Vector A in Zone $i$: $\omega_{o,i}^A_1 \left(1 - \frac{N_i^o}{K_i^o}\right) \zeta_A^i A_i^o$ refers to the production of infected eggs (product of total biting rate, egg production rate, density-dependent correction and vertical transmission rate) while $(\mu_{A}^o + \tau_A^o) A_i^o$ refers to losses through mortality and hatching (in function of El Niño and seasonal flooding through $\tau_A$). Eq 10 describes the rate of change in the uninfected-egg compartment of Vector A in Zone $i$: $\gamma_A^i \left(1 - \frac{N_i^p}{K_i^p}\right) \left[\omega_A^p (1 - \zeta_A^i) A_i^p + (\omega_A^p + \omega_A^i) A_i^o\right]$ refers to the density-dependence corrected production of uninfected eggs both by infected adult vectors (absence of vertical transmission) and uninfected adult vectors while $(\mu_{A}^p + \tau_A^p) A_i^p$ refers to losses through mortality and hatching (in function of El Niño and seasonal flooding through $\tau_A$). Eq 11 describes the rate of change in the uninfected-adult-vector compartment in Zone $i$: $\tau_A^s A_i^p$ refers to the newly ‘hatched’ adults (note that stages intervening between egg and adult are omitted, requiring adjustment of hatching and mortality rates), $\sum_{j \neq i}^{3} \lambda_{ij}^s A_j^p$ refers to the immigration into Zone $i$ and $(\mu_{A} + \omega_A^s + \sum_{j \neq i}^{3} \lambda_{ij}^s) A_i^s$ refers to the losses through mortality, acquisition of infection and emigration out of Zone $i$. Eq 12 describes the rate of change in the infected-adult-vector compartment in Zone $i$: $\tau_A^r A_i^q$ refers to the newly ‘hatched’ infected adult vectors (same remark as for Eq 11), $\omega_A^r A_i^o$ refers to newly infected adult...
vectors, \( \sum_{j=1}^{3} \lambda^A_{ji} A^i_j \) refers to the immigration into Zone \( i \) and \( (\mu_A + \sum_{j=1}^{3} \lambda^A_{ji}) A^i_j \) refers to the losses through mortality and emigration out of Zone \( i \).

Vector B

\[
\frac{dB^i_t}{dt} = \omega^B_{i} \gamma_s \left( 1 - \frac{N^B_i}{K^B_i} \right) C^i_t - (\mu^B_{i} + \tau^B_{i}) B^i_t; \quad (i = 1, \ldots, 3) \tag{13}
\]

\[
\frac{dB^i_t}{dt} = \gamma_s \left( 1 - \frac{N^B_i}{K^B_i} \right) [(\omega^B_{i} + \lambda^B_{ji}) C^i_t] - (\mu^B_{i} + \tau^B_{i}) B^i_t; \quad (i = 1, \ldots, 3) \tag{14}
\]

\[
\frac{dB^i_t}{dt} = \tau^B_{i} B^i_t + \sum_{j=1}^{3} \lambda^B_{ji} B^j_t - (\mu^B_{i} + \sum_{j=1}^{3} \lambda^B_{ji}) B^i_t; \quad (i = 1, \ldots, 3) \tag{15}
\]

\[
\frac{dB^i_t}{dt} = \tau^B_{i} B^i_t C^i_t + \omega^B_{i} C^i_t - (\mu^B_{i} + \sum_{j=1}^{3} \lambda^B_{ji}) B^i_t; \quad (i = 1, \ldots, 3) \tag{16}
\]

The differential equations describing the dynamics of Vector B are identical as those for Vector A, the only difference being the possible presence of dormant eggs in the latter and not in the former.

Vector C

\[
\frac{dC^i_t}{dt} = \gamma_c \left( 1 - \frac{N^C_i}{K^C_i} \right) [(\omega^C_{i} + \lambda^C_{ji}) C^i_t] - (\mu^C_{i} + \tau^C_{i}) C^i_t; \quad (i = 1, \ldots, 3) \tag{17}
\]

\[
\frac{dC^i_t}{dt} = \tau^C_{i} C^i_t + \sum_{j=1}^{3} \lambda^C_{ji} C^j_t - (\mu^C_{i} + \sum_{j=1}^{3} \lambda^C_{ji}) C^i_t; \quad (i = 1, \ldots, 3) \tag{18}
\]

\[
\frac{dC^i_t}{dt} = \omega^C_{i} C^i_t + \sum_{j=1}^{3} \lambda^C_{ji} C^j_t - (\mu^C_{i} + \sum_{j=1}^{3} \lambda^C_{ji}) C^i_t; \quad (i = 1, \ldots, 3) \tag{19}
\]

Vector C differs from Vectors A and B in the absence of vertical transmission and hence the absence of an infected-egg compartment (i.e. no \( \frac{dC^i_t}{dt} \) differential equation). Infected adult vectors can only originate through uninfected adults acquiring infection \( (\omega^C_{i} C^i_t) \) and there is therefore no 'hatching' term in the equation (i.e. no \( \tau^C_{i} C^i_t \) term).
Vector D

\[
\frac{dD_i}{dt} = \gamma_D \left( 1 - \frac{N_i^D}{K^D} \right) \left[ \omega_D D_i' + (\omega_D + \omega_D') D_i - \left( \mu_D + \tau_D \right) D_i' \right] (i = 1, \ldots, 3) \quad (20)
\]

\[
\frac{dD_i}{dt} = \tau_D D_i' + \sum_{j=1}^{3} \lambda_D^{ij} D_j' - (\mu_D + \omega_D D_i + \sum_{j=1}^{3} \lambda_D^{ij}) D_i' (i = 1, \ldots, 3) \quad (21)
\]

\[
\frac{dD_i}{dt} = \omega_D D_i' D_S + \sum_{j=1}^{3} \lambda_D^{ij} D_j' - (\mu_D + \sum_{j=1}^{3} \lambda_D^{ij}) D_i' (i = 1, \ldots, 3) \quad (22)
\]

Vector D is identical to Vector C.

**Auxiliary equations**

**Population totals**

\[
N_i^H = H_i^S + H_i^W + H_i^E + H_i^R; (i = 1, \ldots, 3) \quad (23)
\]

\[
N_i^M = M_i^S + M_i^W + M_i^R; (i = 1, \ldots, 3) \quad (24)
\]

\[
N_i^A = A_i^Q + A_i^P + A_i^S + A_i^I; (i = 1, \ldots, 3) \quad (25)
\]

\[
N_i^B = B_i^Q + B_i^P + B_i^S + B_i^I; (i = 1, \ldots, 3) \quad (26)
\]

\[
N_i^C = C_i^Q + C_i^S + C_i^I; (i = 1, \ldots, 3) \quad (27)
\]

\[
N_i^D = D_i^P + D_i^S + D_i^I; (i = 1, \ldots, 3) \quad (28)
\]

**Vector feeding and infection rates**

Parameters 29–35 are the basic parameters used to compute carrying capacity etc. of a zone vis-à-vis its resident vectors. The present approach is to compare the total number of bites (successful feedings, . . . for sake of brevity referred to as 'bites' from now on) the vectors can inflict upon the hosts per time unit with the total number of number of vector bites the host populations can sustain (given their resistance, evasive behaviour, . . .). The minimum value of these two is used to compute the actual number of bites given per vector and/or the number of bites suffered per host. It is understood that this approach may introduce a number of parameters whose values are only vaguely known at best, but an attempt was made to avoid unrealistic numbers of vectors interacting with a single host, i.e. host numbers determine vector numbers. At the same time, the possibility is offered to include so-called alternative hosts, which can be used by the vectors when the hosts included in the model are insufficient, in order to avoid
vectors disappearing when host population levels are too low.

\[ \epsilon_{kj} = \text{proportion of vector population } \Xi_k \text{ feeding on host } \Lambda_j \sum_j \epsilon_{kj} \leq 1 \] (29)

\[ v_k = \text{average number of bites an individual of vector } \Xi_k \text{ issues per time unit} \] (30)

\[ \eta_j = \text{maximum number of bites host } \Lambda_j \text{ can 'sustain' per time unit, before e.g. taking evasive action or dislodging behaviour} \] (31)

\[ \varphi_{fj} = \text{number of } f \text{ transmitting hosts contacted by receiving host } j \text{ per time unit} \] (32)

\[ \pi_{uv} = \text{probability to transmit infection from } u \text{ to } v \] (33)

\[ \text{with } u \in \{j, k\} \& v \in \{k, j\} \& v \neq u \] (34)

\[ \beta_{sl} = \text{probability to pick up infection from wildlife hosts in general} \] (35)

Parameters 36 and 37 are computed from the simulation output:

\[ N_{\Xi_k} = \text{Population size of vector } \Xi_k \] (36)

\[ N_{\Lambda_j} = \text{Population size of host } \Lambda_j \] (37)

The potential maximum number of vector bites (all vector species) on whole host population \( \Lambda_j \) is computed as:

\[ \Omega_j = \sum_k \epsilon_{kj} N_{\Xi_k} v_k \] (38)

This is compared with the maximum number of bites the same host population can 'sustain' (see above for more details):

\[ \zeta_j = \eta_j N_{\Lambda_j} \] (39)

The 'availability' of host population \( \Lambda_j \) (i.e. the proportion of the potential bites actual inflicted on the host population in question) is the ratio of parameter 39 over parameter 38 with a maximum of unity:

\[ \sigma_j = \min \left(1, \frac{\zeta_j}{\Omega_j} \right) \] (40)

The actual number of bites by vector \( \Xi_k \) on the whole host population \( \Lambda_j \) is thus:

\[ \Omega_{kj} = \epsilon_{kj} N_{\Xi_k} v_k \sigma_j \] (41)

The individual biting rate of vector \( \Xi_k \) on host \( \Lambda_j \) per time unit becomes:

\[ \omega_{kj} = \epsilon_{kj} v_k \sigma_j \] (42)
The total individual biting rate of vector $X_k$ on all host populations per time unit therefore is the sum of the respective $\omega_{kj}$:

$$\omega_k = \sum_j \omega_{kj} \quad (43)$$

The biting rate of vector $X_k$ on alternative hosts (with $\Omega_{alh}$ = number of alternative hosts) is defined as:

$$\omega_{kj} = \frac{\Omega_{alh}}{N_h} \quad (44)$$

The proportion of infection in vector $X_k$ feeding on all modelled hosts species is computed as (the reference to the zone is left out, $I_{\Lambda_j}$ being the number of infective individuals of host $\Lambda_j$; $\beta_{al}$ refers to the infection picked up from game animals and it is added only in the case of Zone-3-dwelling vectors):

$$\beta_k = \min \left( 1, \sum_j \pi_{kj} \frac{I_{\Lambda_j}}{N_{\Lambda_j}} + \beta_{al} \right) \quad (45)$$

The infection rate of host $\Lambda_j$ being subjected to the actual number of bites by the various vectors and/or interacting with other infectious hosts is calculated as ($\phi_{j0}$, $\phi_{j}$ refers to the number of transmitting hosts [domestic animal] met by one receiving host [a person] per time unit; $\frac{\alpha_0}{N_h}$ becomes $\frac{\alpha_{0j}}{N_{\Lambda_j}}$ because $\omega_{kj} = \frac{\alpha_{0j}}{N_{\Lambda_j}}$):

$$\beta_j = -\log \left\{ 1 - \left[ \prod_{k} (1 - \pi_{\Xi_{\Lambda_k}}) \frac{\omega_{kj} I_{\Xi_{\Lambda_k}}}{N_{\Lambda_j}} \right] - \left[ 1 - \prod_{j \neq k} (1 - \pi_{\Lambda_j \Lambda_k}) \right] \frac{\phi_{j0} I_{\Lambda_j}}{N_{\Lambda_j}} \right\} \quad \forall j \neq k \quad (46)$$

The second and third terms of the logarithm function of Eq 46 are currently implemented only for animal-to-human direct transmission.

**Seasonality and El Niño effect**

Simulating an annual (seasonal) animal transhumance between Zone 1 and Zone 2 is possible: animals move to Zone 1 on day $d_1$ and move back to Zone 2 on day $d_2$. This is achieved through the generation of 0/1 indicators, which are to be multiplied with the movement rate:

$$\lambda_{12}^M = \left[ t \equiv d_1 \pmod{360} \right] \quad (47)$$

$$\lambda_{21}^M = \left[ t \equiv d_2 \pmod{360} \right] \quad (48)$$

Hatching of dormant eggs of Vector $A$ can be regulated on a seasonal basis as well as periodically through El Niño events in Zone 1 ($d_3$ and $d_4$ are respectively the start and end of the annual flooding, $\pi_p$ is the proportion proportion of Zone 1 that is seasonally flooded; $d_5$ and $d_6$
Table 2. Seasonal variation in vector egg eclosion.

<table>
<thead>
<tr>
<th>Wet/dry year</th>
<th>Seasonal variation</th>
<th>$\tau_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wet</td>
<td>no</td>
<td>$1$</td>
</tr>
<tr>
<td>wet</td>
<td>yes</td>
<td>$\cos \left( \frac{\pi}{180} \right)$</td>
</tr>
<tr>
<td>dry</td>
<td>no</td>
<td>$\pi_\delta$</td>
</tr>
<tr>
<td>dry</td>
<td>yes</td>
<td>$\pi_\delta \cos \left( \frac{\pi}{180} \right)$</td>
</tr>
</tbody>
</table>

where: $\pi_\delta = \frac{\text{proportion hatching dry season}}{\text{hatching normal season}}, n = \text{number of optimums per annum}, \delta = \text{shift from 1 January}

are respectively the start and end of the El Niño event):

$$\tau_1 = \left[ d_1 \leq t \leq d_1 \text{ (mod 360)} \right] + \pi_\delta \left[ d_2 \leq t \leq d_4 \text{ (mod 3600)} \right]$$

Annual variation (e.g. because of wet and dry years) and seasonal variation in vector egg eclosion ($\tau_s$) in all three zones can be included in the model: the current approach is by penalising hatching rates during dry years (hatching rate becomes a fraction $\pi_\delta$ of normal rates) and by allowing hatching rates in normal and dry years to vary seasonally according to a cosine curve (see the accompanying user’s manual S1 Appendix for examples on different parameter settings). The different possible combinations are as follows in Table 2:

Model—Calibration

The model is calibrated using data that were extracted from two studies in the Kilombero Valley in Tanzania (Morogoro region, [17, 22]): the principal findings of these studies were the presence of inter-epidemic RVFv circulation in human and domestic animal populations and the location of so-called infection ‘hot-spots’ away from the floodplain and in fact closer to forested areas on the plateau. The Kilombero Valley region consists of a seasonally inundated floodplain between the densely forested escarpments of the Udzungwa mountains to the northwest and the grass covered Mahenge mountains to the southeast. The valley receives an average annual rainfall of 1200–1800 mm and the average monthly temperature ranges between 25°C and 32°C. The valley has a diverse ecology and demography with villages consisting largely of numerous distinct groups of houses located on the margins of the floodplain where rice cultivation is the predominant economic activity. Other land use types include hunting, fishing, forestry, pastoral livestock rearing and cultivation of other crops. Several mosquito species inhabit the valley, including known vectors of RVFv, such as Culex spp., *Aedes* spp. and *Mansonia* spp. [17, 22, 40]. The zones, the two mammalian hosts and the four vector populations modelled are in this case:

- **Areas**
  - **Zone 1**: Floodplain (rice cultivation and dry season grazing)
  - **Zone 2**: Residential area (= village) & rainy season grazing area (= pastures)
  - **Zone 3**: Forest (people collect various resources, occasional grazing by cattle)

- **Species**
  - **H**: Human population
• M: Cattle
• A: *Aedes mcintoshi* (residing in the floodplain zone, known RVFv vector with vertical transmission and dormancy in eggs)
• B: *Aedes aegypti* (residing in residential and forest zones, known RVFv vector with vertical transmission)
• C: *Culex* sp.1 (residing in the floodplain, exact species currently unknown in Kilombero Valley)
• D: *Culex* sp.2 (residing in the residential and forest zones, exact species currently unknown in Kilombero Valley)

*Aedes mcintoshi* floodplain populations have vertical transmission and dormant (infected and uninfected) eggs. *A. aegypti* populations also have vertical transmission, but no dormancy in the eggs so only the *A. mcintoshi* eggs sustain the infection during a drought spell. *Culex* populations have neither vertical transmission nor dormancy in the eggs. Mosquito larvæ are ignored in the model (the delay they represent is simulated by means of a lower egg eclosion rate and a higher egg mortality). *Aedes* mosquitoes generally have a lower vector competence for RVFv compared to *Culex* spp. Due to heavy rains (annual flooding and the El Niño phenomenon), the infected *Aedes* mosquito eggs hatch. The infection is quickly taken over by the *Culex* species present in that region, making an epidemic possible.

Parameter values (ranges) for this scenario are given in Tables 3, 4 and 5. The model was run for 27 years, thereby modelling three El Niño events (years 1, 11 and 21) allowing the model to reach quasi-equilibrium conditions and generating output six years after the last ENSO, which could be compared with the observations made during the field studies [17, 22].

**Results**

The graphical output (showing results for the years 20–27) for the simulations over a period of 27 years, using the standard parameter values as shown in Tables 3–5 are presented in Figs 3–14. The graphical output for the *A. mcintoshi* population in zone 1, when this is the only vector and when there is no seasonal flooding of the plains in this zone is shown in Fig 15: the importance of the level of vertical transmission within the *Aedes* population is shown in the respective sub-figures of Fig 15. The seroprevalence levels in the human and cattle population at different years after the El Niño event of year 21 are shown in Table 6.

**Discussion**

A model on RVFv transmission in the Kilombero valley in Tanzania was run for 27 years to include three El Niño events (and thus three RVF epidemics), to allow the model to reach a state of ‘equilibrium’ and to allow model output during a period of 4-7 years after the epidemic to coincide with published observations [17, 22]. The model is a complex interaction of density-dependent birth, death and transmission processes and as such very sensitive to certain parameter values. The model was explored by means of scenarios and no attempt was made to include a sensitivity analysis.

Most parameters could be kept at values within the ranges found in the literature, by adjusting the values of other parameters to acceptable values, based on expert opinion. In this respect, a major influence is exerted by $v$, the maximum number of bites ‘supported’ by an individual host. The value itself directly determines the (e.g.) seroprevalence levels, but this parameter also introduces a competition between the various vector species, as at present it is assumed that the ‘available’ bites are distributed proportionally between the different vectors.
The effect can be seen in Table 6, when comparing lines one and (e.g.) nine: *Culex* on its own, being a more efficient vector, yields higher seroprevalence values than the standard setting, where it must share the biting opportunities with *Aedes*. The exception to the above was the vertical transmission rate (trans-ovarial transmission rate) for *Ae. mcintoshi*. The range found in [50] (0–8.5%) is not sufficient to carry the virus from one epidemic to another in the absence of other vectors to ensure inter-epidemic

### Table 3. Basic model parameters—1.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Roman</th>
<th>Description</th>
<th>Value</th>
<th>References</th>
<th>Comments</th>
</tr>
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<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>year</td>
<td>Number of years (360 days) to run the simulation</td>
<td>27</td>
<td>user-defined</td>
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<tr>
<td></td>
<td>flood_prop</td>
<td>Proportion flooded annually in floodplain</td>
<td>0.025</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ω_alt</td>
<td>Number of bites by all vector species on alternative hosts</td>
<td>0</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β_wl</td>
<td>Wildlife infection rate</td>
<td>0</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>γ_H</td>
<td>Human birth rate</td>
<td>4/(2’50’360)</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ_H</td>
<td>Human mortality rate</td>
<td>= γ_H</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>δ_H</td>
<td>Human RVF-specific mortality rate</td>
<td>1/3’0.01</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>α_H</td>
<td>Human RVF recovery rate</td>
<td>1’3/0.99</td>
<td>[2, 29]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ξ_H</td>
<td>Human immunity loss rate</td>
<td>1/900</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l_{hi}</td>
<td>Human migration rate from zone i to zone j</td>
<td>various’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_{HA}</td>
<td>Probability to transmit infection from person to <em>Ae. mcintoshi</em></td>
<td>0.89 (77–100%)</td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
<tr>
<td></td>
<td>π_{HB}</td>
<td>Probability to transmit infection from person to *Ae. aegypti</td>
<td>0.89 (77–100%)</td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
<tr>
<td></td>
<td>π_{HC}</td>
<td>Probability to transmit infection from person to Culex sp1</td>
<td>0.81 (78–84%)</td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
<tr>
<td></td>
<td>π_{HD}</td>
<td>Probability to transmit infection from person to Culex sp2</td>
<td>0.81 (78–84%)</td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
<tr>
<td></td>
<td>η_H</td>
<td>Maximum number of bites per person per day in zone i</td>
<td>25, 25, 25</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>γ_M</td>
<td>Birth rate non-infected cattle</td>
<td>0.00082</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Proportion abortion due to RVF</td>
<td>0.90</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ_M</td>
<td>Cattle mortality rate</td>
<td>(1 – π_M) × γ_M_i</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n^j_H</td>
<td>Carrying capacity cattle in zone i</td>
<td>500000</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ρ_M</td>
<td>Cattle RVF incubation rate</td>
<td>0.0008</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>δ_M</td>
<td>Cattle RVF-specific mortality rate</td>
<td>1/3’0.05</td>
<td>OIE disease fact sheet RVF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>α_M</td>
<td>Cattle RVF recovery rate</td>
<td>1’3/0.95</td>
<td>[2]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ρ_M</td>
<td>Bovine immunity loss rate</td>
<td>1/900</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ξ_M</td>
<td>Bovine RVF incubation rate</td>
<td>24/3.25</td>
<td>[44]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>δ_M</td>
<td>Bovine RVF-specific mortality rate</td>
<td>1/3’0.05</td>
<td>OIE disease fact sheet RVF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n^j_M</td>
<td>Bovine migration rate from zone i to zone j</td>
<td>various’</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>φ_H</td>
<td>Number of cattle met per person per time unit in zone i</td>
<td>2.5</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Probability to transmit infection from bovine to <em>Ae. mcintoshi</em></td>
<td>0.89 (77–100%)</td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Probability to transmit infection from bovine to *Ae. aegypti</td>
<td>0.89 (77–100%)</td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Probability to transmit infection from bovine to Culex sp1</td>
<td>0.81 (78–84%)</td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Probability to transmit infection from bovine to Culex sp2</td>
<td>0.81 (78–84%)</td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>π_M</td>
<td>Probability to transmit infection from bovine to people</td>
<td>0.001</td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>h_m</td>
<td>Maximum number of bites per bovine per day</td>
<td>50</td>
<td>user-defined</td>
<td></td>
</tr>
</tbody>
</table>

1 Currently: 21 = 0.005; 23 = 0.001; 12 = 0.05; 33 = 0.05; 13 = 0.0001; 31 = 0.005

‡ Currently: 13 = 0; 23 = 0.0001; 32 = 0.0005; 31 = 0; 21 and 12 seasonal movement from plateau to floodplain

https://doi.org/10.1371/journal.pone.0209929.t003

The effect can be seen in Table 6, when comparing lines one and (e.g.) nine: *Culex* on its own, being a more efficient vector, yields higher seroprevalence values than the standard setting, where it must share the biting opportunities with *Aedes*.

The exception to the above was the vertical transmission rate (trans-ovarial transmission rate) for *Ae. mcintoshi*. The range found in [50] (0–8.5%) is not sufficient to carry the virus from one epidemic to another in the absence of other vectors to ensure inter-epidemic...
transmission. As shown in Fig 15, a vertical transmission rate of 0.25 does not suffice to ensure sufficient numbers of infected eggs to trigger an epidemic at the next El Niño event. No other estimates of this parameter could be traced in the literature and it is recommended that the correct values (ranges) of this important parameter are determined experimentally.

### Table 4. Basic model parameters—2.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Roman</th>
<th>Description</th>
<th>Value</th>
<th>Range</th>
<th>References</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \gamma_A )</td>
<td>( g_a ) ( A. ) mcintoshi egg production rate</td>
<td>10</td>
<td>10</td>
<td>expert opinion</td>
<td></td>
</tr>
<tr>
<td>( k_a1 )</td>
<td>( k_a1 )</td>
<td>( A. ) mcintoshi carrying capacity in zone 1</td>
<td>175000</td>
<td>user-defined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \zeta_A )</td>
<td>( z_a )</td>
<td>Probability ( A. ) mcintoshi vertical transmission</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu_{mq} )</td>
<td>( m_{aq1} )</td>
<td>Mortality rate ( A. ) mcintoshi infected eggs in zone 1</td>
<td>0.00001</td>
<td>[46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu_i )</td>
<td>( m_{ap1} )</td>
<td>Mortality rate ( A. ) mcintoshi uninfected eggs in zone 1</td>
<td>0.00001</td>
<td>[46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu_A )</td>
<td>( m_a )</td>
<td>( A. ) mcintoshi adult mortality rate</td>
<td>1/3</td>
<td>1/3</td>
<td>expert opinion</td>
<td></td>
</tr>
<tr>
<td>( e_{AH} )</td>
<td>( e_{ah} )</td>
<td>Proportion of ( A. ) mcintoshi feeding on people</td>
<td>0.1</td>
<td>(0.1–0.9)</td>
<td>[47]</td>
<td>adequate contact</td>
</tr>
<tr>
<td>( e_{AM} )</td>
<td>( e_{am} )</td>
<td>Proportion of ( A. ) mcintoshi feeding on cattle</td>
<td>0.3</td>
<td>(4/13)</td>
<td>[48]</td>
<td>% engorged based on host choice experiments</td>
</tr>
<tr>
<td>( v_A )</td>
<td>( v_a )</td>
<td>Number of bites per ( A. ) mcintoshi mosquito per day</td>
<td>0.5</td>
<td>(0.45–0.7)</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td>( \pi_{AH} )</td>
<td>( p_{ah} )</td>
<td>Probability to transmit infection to person upon ( A. ) mcintoshi bite</td>
<td>0.01</td>
<td></td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td>( \pi_{AM} )</td>
<td>( p_{am} )</td>
<td>Probability to transmit infection to bovine upon ( A. ) mcintoshi bite</td>
<td>0.01</td>
<td></td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td>( \gamma_B )</td>
<td>( g_b )</td>
<td>( A. ) aegypti egg production rate</td>
<td>25</td>
<td></td>
<td>expert opinion</td>
<td></td>
</tr>
<tr>
<td>( k_{b2} )</td>
<td>( k_{b2} )</td>
<td>( A. ) aegypti carrying capacity in zone 2</td>
<td>175000</td>
<td>user-defined</td>
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<td></td>
</tr>
<tr>
<td>( k_{b3} )</td>
<td>( k_{b3} )</td>
<td>( A. ) aegypti carrying capacity in zone 3</td>
<td>175000</td>
<td>user-defined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \zeta_B )</td>
<td>( z_b )</td>
<td>Probability ( A. ) aegypti vertical transmission</td>
<td>0.05</td>
<td>(0–8.5%)</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>( \mu_{aq2} )</td>
<td>( m_{bq2} )</td>
<td>( A. ) aegypti infected egg mortality rate in zone 2</td>
<td>0.005</td>
<td></td>
<td>[46]</td>
<td></td>
</tr>
<tr>
<td>( \mu_{bp2} )</td>
<td>( m_{bp2} )</td>
<td>( A. ) aegypti uninfected egg mortality rate in zone 2</td>
<td>0.005</td>
<td></td>
<td>[46]</td>
<td></td>
</tr>
<tr>
<td>( \mu_{aq3} )</td>
<td>( m_{bq3} )</td>
<td>( A. ) aegypti infected egg mortality rate in zone 3</td>
<td>0.005</td>
<td></td>
<td>[46]</td>
<td></td>
</tr>
<tr>
<td>( \mu_{bp3} )</td>
<td>( m_{bp3} )</td>
<td>( A. ) aegypti uninfected egg mortality rate in zone 3</td>
<td>0.005</td>
<td></td>
<td>[46]</td>
<td></td>
</tr>
<tr>
<td>( \tau_B )</td>
<td>( t_b )</td>
<td>( A. ) aegypti hatching rate</td>
<td>0.2</td>
<td></td>
<td>[51]</td>
<td></td>
</tr>
<tr>
<td>( \mu_B )</td>
<td>( m_b )</td>
<td>( A. ) aegypti adult mortality rate</td>
<td>0.10</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>( e_{BM} )</td>
<td>( e_{bh} )</td>
<td>Proportion of ( A. ) aegypti feeding on people</td>
<td>0.01</td>
<td></td>
<td></td>
<td>[53]</td>
</tr>
<tr>
<td>( v_B )</td>
<td>( v_b )</td>
<td>Number of bites per ( A. ) aegypti mosquito per day</td>
<td>0.5</td>
<td>(0.45–0.7)</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td>( l_{bij} )</td>
<td>( l_{bij} )</td>
<td>( A. ) aegypti migration rate from zone ( i ) to zone ( j )</td>
<td>0</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>( \pi_{BH} )</td>
<td>( p_{bh} )</td>
<td>Probability to transmit infection to person upon ( A. ) aegypti bite</td>
<td>0.01</td>
<td></td>
<td>[42, 43]</td>
<td>Based on Hamster model</td>
</tr>
<tr>
<td>( \pi_{BM} )</td>
<td>( p_{bm} )</td>
<td>Probability to transmit infection to bovine upon ( A. ) aegypti bite</td>
<td>0.01</td>
<td></td>
<td>[42, 43]</td>
<td>Based on Hamster model</td>
</tr>
</tbody>
</table>

\[1\] Values within the published range [0–8.5%, [50]] did not allow infection to be carried by dormant \( A. \) mcintoshi eggs from one El Niño event to the next.
Table 5. Basic model parameters—3.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Roman</th>
<th>Description</th>
<th>Value</th>
<th>Range</th>
<th>References</th>
<th>Comments</th>
</tr>
</thead>
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<tr>
<td>$\gamma_{\text{C}}$</td>
<td>$g_c$</td>
<td>Culex sp.1 egg production rate</td>
<td>25</td>
<td></td>
<td>expert opinion</td>
<td></td>
</tr>
<tr>
<td>$k^{\text{C}}_{\text{c1}}$</td>
<td>$k_{\text{c1}}$</td>
<td>Culex sp.1 carrying capacity in zone 1</td>
<td>1750</td>
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<td>user-defined</td>
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<tr>
<td>$\mu^{\text{C}}_{\text{m1}}$</td>
<td>$m_{\text{sp1}}$</td>
<td>Culex sp.1 egg mortality rate in zone 1</td>
<td>0.002</td>
<td></td>
<td>user-defined</td>
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</tr>
<tr>
<td>$\tau_{\text{C}}$</td>
<td>$t_{\text{c}}$</td>
<td>Culex sp.1 hatching rate</td>
<td>0.2</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\mu^{\text{C}}_{\text{m}}$</td>
<td>$m_{\text{c}}$</td>
<td>Culex sp.1 adult mortality rate</td>
<td>0.10</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\epsilon^{\text{CH}}_{\text{e_ch}}$</td>
<td>$e_{\text{ch}}$</td>
<td>Proportion of Culex sp.1 feeding on people</td>
<td>0.005</td>
<td></td>
<td>[47]</td>
<td>depends on host availability</td>
</tr>
<tr>
<td>$\epsilon^{\text{CM}}_{\text{e_cm}}$</td>
<td>$e_{\text{cm}}$</td>
<td>Proportion of Culex sp.1 feeding on cattle</td>
<td>0.02 (0–0.9)</td>
<td></td>
<td>[47, 48]</td>
<td>host availability and host choice experiments</td>
</tr>
<tr>
<td>$v^{\text{C}}_{\text{v_c}}$</td>
<td>$v_{\text{c}}$</td>
<td>Number of bites per Culex sp.1 mosquito per day</td>
<td>1</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\pi^{\text{CH}}_{\text{p_ch}}$</td>
<td>$p_{\text{ch}}$</td>
<td>Probability to transmit infection to person upon Culex sp.1 bite</td>
<td>0.07 (7–37%)</td>
<td></td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
<tr>
<td>$\pi^{\text{CM}}_{\text{p_cm}}$</td>
<td>$p_{\text{cm}}$</td>
<td>Probability to transmit infection to bovine upon Culex sp.1 bite</td>
<td>0.07 (7–37%)</td>
<td></td>
<td>[42, 43]</td>
<td>based on hamster model</td>
</tr>
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</table>

Culex sp.2

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Roman</th>
<th>Description</th>
<th>Value</th>
<th>Range</th>
<th>References</th>
<th>Comments</th>
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<tbody>
<tr>
<td>$\gamma^{\text{D}}_{\text{g_d}}$</td>
<td>$g_d$</td>
<td>Culex sp.2 egg production rate</td>
<td>25</td>
<td></td>
<td>expert opinion</td>
<td></td>
</tr>
<tr>
<td>$k^{\text{D}}_{\text{d2}}$</td>
<td>$k_{\text{d2}}$</td>
<td>Culex sp.2 carrying capacity in zone 2</td>
<td>17500</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$k^{\text{D}}_{\text{d3}}$</td>
<td>$k_{\text{d3}}$</td>
<td>Culex sp.2 carrying capacity in zone 3</td>
<td>17500</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\mu^{\text{D}}_{\text{m2p}}$</td>
<td>$m_{\text{dp2}}$</td>
<td>Culex sp.2 egg mortality rate in zone 2</td>
<td>0.002</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\mu^{\text{D}}_{\text{m3p}}$</td>
<td>$m_{\text{dp3}}$</td>
<td>Culex sp.2 egg mortality rate in zone 3</td>
<td>0.002</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\tau^{\text{D}}_{\text{t_d}}$</td>
<td>$t_{\text{d}}$</td>
<td>Culex sp.2 hatching rate</td>
<td>0.2</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\mu^{\text{D}}_{\text{m}}$</td>
<td>$m_{\text{d}}$</td>
<td>Culex sp.2 adult mortality rate</td>
<td>0.10</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\epsilon^{\text{DH}}_{\text{e_dh}}$</td>
<td>$e_{\text{dh}}$</td>
<td>Proportion of Culex sp.2 feeding on people</td>
<td>0.005 (0–0.9)</td>
<td></td>
<td>[47]</td>
<td></td>
</tr>
<tr>
<td>$\epsilon^{\text{DM}}_{\text{e_dm}}$</td>
<td>$e_{\text{dm}}$</td>
<td>Proportion of Culex sp.2 feeding on cattle</td>
<td>0.12 (0–0.9)</td>
<td></td>
<td>[47, 48]</td>
<td>host availability and host choice experiments</td>
</tr>
<tr>
<td>$v^{\text{D}}_{\text{v_d}}$</td>
<td>$v_{\text{d}}$</td>
<td>Number of bites per Culex sp.2 mosquito per day</td>
<td>1</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\lambda^{\text{D}}_{\text{l_d[ij]}}$</td>
<td>$l_{\text{d[ij]}}$</td>
<td>Culex sp.2 migration rate from zone $i$ to zone $j$</td>
<td>0</td>
<td></td>
<td>user-defined</td>
<td></td>
</tr>
<tr>
<td>$\pi^{\text{DH}}_{\text{p_dh}}$</td>
<td>$p_{\text{dh}}$</td>
<td>Probability to transmit infection to person upon Culex sp.2 bite</td>
<td>0.07</td>
<td></td>
<td>[42, 43]</td>
<td></td>
</tr>
<tr>
<td>$\pi^{\text{DM}}_{\text{p_dm}}$</td>
<td>$p_{\text{dm}}$</td>
<td>Probability to transmit infection to bovine upon Culex sp.2 bite</td>
<td>0.07</td>
<td></td>
<td>[42, 43]</td>
<td></td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0209929.t005

Fig 3. Standard parameters: Human—Zone 1.

https://doi.org/10.1371/journal.pone.0209929.g003
A low level of RVFv transmission was predicted by the model (Table 6). Using the standard values, predicted seroprevalence levels in humans and cattle at different times after the El Niño event were comparable to those observed. Seroprevalence is estimated to be 13.2% in people and 12.3% in cattle, six years after an El Niño event. The field studies found similar overall seroprevalence levels of 11.7% in people and 11.3% in cattle, five to six years after the 2006/07 RVF epidemic in the area [17, 22]. The results are also in line with previous studies across Africa with evidence of inter-epidemic transmission of RVF [1, 15, 16]. The dynamics of levels of seroprevalence are of course in the first place dependent on the value employed for the loss-of-serotitre rate: currently a daily value of 1/900 is used, based on a single, rather vague
reference [41]. Inclusion of a wildlife reservoir (Table 6, second line) did not have a significant effect on the predicted levels of seroprevalence.

The simulated seroprevalence levels in Table 6 in both the human and livestock populations show a gradual decline during the years after an epidemic event (El Niño), which seems to imply low numbers of infective bites during inter-epidemic periods, reflecting the generally low numbers of mosquitoes in the absence of heavy rainfall associated with the El Niño events. People and cattle transiting in the forest (zone 3, Figs 5 and 8) are exposed to infectious bites every year from the *Ae. aegypti* and *Culex* sp.2 populations (Figs 11 and 14): the mosquitoes are constantly infected from the wildlife reservoir [56]. People and cattle remaining in the villages

Fig 6. Standard parameters: Cattle—Zone 1.

https://doi.org/10.1371/journal.pone.0209929.g006

Fig 7. Standard parameters: Cattle—Zone 2.

https://doi.org/10.1371/journal.pone.0209929.g007
(zone 2, Figs 4 and 7) and/or the floodplains (zone 1, Figs 3 and 6) are minimally exposed on an annual basis with high exposure rates occurring only every ten years (Figs 9, 10, 12 and 13). Infection thus principally spreads to the villages and floodplains by humans and cattle temporarily residing in the forest zone.

The *Ae. mcintoshii* population in the floodplains (Fig 9) is the one maintaining the infection inside the dormant eggs. Adult mosquitoes do not survive the drier period following the El Niño event and only some eggs hatch every year during the partial seasonal flooding of the plain. Substantial hatching occurs during flooding related to the El Niño event in the East African region, releasing the infection and starting the epidemics. The infection is picked up by
Culex sp.1 present in this area. The human population acquires the infection first, followed by the cattle population. From there on, the epidemic spreads to the village and the forest with migrating cattle and people.

As indicated by lines three and four of Table 6 (with the current standard parameter settings), *Ae. mcintoshi* on its own is not able to explain the high seroprevalence found in both humans and cattle [17, 22], not even when including annual partial flooding of zone 1 accompanied by eclosion of part of the dormant eggs. The same can be said for *Ae. aegypti*, despite it being resident in the village and forest zones, although it must be understood that in this case the low values for vertical transmission were maintained.

Fig 10. Standard parameters: *A. aegypti*—Zone 2.
https://doi.org/10.1371/journal.pone.0209929.g010

Fig 11. Standard parameters: *A. aegypti*—Zone 3.
https://doi.org/10.1371/journal.pone.0209929.g011
Lines six to nine of Table 6 examine different scenarios with an efficient *Culex* vector in the village and forest zones. Introduction of infection, either by means of a wildlife reservoir (line seven) or through the introduction of an infective animal, allows for maintenance of the infection within the host and vector populations. Because of the interaction between the different vectors for host-feeding opportunities, the more efficient *Culex* vector on its own (without competition from *Aedes* species) results in higher infection transmission and higher seroprevalence levels. Again, a lot more detailed observations are required to properly quantify this aspect of the transmission dynamics.

Mosquito species in the forested environment (*Ae. aegypti* and *Culex* sp.2) (Figs 11 and 14) had high annual infection rates. On the other hand, mosquitos in the residential area...
Fig 14. Standard parameters: Culex sp.2—Zone 3.
https://doi.org/10.1371/journal.pone.0209929.g014

Fig 15. Aedes mcintoshi as only vector, no seasonal flooding of zone 1. A: Vertical transmission rate = 0.25; B: Vertical transmission rate = 0.50.
https://doi.org/10.1371/journal.pone.0209929.g015
(Ae. \textit{egypti} and \textit{Culex} sp.2) and in the floodplain (Ae. \textit{mcintoshi} and \textit{Culex} sp.1) have low infection rates (Figs 9, 10, 12 and 13) with peak rates occurring only during or immediately after an El Niño event and subsequent RVF epidemics in the East African region [57].

The model presented here needs further calibrating with datasets from other regions where there are similar or dissimilar ecologies compared to our study area in order to extend and/or improve usability of the model in different geographical, climatic settings. This model, being built with open-source software and with an easy to use interface, can be adapted by researchers and program managers to their specific needs by plugging in new parameters relevant to their situation and locality. Its use can be further expanded by including disease prevention and control interventions to model potential impact of these veterinary and public health measures on disease in people and domestic animals, for example vaccination, quarantining and vector control programs.

Supporting information

S1 Appendix. User manual. (PDF)

S2 Appendix. Program R code. (PDF)

Author Contributions

Conceptualization: Robert Sumaye, Famke Jansen, Etienne Thiry.

Data curation: Robert Sumaye, Famke Jansen.

Table 6. RVF seroprevalence levels (proportion) in people and cattle at different times after an El Niño event.

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EN+2</td>
<td>EN+4</td>
</tr>
<tr>
<td>Standard</td>
<td>0.209</td>
<td>0.147</td>
</tr>
<tr>
<td>Standard + wl</td>
<td>0.209</td>
<td>0.147</td>
</tr>
<tr>
<td>only Aemc (100 A1L + 9900 A1U) - flood</td>
<td>0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>only Aemc (100 A1L + 9900 A1U) + flood</td>
<td>0.136</td>
<td>0.093</td>
</tr>
<tr>
<td>only Aeae (100 B1L + 9900 B1U)</td>
<td>0.048</td>
<td>0.041</td>
</tr>
<tr>
<td>only Cu2 (1000 D2U)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>only Cu2 (1000 D2U) + wl</td>
<td>0.130</td>
<td>0.138</td>
</tr>
<tr>
<td>only Cu2 (1000 D2U)</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

† EN+2/4/6 = year 2/4/6 after El Niño event

- Standard: 1000 H2S, 2500 M2S, 100 A1Q, 9900 A1P, 10 B3P, 100 C1P, 1000 D2S, 1000 D3P
- Standard + wl: as above + wildlife reservoir (infection rate for vectors = 1e⁻⁵)
- only Aemc (100 A1L + 9900 A1U) - flood: \textit{Ae. mcintoshi} 100 infected eggs, 9900 uninfected eggs in zone 1, no annual partial flooding of zone 1
- only Aemc (100 A1L + 9900 A1U) + flooding: as above + annual partial flooding of zone 1
- only Aeae (100 B1L + 9900 B1U): \textit{Ae. egypti} 100 infected eggs, 9900 uninfected eggs in zone 2
- only Cu2 (1000 D2U): \textit{Culex} sp.2 1000 eggs in zone 3
- only Cu2 (1000 D2U): as above + wildlife reservoir (infection rate for vectors = 1e⁻⁵)
- only Cu2 (1000 D2U): \textit{Culex} sp.2 1000 eggs in zone 2
- only Cu2 (1000 D2U) + introduction of 1 M2I: as above with introduction of one infective bovine in Zone 2

https://doi.org/10.1371/journal.pone.0209929.t006

(Æ. \textit{egypti} and \textit{Culex} sp.2) and in the floodplain (Æ. \textit{mcintoshi} and \textit{Culex} sp.1) have low infection rates (Figs 9, 10, 12 and 13) with peak rates occurring only during or immediately after an El Niño event and subsequent RVF epidemics in the East African region [57].

The model presented here needs further calibrating with datasets from other regions where there are similar or dissimilar ecologies compared to our study area in order to extend and/or improve usability of the model in different geographical, climatic settings. This model, being built with open-source software and with an easy to use interface, can be adapted by researchers and program managers to their specific needs by plugging in new parameters relevant to their situation and locality. Its use can be further expanded by including disease prevention and control interventions to model potential impact of these veterinary and public health measures on disease in people and domestic animals, for example vaccination, quarantining and vector control programs.
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Investigation: Robert Sumaye, Famke Jansen.
Methodology: Dirk Berkvens, Bernard De Baets.
Project administration: Dirk Berkvens, Eveline Geubels, Etienne Thiry.
Resources: Robert Sumaye, Eveline Geubels.
Software: Meryam Krit.
Supervision: Dirk Berkvens, Meryam Krit.
Validation: Bernard De Baets.
Writing – original draft: Robert Sumaye, Famke Jansen.
Writing – review & editing: Dirk Berkvens, Meryam Krit.

References
CHAPTER 6

General discussion
6.1 Introduction

The transmission dynamics of RVF both during the epidemics and inter-epidemic periods can be complex and might be uniquely different at fine geographical scales. Epizootics in animals, sometimes accompanied by epidemics in humans, have been observed in diverse ecologies across Africa (Clements et al., 2007; Eisa et al., 1977; Gear et al., 1955; Hoogstraal et al., 1979; McIntosh et al., 1980b; Ringot et al., 2004; Woods et al., 2002; Zeller et al., 1995). Africa’s Indian ocean islands (Morvan et al., 1992; Sissoko et al., 2009) and the Arabian Peninsula (Abdo-Salem et al., 2006; Shoemaker et al., 2002).

The introduction of RVF to naïve regions has been linked with movement of infected/incubating animals from endemic areas (Balkhy and Memish, 2003; Rolin et al., 2013; Soumare et al., 2007). Serological evidence suggests presence of low-level RVF transmissions in animal and human populations that occur regularly throughout much of the Africa continent (Heinrich et al., 2012; LaBeaud et al., 2011, 2008; Labeaud et al., 2007; Pourrut et al., 2010; Ringot et al., 2004; Sindato et al., 2013; Zeller et al., 1995), Chapters 2 and Chapter 3 of this thesis.

However, there is lack of adequate surveillance during inter-epidemic periods in endemic areas, and therefore little or no new data is generated to update on the changing epidemiology regarding presence of RVFV activity and its geographical extent during inter-epidemic periods. RVF virus activity during inter-epidemic periods and its effect on either epidemic transmission or likelihood of epidemics occurring is not fully understood. Better understanding of the complexity of transmission dynamics of RVF in both human and livestock populations is necessary for preparedness planning in combating future epidemics through evidence-based control and preventive strategies such as proper allocation of resources, and ultimately reducing negative consequences.

The goal of this work was therefore to investigate presence of recent and longer standing infection/exposure with RVF in people and livestock during the inter-epidemic period and to explore the relative importance of the interaction between spatial environment and human behaviour on the RVF exposure risk factors in the seasonal flood plains of Kilombero river valley in Tanzania, which mimics unusual high precipitation increase on annual basis.
6.2 Rift Valley fever exposure status in livestock and human populations

The past exposure to RVF virus was determined through detection of IgM and IgG antibodies to RVFV in serum samples collected from animals and people. The study findings from both livestock and humans as reported in Chapters 3 and 4, indicate the two populations were exposed to RVF infection at various stages prior to this study. Post epidemic exposure was determined through either detection of IgM antibodies (which is short lived class of antibodies with median detection duration of about two months (Morvan et al., 1992; Paweska et al., 2003), or detection of neutralizing antibodies in serum samples from animals or people who were born after RVF epidemic of 2006/07.

In this study, RVFV neutralizing antibodies have been detected in animal sera collected from animals as young as one year old and also IgM antibodies were detected in animal sera. Human sera analysis revealed presence of neutralizing antibodies in individuals born before the 2006/07 epidemics, and also IgM antibodies were detected in serum samples. Detection of IgM antibodies to RVFV and neutralizing antibodies in individuals born after epidemics in this study indicate presence of post epidemic transmission of RVF to humans and livestock in the Kilombero valley.

The findings from Kilombero valley are also supported by other studies in livestock during the same period in Tanzania (Kifaro et al., 2014; Sindato et al., 2013) but from dissimilar climatic and ecological conditions. The current observation in Kilombero valley adds to the increasing body of serological evidence (Chevalier et al., 2011; Hussein et al., 1985; LaBeaud et al., 2011, 2008) and virological evidence (Linthicum et al., 1985; Popova et al., 2010) pertaining to RVFV transmission during the inter-epidemic periods in parts of Africa. Detection of inter-epidemic transmissions in human and livestock populations has been the result of active surveillance and laboratory testing, which indicate a high likelihood that in absence of active disease detection inter-epidemic RVFV activity would pass undetected. The challenge experienced in passive clinical detection of RVF activity during inter-epidemic period might be due to sub-clinical nature of RVF and/or mistaken clinically with other febrile conditions that present with similar features but also lack of awareness of RVF among health workers (Heinrich et al., 2012; Olaleye et al., 1996; Pourrut et al., 2010).
6.3 Temporal and spatial risk factors for recent and long-standing RVF sero-positivity in livestock

The exposure to RVF virus in livestock has been detected both in animals born prior to the 2006/07 RVF epidemic and in those born post 2006/07 epidemic in the study area. The linear increase in the proportion of sero-converted animals from yearlings to 5 years (i.e. born after the last epidemic) implies an annual exposure to infectious mosquito bites in livestock populations post 2006/07 epidemic. This is also reflected in the high sero-prevalence observed in female animals which due to their reproduction roles stay longer in the herds.

Herds located away from the main Kilombero floodplains and probably close to the forests had an increased RVFV exposure risk. This was unexpected in particular where floodwater *Aedes* mosquitoes are thought to be the main drivers of RVFV transmission. In this situation other non-flood water *Aedes* mosquitoes, such as *Aedes aegypti* a common mosquito in the study area and *Aedes albopictus*, which might be playing a role, but also movement of the livestock hosts at different season between the floodplains (during the dry season) and higher grounds (during the rainy seasons).

The long-standing exposure as demonstrated by detection of neutralizing antibodies to RVF virus was seen across study villages. However spatial analysis indicated presence of four main areas of transmission hotspots (Chapter 3) in livestock in the Kilombero valley. Within two hotspot locations IgM antibodies were detected in serum samples which indicate presence of active foci of recent transmission preceding the sero-survey.

6.4 The demographic, behavioural, temporal, occupational and spatial risk factors in people

Risk factors for past exposure to RVF virus in people are reported in Chapter 4. Households keeping livestock (a high proportion of which are agro-pastoralist families) had a high chance for their members to have past exposure to RVF virus. This is expected due to possible close contact with sick animals in livestock keeping households which includes among others animal slaughter at home, milking animals and assisting animal birthing. The findings from the current study indicate people who ate raw meat (including blood and internal organs such as kidneys and liver) and those who milked animals were more likely to have evidence of past RVF infection. It is known that, given a quasi-constant risk of exposure, the seroprevalence increases with age. This was also borne out by our observations (unpublished results). Also older individuals were more likely to have evidence of past epidemics, e.g. the last RVFV epidemics of 2016/07 or
earlier. There was no spatial difference among villages where study was conducted, but this could be as a result of selection of livestock hotspot areas for human survey.

6.5 Direct mosquito-borne transmission vs. zoonotic transmission in people

Establishing the relative importance of risk of acquiring RVF virus through infectious mosquito bite as compared to zoonotic transmission is key both during epidemics and inter-epidemic periods. The zoonotic route (direct contact with infected animals, body fluids or their infectious products) is considered the main transmission route of RVF virus to people. The findings in the current study indicate the two transmission routes carry equal weight. This observation is likely key feature for RVF in the Kilombero valley and inter-epidemic period scenario given its ecology which favours breeding of the mosquitoes, some of which are vectors of RVF virus. It is of importance to substantiate the Kilombero valley situation by conducting further epidemiological studies in the human population in other ecologies dissimilar to Kilombero flood prone.

6.6 Rift Valley fever virus transmission dynamics at a fine scale ecologies

In an attempt to explain further the findings we developed a mathematical model, Chapter 5. Our model predicts low level transmission of RVF which is in line with epidemiological studies in this study area. Our model also indicate that wildlife reservoir play an important role in maintenance of RVF in the study area. The main elements in our simulation have been both the dynamics and composition of vector species in three ecological zones. The model presented here with further calibration using datasets from other settings where there is similar or dissimilar ecologies with our study areas will expand usability in different geographical areas. This is subject of an ongoing work by our group.

This model, being built with openSource software and with an easy to use interface, can be adapted by researchers and program managers to their specific scenarios by plugging in new parameters relevant to their situation and localities. Its usage can be further expanded by including disease prevention and control interventions to model potential impact of these veterinary and public health measures on disease in cattle and humans, for example vaccination, quarantining and vector control programs.
6.7 Dynamics of RVF transmission and associated disease surveillance challenges

6.7.1 Transmission to susceptible hosts

Transmission of RVFV to animals and people is primarily through infectious mosquito bites. Potential mosquito vectors include more than 30 species from which RVF virus has been detected, (Table 1). *Aedes species* are regarded as the main vectors of RVF that also maintain the virus between epidemics, and have been incriminated in several RVF outbreaks, however other mosquito species including *Anopheles, Culex, Eratmopodites, Mansonia, Mansonoides* and *Coquillettidiae* may also play important role in either disease maintenance or perpetuation of the epidemics (Gad et al., 1987; Hanafi et al., 2011; Seufi and Galal, 2010). Other arthropod vectors including *Glossina, Stomoxys, Phlebotomes*, and *Culicoides species* may transmit the disease mechanically, usually through contaminated mouthparts (Hoch et al., 1985; Turell et al., 1990, 2010). This phenomena of multi-vector involvement can dramatically increase the number of potential vectors during epidemics.

In addition human infections have resulted from contact with infected animals or their products in the course of executing occupational activities e.g. during treating or butchering RVF infected animals, also by inhalation of infectious aerosols released during animal slaughter or in the laboratory environment (Abu-Elyazeed et al., 1996; Archer et al., 2011; Hoogstraal et al., 1979; Morita, 1988; Smithburn et al., 1949). Close contact with infected animals through common shelter or through consumption of raw animal products such as milk, meat and blood from infected animals has shown to be an important risk factor for past exposure to the disease (LaBeaud et al., 2008; Woods et al., 2002). Vertical transmission in humans has also been documented through demonstration of IgM antibodies against RVFV in both newborn and the mother (Adam and Karsany, 2008; Arishi et al., 2006); also experimental studies in sheep have indicated vertical transmission even in absence of detectable viraemia in the ewe (Antonis et al., 2013). The vertical acquisition of RVF presents a new paradigm that warrants further exploration during epidemics and experimental settings to further the understanding on the transmission dynamics in people and animals and its epidemiological implications.

6.7.2 Host immune response and role of herd immunity

The immune response in the form of neutralizing antibodies that mammalian hosts mount following an infection by RVFV, clears the virus from the mammalian hosts and also prevents
subsequent infection by the same virus. The immune response therefore plays two immediate roles in the transmission cycle in particular during epidemics, one is creating protective herd immunity (Fine et al., 2011) and secondly through reducing the source of infectious agents for secondary vectors to pick RVFV and transmit to other susceptible hosts. Since viral perpetuation relies on the infecting new susceptible hosts for completing the transmission cycle, subsequent cycles can easily be stopped when there is high proportion of exposed animals within the herd. However, low-level transmission may still occur where ecologies of an area support maintenance vectors and where hatching of infected vectors occurs. The infected vectors may still transmit the disease to few available susceptible hosts, but many of the infectious bites may hit a dead end (immune hosts) in the population thus infection is blocked. In such scenarios low-level transmission would lead to sero-conversion in the population but would not trigger an epidemic. Whether or not a seroprevalence of $\pm 11\%$ constitutes herd immunity in the endemic areas remains to be investigated.

In conclusion, the difference between inter-epidemic and epidemic transmission appears to be one of scale, with epidemics increasing the numbers of vectors and numbers of hosts involved. Epidemics typically involve mass-scale emergence of dormant, infected vectors whereas the inter-epidemic transmission may or may not involve small-scale emergence.

### 6.7.3 Environmental and climatic drivers

The environment and climate have a direct role on mosquito borne diseases as they affect vector population dynamics through provision of essential life resources and influence of temperature on infectious agent extrinsic incubation period (Turell, 1993; Turell et al., 1985). In years with high precipitation, sustained flooding can reach the edges of the previous dambo’s limits and thus hatching of infected eggs can occur and therefore trigger an epidemic upon feeding to susceptible hosts. Depending on the area’s physical characteristic and rainfall pattern, the cycle between normal and unusual high rainfall can be annual, a few years or even decades as seen in the Eastern Africa (Davies et al., 1985).

Vegetation types may modify vector population dynamics depending on species adaption to a particular environment. For example, in forested environments some species of Aedes can lay eggs on water bodies collected in tree-holes and plant axils; in such an environment annual emergence of vectors can occur as the tree-holes and plant axils would require relatively low precipitation to accumulate enough water for submerging Aedes eggs, but also the forested areas receive relatively high precipitation. In such situations, the cycle/dynamics of transmission
might be more frequent if there is also presence of either susceptible livestock, people or wildlife (Anderson and Rowe, 1998; Evans et al., 2008; LaBeaud et al., 2011).

In relatively wet areas (high precipitation), following epidemics, a high proportion of infected Aedes eggs may be laid in the dambos. However due to annual weather fluctuation, in the subsequent years relatively few dambos will be filled up completely. In such situation hatching of dormant infected Aedes eggs can therefore occur by instalment each year. These emerging infected Aedes would infect few available susceptible hosts due to herd immunity arising from previous epidemic, but also will continue to keep the herd immunity high. The implication here would be gradual depletion with little replenishment of the infectious Aedes eggs in the environment to trigger a new epidemic in the event of abnormal high rainfall weather event. The above scenario would hold especially if the vertical transmission stops at the F1 generation, i.e. different to how LaCrosse virus behaves in mosquito vectors (Tesh and Gubler, 1975).

On the other hand, in arid and semi-arid areas following unusual rainfall that triggers a RVF epidemic, a good proportion of Aedes mosquitoes would also lay infected eggs. In the subsequent years which are normally characterized by low rainfall, this will not allow for dambos to be filled up and therefore much of the infected eggs will possibly remain dormant until an abnormal high rainfall weather event causes a sustained flooding leading to massive hatching of infected eggs, thus triggering another epidemic. The epidemics in the relatively drier areas may also partly be contributed to build-up of a naïve population due to lack of frequent challenge, lack of maternal immunity in young animals and population turnover (offtake) in livestock and therefore presence of amplifying hosts.

Unusual high rainfall years have been shown to be associated with RVF epidemics in retrospectively analysed precipitation patterns (Anyamba et al., 2001; Davies et al., 1985). This relationship has made prediction of RVF outbreaks in East Africa possible using remotely sensed data (satellite imagery and ENSO cycles) (Anyamba et al., 2009; Linthicum et al., 1999). The use of such information has enabled RVF prediction with sufficient lead time that would allow for preparedness planning (Anyamba et al., 2006). Climate change associated flooding will likely play a major role in RVF epidemics in the future.

6.7.4 Inter-epidemic vs. epidemic transmission

The main known mechanism by which RVFV is maintained between epidemics is through infected eggs of the floodwater Aedes mosquitoes. The infected eggs can lay dormant in dry soils for years and hatch when submerged in flood water and subsequently produce infected
vectors. Among arboviruses, the vertical transmission is not unique to RVFV only, it has also be described in LaCrosse virus as overwintering mechanism (Gerhardt et al., 2001; Miller et al., 1977; Tesh and Gubler, 1975), Dengue fever virus (Freier and Rosen, 1987) and West Nile virus (Dohm et al., 2002; Goddard et al., 2003; Miller et al., 2000). In experimental studies, the vertical transmissions have been shown to pass the LaCrosse virus up to 8th generation of arthropod without involvement of vertebrate hosts, a scenario not well described for RVFV. The evidence for trans-ovarian transmission in RVF was first recorded in Kenya through isolation of RVFV in unfed female and male mosquitoes reared from field collected larvae and pupae (Linthicum et al., 1985). Such a phenomenon has been further demonstrated in a laboratory experiment through injecting RVFV into mosquito haemocoel which resulted in infection of reproductive tissues (follicular epithelia and oocyte) which is a key stage in establishing vertical transmission (Romoser et al., 2011). These features observed in *Aedes spp* have been linked to both endemic maintenance of RVF and also acting as reservoir during inter-epidemic period. The *Culex pipiens*, *Aedes aegypti* and *Aedes circumbuteolus* have also been shown experimentally as being capable of acquiring infection at immature stages and transmitting the disease to mammalian host as adult mosquitoes (Turell et al., 1990).

The epidemics in the Eastern Africa have been triggered by sustained floods over large areas resulting from unusual weather events such as El Niño (Anyamba et al., 2009; Linthicum et al., 1999). The floods allow for emergence of trans-ovarian infected mosquitoes which initiate the transmission but also for massive secondary vector reproduction that sustains high level transmission among animals (Linthicum et al., 1985). Thus a prerequisite for epidemic is that there is massive vector reproduction in presence of sufficient susceptible hosts, which makes it possible for vectors to keep the infection going before herd immunity comes into play (Fine et al., 2011). This partly explains the sharp epidemic curve observed in RVF outbreaks (Archer et al., 2013), in a naïve population massive transmission to susceptible individuals which develop neutralizing antibodies, the RVFV is cleared and therefore the source of infection to secondary vectors is depleted, but also due to massive transmission high proportion of individuals in the susceptible population is quickly covered and survivors remain immune.

We hypothesize that once the epidemic has subsided, a high proportion of the population will have been exposed and therefore is protected against subsequent attacks by the disease agent. The immune female animals after epidemics will also give birth to immune offspring. By the time maternal antibodies wane they are no longer highly susceptible and on exposure
to infectious mosquito bites they might suffer only mild clinical disease and develop protective
immunity. Since abnormal weather events that trigger emergence of RVFV occur in a cycle
of 5-15 years on average, for example in Eastern Africa, the next such event will likely find a
good proportion of naïve livestock population given the newborns and selling/slaughter of older
individuals in the livestock populations.

In contrast, during the inter-epidemic period, RVFV transmission will still be started by the
main vectors through trans-ovarian infected mosquitoes or through ongoing transmission, but
fail to infect high proportion of the population mainly due to low number of both primary and
secondary vectors. Also, if the population has come out of epidemic in the recent past, the herd
immunity will as well contribute to preventing an epidemic from happening. The herd immunity
would also likely play a role even in situation of massive vector reproduction as the infectious
bites will meet with the protected mammalian hosts and thus hitting the dead end.

Such a situation can occur in areas where there is annual RVFV challenge given the weather
and ecology that allow for modest reproduction of vertically infected mosquitoes and of the
secondary vectors. The annual challenge’s other role would be to widen the base of protected
members of the population by producing either immune female animals or challenge to the new-
borns with maternal immunity such that they could develop long term protective immunity.
This can be observed in a population in which the percent of protected individuals is low but
maintained over long period.

6.8 The need for novel approaches in surveillance and diagnostics

In both livestock and humans, diagnosis of RVF is mainly based on clinical features, patho-
logical changes at post mortem examinations and laboratory testing of samples. RVF should
be considered whenever a high rate of abortions among pregnant animals and death of young
animals occur, that coincides with unusually heavy rainfall and sustained flooding. The sus-
picion should be higher in situations where the clinical signs in animals are accompanied with
influenza-like, febrile illness in people with close contact with sick animals, for example livestock
keepers and farm workers (Davies and Martin, 2003).

Laboratory diagnosis is however necessary to confirm the clinical diagnosis in the field as
there are several diseases which may present with similar clinical features in both livestock
and humans. In livestock the following diseases should be considered as differential diagnosis;
Nairobi sheep disease, Bluetongue, Heartwater, Ephemeral fever, Wesselbron, Toxoplasmosis,
Leptospirosis, Brucellosis, Q fever, Salmonellosis, Peste des petits ruminants and Foot-and-mouth disease. In people the disease may be confused with other febrile illnesses, including malaria and diseases causing ocular infection and haemorrhagic syndromes.

The diagnostic techniques include those detecting the aetiological agent and immune responses upon exposure to the RVFV in mammalian hosts. Serological tests which detect immune response are good to indicate past exposures, so they can be useful both during epidemics and inter-epidemic periods, but during the inter-epidemic period the serological tests could prove to be an indispensable tool due to short detection window of RVFV.

The aetiological agent can be detected by either virus isolation or molecular detection of RVFV antigen in clinical specimens. These are best suited during epidemics as viraemic specimens from clinical cases are easy to get, but would require to be tailored to be effective during the inter-epidemic period. This is due to quick clearance of the virus from mammalian host and difficulty in detecting clinical cases as most would easily be confused with other fever causing infections. Recent advances in molecular detections including Loop-mediated isothermal amplification (LAMP, Mori and Notomi (2009)), single tube polymerase chain reaction (PCR), and affordable laboratory equipment have allowed for development of laboratory techniques that can be applied under field conditions. Virus isolation on the other hand would require specialized biosafety level 3 (BSL-3) laboratories which are lacking in most RVF endemic areas. But all these still remain of research/academic importance rather than routine disease monitoring, such as for transmission occurring during the inter-epidemic period.

They can however be useful during IEP if appropriate/innovative surveillance techniques are adopted to capture active infections in mammalian hosts. Therefore techniques like rapid tests in both animal and humans would address the diagnosis challenges better and in particular those that would allow integrated monitoring of other diseases such a rapid test that are widely used in malaria diagnosis in areas affected/endemic to RFV as well.

The transmission dynamics of RVF both during the epidemics and epidemic free periods are far more complex and may be uniquely different at fine geographical scales. This calls for a new approach in monitoring the disease in both situations which should include both livestock and human populations as RVFV circulation in people can be independent from that in the livestock populations.

Surveillance relying on sentinel livestock as the only indicator population could miss potential viral circulation that might be independent of animal population, therefore a need to also include
humans as sentinel population. In this case, the surveillance approach should target people presenting with fever in health facilities. New surveillance tools, including rapid diagnostic tests (RDTs), that are either available but used for other diseases and that can be adopted and modified, or improved by addition of genetic material preservatives e.g. RNA later to capture infectious agent’s RNA in widest forms and opportunities. New approaches to diagnosis and surveillance need to be devised in order to deal with the observed challenges in the transmission dynamics.

Monitoring infection in vector populations would serve as an important indicator of possible viral circulation. This can be programmatic and logistically feasible where dry preserved RVF vectors can be utilized (Bangs et al., 2007; Mavale et al., 2012). However, this would need to be preceded by studies on possibilities of detecting RVF aetiological agent or genetic material in dried mosquito samples of different genera known to be vectors of RVFV in diverse geographical areas. Thereafter, standards that allow utilization of sentinel samples that are dry preserved can be set and used in surveillance of RVFV in vector populations. Such development would make it possible for embedding RVF surveillance within other vector monitoring studies including those for diseases such as Malaria and Lymphatic filariosis as these diseases may share the same vectors with RVF (Hassan et al., 2011) and therefore present a possibility of utilizing the same logistics and infrastructure for field sampling.

6.9 Limitations of the study

The study aimed to detect inter-epidemic transmission of RVF virus in the flood prone Kilombero valley that mimics flooding associated with heavy rainfall elsewhere. This to a large extent has been demonstrated in two populations (livestock and people) but could not be demonstrated in mosquito vectors which is an important link in the transmission cycle and epidemiology of the disease. The gap still need to be addressed and with the findings in livestock and people as supporting hypothesis, a rigourous vector study in particular mosquito vectors starting with main vectors can be set to elucidate what is happening in the vector population.

Vaccination in a human population has never been done in Tanzania. However, in the livestock population, there have been vaccination rounds in Tanzania following the RVF epidemics of the 2006/07. These vaccinations were conducted even after the epidemic waves and this could have had an effect in the reported findings in Chapter 3: some of the vaccinated animals could still have been present in the herds, which we sampled animals from. We did enquire about
previous vaccination prior to sampling, and all herd owners reported no animal vaccination. However, there is no method to distinguish vaccinated animals from unvaccinated ones. Thus, the reported seroprevalence may to some extent be an overestimate.

The simulation model as developed in Chapter 5 has several limitations, typical for preliminary models. Amongst them is the obvious large number of parameters included to ensure flexibility in different ecological settings. It is understood that this automatically entails that finetuning one or some parameters is greatly influenced by the values of at least some of the other parameters. It is therefore stressed once more that the current settings of the model allow in the first place scenario analysis (i.e. evaluating outcomes in function of broad settings of a large number of parameters), rather than finetuning specific parameters. A lot more precise information about key parameters (i.a. vertical transmission rates in vector species, vector population densities, carrying capacities, etc.) is needed before the latter exercise can be envisaged.

The expert opinion currently included in the model is often based on a single expert’s advice. It is hoped that publishing this information as such will generate a discussion to arrive at better prior point estimates and ranges. Note that this in no way expresses a doubt about the expert’s opinion.

6.10 Conclusion

As a result of lack of or inadequate surveillance during the inter-epidemic period in RVF endemic areas, little or no new evidence is generated to update on the changing epidemiological situations regarding presence of RVFV activity and its geographical extent during inter-epidemic periods. The findings from this study, have managed to register evidence regarding post-epidemic transmission of RVFV in both livestock and human populations in the Kilombero river valley that preceded the sero-survey (Chapter 3 and Chapter 4).

The findings also indicate annual persistence of RVF virus circulation in the two populations in the study area with interaction with livestock being important risk factor for human RVFV exposure. The linear increase in prevalence of RVFV antibodies in the post-epidemic annual cohorts implies a constant exposure and presence of active foci of recent transmission preceding the survey. Such information is of significance in dealing with challenges regarding transmission dynamics in endemic countries and epidemic prone areas for RVF epidemic preparedness planning.

Active surveillance in this study during inter-epidemic period in both human and animal
populations has allowed detection of low level transmission. Such surveillances could also be extended to include main vectors of RVF in particular geographical locations, and where applicable the wildlife population. However, during inter-epidemic period it has not been possible for livestock and health authorities in endemic countries to conduct meaningful routine surveillance and laboratory testing due to high costs involved. Such a challenge could be dealt with through novel approaches in surveillance and diagnostics that can cut costs dramatically and therefore encourage their implementation. This in turn will assist in updating the changing epidemiological situations in endemic countries.

In the wake of declining malaria cases (WHO, 2013), these findings underscore the need for clinicians to consider other causes of fever such as RVF for Kilombero valley inhabitants in the differential diagnosis for febrile illnesses. All too often, febrile patients are considered malaria positive (even in the absence of a positive confirmatory test, due to the known low test sensitivity) and the resulting apparent malaria persistence is blamed on failure of control measures, rather than clinicians considering other causes for febrile illnesses (such as RVF). This also means that control of other infections is not initiated.

The findings also suggest the opportunity and need to further investigate the circulating RVFV strain as the study area has shown to support transmission of RVF during the inter-epidemic period both in people and livestock, with direct mosquito transmission also playing an important role in human. The traditional epidemic risk factors were not evident in this study which indicate different mechanism of maintenance during inter-epidemic period.

These findings have implications for the Rift Valley fever control programmes in Tanzania and beyond, whose control and prevention strategies rely/based on traditional transmission dynamics. At present contingency plans are based on epidemic models of the disease typical of the Eastern Africa region. Finally, transmission dynamics studies should be carried out in other regions of Tanzania to provide data necessary for the contingency plan of countrywide RVF control, as well as to identify and monitor potential RVFV vector populations. These, coupled with new scientific advances that allow earlier forecasting of climatic patterns conducive to RVF outbreaks and vaccines will help endemic countries to better confront RVF epidemics (Geering et al., 2002) situations and thus reducing human toll as well as socio-economic consequences of the epidemics.


Anderson EC, Rowe LW. The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, rift valley fever, ephemeral fever and bluetongue and to Leptospira sp. in free-ranging wildlife in Zimbabwe. Epidemiology & Infection. 1998; 121:441–449.


Davies FG, Martin V. Recognizing Rift Valley fever. FAO; 2003.


McCarthy M. Zika virus was transmitted by sexual contact in Texas, health officials report. British Medical Journal Publishing Group; 2016.


Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008; 102(5):400–408.


Appendices
APPENDIX A

Model user manual
RVF transmission dynamics
User manual

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1 Introduction

Two versions of the simulation Rift Valley fever virus (RVFv) transmission dynamics package are offered. They are, on the one hand, a faster, less user-friendly compromise and, on the other, a completely open, easily adapted but rather slow version (about 60 times slower than the first version). The compromise version has the actual model code written in C++ with input and output through RStudio shiny. The completely open version has the entire code (model computations, input and output) written in R.
2 Installation

2.1 Installation of R™ and RStudio™

1. Obtain and install the most recent versions of R™ and RStudio™

⚠️ Attention!

It is important to always ensure both R and RStudio are up-to-date as conflicts between different packages may occur if this is not the case.

2. Ensure the latest version of each of the following packages is downloaded and installed:
   - shiny
   - deSolve
   - Rcpp

⚠️ Attention! If C++ is not installed

Test to see if C++ language components are correctly installed by first running the short script test.R (Listing 1). If C++ is properly installed the message Rcpp installed 0 will appear. If not:

- MacOS: users must have local copies of the xcode developer line tools. If not installed, follow the instructions at: https://thecoatlessprofessor.com/programming/r-compiler-tools-for-rcpp-on-os-x/
- Windows: users must have a local install of Rtools. If not installed, when running Listing 1 the ensuing error will automatically open the following wizard. Follow the instructions.

Listing 1: Test C++ installation

```r
1 require(Rcpp)
2 cppFunction('void test(int m) { std::cout<<"Rcpp installed "<<m
<<std::endl;}
3 test(0)
```
2.2 Installation of the RVF components

2.2.1 Fast C++ version

Ensure the following files are present, all in the same folder/directory:

- RVF.R
- CppFunctions.cpp
- CppFunctions.h

⚠️ Attention!

Any name, allowed by the respective operating system, can be used for the enclosing directory/folder. However, it is essential **not to change the names** of the individual files. An error will occur when any of the file names are changed. An error also occurs when the three files are **not in the same directory/folder**.

2.2.2 Slow open version

Ensure the RVF_nocpp.R is installed.
3 Starting the program

1. Open RVF.R (or RVF_nocpp.R) in RStudio

2. Select RVF.R (or RVF_nocpp.R): the top right of the script pane (which is the top left window of RStudio in the standard set-up) should show a green arrow with the text Run App

3. Click the small triangle to the right of Run App and select Run in Viewer Pane from the drop-down menu\(^1\)

4. Click the Viewer tab in the right bottom window pane and maximise its size by moving the horizontal divider to the top and the vertical divider as far left as possible, making sure the green arrow in the script pane remains visible (once the program runs, the divider can be moved further left to increase the size of the Viewer pane even more)

5. Click the green arrow in the script pane to start the program

\(^1\)It may happen that you have to try and select Run in Viewer Pane several times. If the \(\checkmark\) does not appear, select first Run External and then again Run in Viewer Pane.
4 Setting up the simulation

4.1 Introduction

Parameter and initial population values can be defined by means of numeric entry fields, sliders and checkboxes, divided over the different tab panes. Sliders can be moved by selecting the slider knob with the cursor and moving it. When the slider knob has been selected it can also be moved by means of the left and right arrows on the keyboard. The individual tab panes can be selected from the tab menu at the top of the Viewer Pane.

The time-unit of the actual model is one day. For practical reasons, simulation length is set in terms of years. Years start of 1 January and are subdivided in twelve months of 30 days each, e.g. 1 March is day 61, 1 June is day 181 and 31 December is day 360.

4.2 Run simulation & select plots

Select the plots to be generated, select the type of x-axis (either labels for each month or a continuous variable showing the number of days lapsed since the start of the plot), indicate the range to be plotted (first and last days), set the number of years over which the simulation is to be run, if desired set the random number seed and run the simulation (of course, after having set whatever parameters to the desired values).

4.3 Initial state

Set the initial values (the number of individuals in a particular compartment) for the various compartments of the model. The first letter indicates the organism (human/-mammal/vector)

- H = people
- M = domestic animals
- A = vector A
- B = vector B
- C = vector C
- D = vector D

The second letter indicates the compartment the individuals are in:

- S = susceptible (adult in case of vector)
- E = exposed
- I = infective (adult in case of vector)
- R = recovered
• Q = infected eggs
• P = uninfected eggs
All compartments may be selected for the three zones.

4.4 General model information

The overall characteristics of the simulation is determined by means of checkboxes and numerical input fields. The different options are:

• **El Niño flooding**: yes/no checkbox and, if selected, starting and ending dates of flooding
  El Niño rains causing full flooding of Zone 1 is at present set to occur every ten years (year 1, year 11, year 21, ...)

• **Annual flooding**: yes/no checkbox and, if selected, starting and ending dates plus the proportion of Zone 1 flooded
  This type of partial flooding occurs annually at the same period

• **Seasonal effect hatching**: yes/no checkbox, peak shifting from away 1 January and number of peaks
  The standard setting (if selected) generates a single cosine curve per year with peak on 1 January. The following graphs show the output with different settings of delay and number:
  - (a) delay = 0; number = 1; (one peak on 1 January)
  - (b) delay = -90; number = 1; (one peak on 1 April)
  - (c) delay = 90; number = 1; (one peak on 1 September)
  - (d) delay = 0; number = 2 (two peaks, one on 1 January and one on 1 June)
4. SETTING UP THE SIMULATION

- **Annual variation climate**: yes/no checkbox and, if selected, number of dry years in a period of total years and effect on hatching (*i.e.* proportion hatching during dry year compared to normal year)
  
  This control option allows for inter-year climatic variability to be included, creating ‘wet’ and ‘dry’ years. A random number between zero and one is generated for every year in the simulation. If this random number is smaller than the ratio dry years/total years then the corresponding year suffers from drought. The hatching rate during a dry year is the normal hatching rate times the value entered by the Minimum slider.

- **Annual transhumance**: yes/no checkbox and, if selected, date of Zone 1 to Zone 2 movement and date of return movement
  
  Transhumance is supposed to be an annual event currently taking place each year on the same dates. Transhumance is programmed to be from wet-season grazing around the homestead (Zone 2) to dry-season grazing on seasonally inundated pasturelands (Zone 1).

- **Increased susceptibility of animals**: yes/no checkbox and, if selected, starting and ending dates of period of increased susceptibility and factor of increase
  
  This option allows for the inclusion of an annual period where the animals are more prone to (*e.g.*) being bitten\(^2\) by a vector, for instance after shearing of sheep.

- **Infection rate wildlife**: infection rate of wildlife in Zone 3
  
  Vector species residing in Zone 3 can become infected from ‘wildlife’ species and in turn infect visiting people and domestic animals.

\(^2\)Bite/biting etc. are used throughout. Replace by sting/stinging or equivalent where necessary.
- **Number alternative hosts**: number of alternative hosts (i.e. other than people and domestic animals) available for the vectors (per zone). This number represents the number of alternative hosts for the vectors that can be bitten if there are not enough people and/or animals to accommodate the total number of vector bites per unit in the respective zone. Increasing this number increases the survival rate of the vectors in case of insufficient hosts, but decreases infection rates of the vectors as these alternative hosts are assumed refractory to RVFv.

### 4.5 People

Values for parameters pertaining to the human population are entered in this pane.

- Birth rate and natural mortality rate (assumed to be the same in all zones)
  Enter the same value for birth rate and natural mortality rate to obtain a constant population size in absence of disease.

- Incubation and infective periods and disease-specific mortality rate

- Migration rates between the different zones
  Put repetitive migration rates and initial state compartment values to zero to confine people to certain zones.

- RVFv transmission probabilities from people to the different vectors
  This is the probability to transmit the virus when a vector successfully bites an infective person.

- Contact rates with animals per time unit (day)
  This value represents the number of (different) animals a person contacts per day, sufficiently close for infection to be acquired from the animal, if the latter is infective.

- Maximum daily biting rates
  The maximum number of bites a person ‘supports’ per time unit.

### 4.6 Animal

The domestic animal population parameters are entered in this pane. They mostly correspond to what has been discussed in **subsection 4.5** and only the differences are discussed.

- The standard birth rate pertains to non-infected animals. The birth rate for infective animals is adjusted taking into account the abortion rate due to infection.

- There is a so-called animal population carrying capacity for the different zones. Although this affects the reproduction in a density-dependent way by reducing birth rates to zero as the number of individuals approach the carrying capacity, this upper limit can also be used to limit the animal population to a certain number, thereby (e.g.) simulating sales of animals in function of number of animals present.
4.7 Vector A

Vector A is characterised by both the ability of eggs to remain dormant during inimical conditions (e.g. between El Niño events or between annual flooding) and the possibility of vertical transmission of RVFv, i.e. infected females produce infected eggs resulting in a new generation of infective adults. The following parameters can be set:

- Oviposition, vertical transmission and hatching rates
  The hatching rate in Zone 1 can be controlled by manipulating flooding (El Niño and annual) and by means of annual and seasonal variability in the three zones
- Mortality rates for adults and the two types of eggs in the three zones
- Daily maximum biting rate, feeding distribution\(^3\) and RVFv transmission probabilities upon successful biting
  The feeding distribution represents the proportion of bites given to a particular host
- Carrying capacities and migration rates
  See subsection 4.5 on how to constrain vector populations to certain zones

4.8 Vector B

The parameters to be entered for this vector are essentially the same as for Vector A. The difference is that there is no dormancy in the eggs stage for this species.

4.9 Vector C

This species differs from Vector B in that there is no vertical transmission. The other parameters are equivalent.

4.10 Vector D

See subsection 4.9

4.11 Summary tables

A summary of the different compartment sizes, seroprevalence levels, maximum epidemic size etc are provided. At present, there is a table summarising compartment sizes for humans and domestic animals (first lines), as well as maximum epidemic sizes (Infective second line) and average seroprevalence (Recovered second line). A second table shows the average compartment sizes of the various vector populations. A third table provides details of the seroprevalence in human and domestic animal populations, three, five and seven years after the last El Niño event in the simulation.

\(^3\)It is the user’s responsibility to ensure that the sum of the proportions feeding on the different hosts is less than or equal to unity
5 Running the simulation

1. Set up the parameters as required

2. Click the Run simulation button in the General and plot tab pane

3. The differential equation model runs for the required time period and creates the requested sets of graphs in the Plots tab pane of the RStudio output pane. The graphs are created in individual plot windows that can be accessed by means of left and right arrows at the top left of the Plots pane. Which graphs are produced is selected by means of checkboxes in the General and plot tab pane.

⚠️ Attention!

Although it is possible to remove the graphs in the Plots pane (by clicking ✅), this feature is apparently not available when the App is running. It can thus apparently happen that RStudio runs out of memory and hangs if ‘too’ many graph windows have been created. It is therefore recommended to leave the application every now and then, clear the plots and start it up again. It is also recommended to only plot the graphs that are of interest when comparing different scenarios.

4. To go back to the input and output window click the Viewer tab.
6  Saving the graph output

Individual graphs can be saved in various formats. Click the small black arrow to the right of Export and select the desired format. Make sure that text is reproduced correctly, as especially legend text is sometimes saved incompletely. The safest option is to select Copy to Clipboard... (make sure is selected and do not forget to click Copy plot).

7  Stopping the program

The program is stopped by clicking either in the Console or in the output pane.
8 Sample output

The following output is produced with the standard settings (Figure 2 to Figure 14).

**Figure 2:** Standard parameters: Human - zone 1

**Figure 3:** Standard parameters: Human - zone 2
Figure 4: Standard parameters: Human - zone 3

Figure 5: Standard parameters: Animal - zone 1

Figure 6: Standard parameters: Animal - zone 2
Figure 7: Standard parameters: Animal - zone 3

Figure 8: Standard parameters: Vector A - zone 1

Figure 9: Standard parameters: Vector B - zone 2
Figure 10: Standard parameters: Vector B - zone 3

Figure 11: Standard parameters: Vector C - zone 1

Figure 12: Standard parameters: Vector D - zone 2
Figure 13: Standard parameters: Vector D - zone 3

Figure 14: Standard parameters: Summarising output table
RStudio RVFV transmission code
library(shiny)
require(deSolve)

# Define the UI for the application
ui <- navbarPage("Rift Valley Fever",
  mainPanel(fluidRow(br()),
    fluidRow(column(6, actionButton("run", label = h2("Run simulation")), br(), br()),
      fluidRow(column(6, numericInput("year", label = "Number of years to run simulation",
        min = 1, max = 50, value = 27, step = 1), align="center"),
        column(6, br(), br()),
      fluidRow(column(6, numericInput("plotStart", label = "Start plotting at (day):", value = 7201),
                  numericInput("plotEnd", label = "Stop plotting at (day):", value = 9720)))))
  ) )

#--------------- Call Cpp functions
ui = navbarPage( "Rift Valley Fever",
  mainPanel (flowPanel( "Run simulation & select plots",
    mainPanel (fluidRow (br()),
      fluidRow (column(6, actionButton("run", label = h2("Run simulation")), br(), br()),
        fluidRow (column(6, numericInput("year", label = "Number of years to run simulation", min = 1, max = 50, value = 27, step = 1)), align="center"),
          column(6, br(), br()),
        fluidRow (column(6, numericInput("plotStart", label = "Start plotting at (day):", value = 7201),
                      numericInput("plotEnd", label = "Stop plotting at (day):", value = 9720)))))
  ) )

#-------------------- RVF.R : R interface to be used in conjunction with CppFunctions

Listing 1: R VF.R: R interface to be used in conjunction with CppFunctions

1 library (shiny)
2 require (deSolve)
3 ... value = 0), hr(),
91 numericInput( "BQ2", NULL, value = 0), hr(), numericInput( "BP2", NULL, value = 10), hr(),
Page 1
[0x-57]Listing 1: R VF.R: R interface to be used in conjunction with CppFunctions
1 library (shiny)
2 require (deSolve)
3 ... value = 0), hr(),
91 numericInput( "BQ2", NULL, value = 0), hr(), numericInput( "BP2", NULL, value = 10), hr(),
Page 1
[64x86]91 numericInput( "BQ2", NULL, value = 0), hr(), numericInput( "BP2", NULL, value = 10), hr(),
Page 1
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

```
numericInput("BS2", NULL, value = 0), hr(), numericInput("BI2", NULL, value = 0), hr(),
numericInput("CI2", NULL, value = 0), hr(), numericInput("DP2", NULL, value = 0), hr(),
numericInput("BS2", NULL, value = 0), hr(), numericInput("BI2", NULL, value = 0), hr(),
numericInput("CP2", NULL, value = 0.0008), hr(),
```

```
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions
```
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

column(6, numericInput("p_mh0", "Infection transfer rate animal -> human", value = 0.001),
numericInput("p_m", "Infection transfer rate animal vector A", value = 0.89),
numericInput("p_m", "Infection transfer rate animal vector C", value = 0.81),
numericInput("p_m", "Infection transfer rate animal vector B", value = 0.81),
hr(),
numericInput("p_m", "Maximum supported biting rate", value = 50),
hr(),
align="left")

\>

} # mainPanel

\} # mainPanel

\} # fluidRow

\} # mainPanel

\} # fluidRow

\} # fluidRow

\} # fluidRow

\} # fluidRow

\} # fluidRow

\} # fluidRow
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions
# Initialization of all the parameters and the initial values of the compartments per species

Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

## General initializations

- **Initialization of maximum rate allowed in GDE to avoid negative values**
  - If problems are encountered lower max_rate to 0

- **Timeframe**
  - Start and end years
  - Dry years: 0

- **Initial habitat**
  - State of activity

### Parameters to be passed to GDE function

- **Parameters**
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`
  - `input $plotD3`

- **Initial habitat**
  - State of activity
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

```r
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

```
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

542 if(plotA1) drawPlot4b((times[ps:pe] - ps2), out$AP1[ps:pe], out$AS1[ps:pe], out$AQ1[ps:pe], out$AI1[ps:pe], ps, pe, year)
543 "Vector A zone 1", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
544 if(plotA2) drawPlot4b((times[ps:pe] - ps2), out$AP2[ps:pe], out$AS2[ps:pe], out$AQ2[ps:pe], out$AI2[ps:pe], ps, pe, year)
545 "Vector A zone 2", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
546 if(plotA3) drawPlot4b((times[ps:pe] - ps2), out$AP3[ps:pe], out$AS3[ps:pe], out$AQ3[ps:pe], out$AI3[ps:pe], ps, pe, year)
547 "Vector A zone 3", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
548 if(plotB1) drawPlot4b((times[ps:pe] - ps2), out$BP1[ps:pe], out$BS1[ps:pe], out$BQ1[ps:pe], out$BI1[ps:pe], ps, pe, year)
549 "Vector B zone 1", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
550 if(plotB2) drawPlot4b((times[ps:pe] - ps2), out$BP2[ps:pe], out$BS2[ps:pe], out$BQ2[ps:pe], out$BI2[ps:pe], ps, pe, year)
551 "Vector B zone 2", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
552 if(plotB3) drawPlot4b((times[ps:pe] - ps2), out$BP3[ps:pe], out$BS3[ps:pe], out$BQ3[ps:pe], out$BI3[ps:pe], ps, pe, year)
553 "Vector B zone 3", "UNINFECTED EGGS, SUSCEPTIBLE ADULTS", "INFECTED EGGS, INFECTED ADULTS"
554 }}
555 output$summary3 = renderTable(summ3, digits = 3, rownames = T, na = ""
559 output$summary2 = renderTable(summ2, digits = 2, rownames = T, na = ""
560 summ2[4,4] = mean(out$DI1) + mean(out$DI2) + mean(out$DI3)
561 summ2[4,3] = mean(out$DS1) + mean(out$DS2) + mean(out$DS3)
562 summ2[3,3] = mean(out$CS1) + mean(out$CS2) + mean(out$CS3)
563 summ2[2,4] = mean(out$BI1) + mean(out$BI2) + mean(out$BI3)
564 summ2[2,3] = mean(out$BS1) + mean(out$BS2) + mean(out$BS3)
565 summ2[2,1] = mean(out$BP1) + mean(out$BP2) + mean(out$BP3)
566 summ2[1,4] = mean(out$AI1) + mean(out$AI2) + mean(out$AI3)
567 summ2[1,2] = mean(out$AQ1) + mean(out$AQ2) + mean(out$AQ3)
568 summ2[1,1] = mean(out$AP1) + mean(out$AP2) + mean(out$AP3)
569 summ2 = as.data.frame(array(NA, c(4,4), dimnames = list(c("Vector A", "Vector B", "Vector C", "Vector D"), c("Clean eggs", "Infected adult ", "Infected adult ", "Infected adult ")))
570 output$summary = renderTable(summ, digits = 2, rownames = T, na = ""
571 summ[4,3] = max(out$MI1 + out$MI2 + out$MI3)
572 summ[3,4] = mean(out$MR1) + mean(out$MR2) + mean(out$MR3)
573 summ[3,1] = mean(out$MS1) + mean(out$MS2) + mean(out$MS3)
574 summ[2,4] = mean(out$HR1+out$HR2+out$HR3)/mean(out$HS1+out$HS2+out$HS3+out$HE1+out$HE2+out$HE3+out$HI1+out$HI2+out$HI3+out$HR1+out$HR2+out$HR3)
575 summ[2,3] = max(out$HI1 + out$HI2 + out$HI3)
576 summ[1,4] = mean(out$HR1) + mean(out$HR2) + mean(out$HR3)
577 summ[1,1] = mean(out$HS1) + mean(out$HS2) + mean(out$HS3)
578 ss = ((year-7) %/% 10) * 36000; s3 = ss + 2 * 3600 + 1; e3 = ss + 3 * 3600; s5 = ss + 4 * 3600 + 1; e5 = ss + 5 * 3600; s7 = ss + 6 * 3600 + 1;
579 if(year>=7){
580 summ[4,2] = mean(out$DI1) + mean(out$DI2) + mean(out$DI3)
581 summ[3,3] = mean(out$CS1) + mean(out$CS2) + mean(out$CS3)
582 summ[2,4] = mean(out$BI1) + mean(out$BI2) + mean(out$BI3)
583 summ[2,3] = mean(out$BS1) + mean(out$BS2) + mean(out$BS3)
584 summ[2,1] = mean(out$BP1) + mean(out$BP2) + mean(out$BP3)
585 summ[1,4] = mean(out$AI1) + mean(out$AI2) + mean(out$AI3)
586 summ[1,2] = mean(out$AQ1) + mean(out$AQ2) + mean(out$AQ3)
587 summ[1,1] = mean(out$AP1) + mean(out$AP2) + mean(out$AP3)
588 summ[3,2] = mean(out$ME1) + mean(out$ME2) + mean(out$ME3)
589 summ[3,1] = mean(out$MR1) + mean(out$MR2) + mean(out$MR3)
590 summ[4,4] = max(out$MI1 + out$MI2 + out$MI3)
591 summ[3,4] = mean(out$MR1) + mean(out$MR2) + mean(out$MR3)
592 summ[3,1] = mean(out$MS1) + mean(out$MS2) + mean(out$MS3)
593 summ[2,4] = mean(out$HR1) + mean(out$HR2) + mean(out$HR3)
594 summ[2,3] = max(out$HI1 + out$HI2 + out$HI3)
595 summ[2,1] = mean(out$BP1) + mean(out$BP2) + mean(out$BP3)
596 summ[1,4] = mean(out$AI1) + mean(out$AI2) + mean(out$AI3)
597 summ[1,2] = mean(out$AQ1) + mean(out$AQ2) + mean(out$AQ3)
598 summ[1,1] = mean(out$AP1) + mean(out$AP2) + mean(out$AP3)
599 summ[4,3] = max(out$MI1 + out$MI2 + out$MI3)
600 summ[3,3] = mean(out$HR1) + mean(out$HR2) + mean(out$HR3)
601 summ[3,2] = mean(out$ME1) + mean(out$ME2) + mean(out$ME3)
602 summ[3,1] = mean(out$MR1) + mean(out$MR2) + mean(out$MR3)
603 summ[2,4] = mean(out$DI1) + mean(out$DI2) + mean(out$DI3)
604 summ[2,3] = mean(out$DS1) + mean(out$DS2) + mean(out$DS3)
605 summ[2,1] = mean(out$BP1) + mean(out$BP2) + mean(out$BP3)
606 summ[1,4] = mean(out$AI1) + mean(out$AI2) + mean(out$AI3)
607 output$summary2 = renderTable(summ2, digits = 2, rownames = T, na = ""
608 output$summary3 = renderTable(summ3, digits = 3, rownames = T, na = ""
609 )
610 if(year>7) {
611 output$summary = renderTable(summ, digits = 2, rownames = T, na = ""
612 output$summary2 = renderTable(summ2, digits = 2, rownames = T, na = ""
613 output$summary3 = renderTable(summ3, digits = 3, rownames = T, na = ""
614 })
615 print(output$summary)
616 if(inputFrailty)
617 } # observe
618 561 output$summary3 = renderTable(summ3, digits = 3, rownames = T, na = ""
619 ) # observe
Listing 1 (Cont.): R VF.R: R interface to be used in conjunction with CppFunctions

```r

\begin{verbatim}

drawPlot4 = function (tt, g1, g2, g3, g4, ttl, y_ax1, y_ax2, legText, ps, pe, year)
{
  \textbf{if}(ps%%30==0) ps = ps - 1
            \text{year+1})[(ps%%360)%/30+1);

  drawPlot3 = function (tt, g1, g2, g3, ttl, y_ax1, y_ax2, legText, ps, pe, year)
{
  \textbf{if}(ps%%30==0) ps = ps - 1
            \text{year+1})[(ps%%360)%/30+1);

  \}
}
\end{verbatim}
```
APPENDIX C

C++ additional code
#include <Rcpp.h>
#include <math.h>
using namespace Rcpp;

// Function to print vector in cpp to check correctness
void PrinVect(NumericVector x);

// Function to replicate number equivalent to rep in Rcpp
NumericVector rep_N(long double x, int n);

// Function max of two long double
long double Sup(long double a, long double b);

// Function min of two long double
long double Inf(long double a, long double b);

// Function returning the infection rate of cattle
long double b_cattle(NumericVector x);

// Function returning the infection rate for people
long double b_people(NumericVector x);

//Function returning the proportion infection of mosquitoes
long double b_mos(NumericVector x);

//Function to perform the calculus of the rates for people, cattle and mosquitoes
NumericVector Rates_Updates(NumericVector A, NumericVector subparam);

//Function combining vectors in a list to return one vector after concatenation of the others
NumericVector combine(const List& list);

// transhumance flood shearing
NumericVector transhumance(NumericVector t, NumericVector p, double val);

// return sinusoidal function of t for dry years
NumericVector SinFun(NumericVector t, NumericVector p);

//Function with the ODEs to be solved
List ODE(NumericVector t, NumericVector state, NumericVector param);
#include <Rcpp.h>
#include <math.h>
#include <iostream>
#include <limits>
using namespace std;
using namespace Rcpp;

/* Function to print vector in cpp to check correctness */
void PrinVect(NumericVector x) {
  int i = 0;
  for (i = 0; i < x.size(); i++) {
    Rcpp::Rcout << x(i) << std::endl;
  }
}

/* Function to replicate number equivalent to rep in Rcpp exist for NumericVector */
NumericVector rep_N(long double x, int n) {
  NumericVector v(rep(NumericVector::create(x), n));
  return v;
}

// [[Rcpp::export]]
/* Function max of two long double */
long double Sup(long double a, long double b) {
  return double (a <= b ? b : a);
}

// [[Rcpp::export]]
/* Function min of two long double */
long double Inf(long double a, long double b) {
  return (a <= b ? a : b);
}

// [[Rcpp::export]]
// Function returning the infection rate of cattle
long double b_cattle(NumericVector x) {
  long double a = x[0], b = x[1], c = x[2], d = x[3], e = x[4], f = x[5], g = x[6], h =
  x[7], i = x[8], j = x[9], k = x[10], l = x[11], n = x[12], m = x[13];
  long double x1 = -std::log (std::pow((1.0 - b), (c * d / e)) * std::pow((1.0 - f), (g * h / e)) *
  std::pow((1.0 - i), (j * k / e)) * std::pow((1.0 - l), (m * n / e)));
  x1 = (std::isinf(x1) ? a : x1);
  b = (e == 0.0 ? 0.0 : Inf(a, x1));
  //std::cout("b cattle " << b << " " << x1 << " " << a << std::endl;
  return b;
}

// [[Rcpp::export]]
// Function returning the infection rate for people
long double b_people(NumericVector x) {
  long double a = x[0], b = x[1], c = x[2], d = x[3], e = x[4], f = x[5], g = x[6], h = x[7],
  i = x[8], j = x[9], k = x[10], l = x[11];
  long double m = x[12], n = x[13], o = x[14], p = x[15], q = x[16], r = x[17];
  long double aa = 0.0, bb = 0.0;
  aa = (e == 0.0 ? 0.0 : 1.0 - std::pow((1.0 - b), (c * d / e)) * std::pow((1.0 - f), (g * h / e)) *
  std::pow((1.0 - i), (j * k / e)) * std::pow((1.0 - l), (m * n / e)));
  bb = (q + r == 0.0 ? 0.0 : 1.0 - std::pow((1.0 - o), (p * q / (q + r))));
  long double x1 = -std::log (1.0 - (aa + bb - aa * bb));
  x1 = (std::isinf(x1) ? a : x1);
  b = Inf(a, x1);
  return b;
}
// [Rcpp::export]
// Function returning the proportion infection of mosquitoes
long double b_mos(NumericVector x) {
    long double a = x[0], b = x[1], c = x[2], d = x[3], e = x[4], f = x[5], g = x[6];
    long double x1=(c==0.0 ? 0.0 : a*b/c)+(e+f==0.0 ? 0.0 : d*e/(e+f)) + g; // ici b_wl is outside ifelse here
    return (x1<=1.0 ? x1 : 1.0);
}

// [Rcpp::export]
// Function to perform the calculus of the rates for people, cattle and mosquitoes
NumericVector Rates_Updates(NumericVector A, NumericVector subparam1, NumericVector subparam2){
    long double AA = AS + AI, BA = BS + BI, CA = CS + CI, DA = DS + DI;
    long double O_ = subparam1[0]*(AA)*subparam1[1] + subparam1[2]*(BA)*subparam1[3] +
                   subparam1[4]*(CA)*subparam1[5] + subparam1[6]*(DA)*subparam1[7],
               chi_ = subparam1[8]*NH, temp=chi_/_Sup(O_,0.000001), s_ = Inf(1.0,temp);
    long double o_ah = subparam1[0] * subparam1[1] * s_, o_bh = subparam1[2] *
                       subparam1[6]*subparam1[7] * s_;
    long double O_m= subparam1[9]*(AA)*subparam1[1] + subparam1[10]*(BA)*subparam1[3]+
               chi_m = subparam1[14]*subparam1[13]*(NM+MI);
    temp=chi_m/_Sup(O_m,0.000001);
    long double s_m = Inf(1.0,temp), o_am = subparam1[9] * subparam1[1] * s_m;
    long double o_bm = subparam1[10] * subparam1[3] * s_m, o_cm=subparam1[5] * s_m,
                 o_dm=subparam1[12] * s_m; // o_a1 o aa2 o_aaa3
    long double o_a = o_ah + o_am, o_b = o_bh +o_bm, o_c= o_ch + o_cm, o_d= o_dh +o_dm;
    // o_ab1 o_abl_2 o_ab1_2 o_d1_2
    long double o_aa = supparam1[15]/Sup(0.000001,AA), o_ra =
                       supparam1[15]/Sup(0.000001,CA), o_da =
                       supparam1[15]/Sup(0.000001,DA);

    NumericVector p=NumericVector::create(subparam1[16], HI, NH, subparam1[17], MI, NM,
                                           subparam1[15]);
    long double b_a = b_mos(p);
    p=NumericVector::create(subparam1[18], HI, NH, subparam1[19], MI, NM,
                              subparam1[15]);
    long double b_b = b_mos(p);
    // b_ab[b_c] b_d[b_h1 b_m1]
    p=NumericVector::create(subparam2[0], HI, NH, subparam2[1], MI, NM, subparam2[15]);
    long double b_c = b_mos(p);
    //p=NumericVector::create(subparam2[2], HI, NH, subparam2[3], MI, NM, subparam2[15]);
    long double b_d=b_mos(p);
    //b_pe1 b_pe2 b_pe3
    p=NumericVector::create(subparam2[4], subparam2[5], o_ah, AI, NH, subparam2[6],
                   o_bh, BI, subparam2[7], o_ch,
               CI,subparam2[8],o_dh,DI,subparam2[9],subparam2[10],HI,NM);
    long double b_h = (b_people(p));
    // b_cal b_ca2 b_ca3
    p=NumericVector::create(subparam2[4], subparam2[11], o_am, AI, NH, subparam2[12],
                   o_bm, BI, subparam2[13], o_cm, CI,subparam2[14], o_dm, DI);
    long double b_m = ( b_cattle(p));
    NumericVector L(14);
    L=NumericVector::create(o_a, o_b, o_c, o_d, o_aa, o_ba, o_ca, o_da, b_a, b_b, b_c,
                       b_d, b_h, b_m);
    return(L);
}
NumericVector combine(const List& list)
{
    std::size_t n = list.size();
    // Figure out the length of the output vector
    std::size_t total_length = 0;
    for (std::size_t i = 0; i < n; ++i)
        total_length += Rf_length(list[i]);
    // Allocate the vector
    NumericVector output = no_init(total_length);
    // Loop and fill
    std::size_t index = 0;
    for (std::size_t i = 0; i < n; ++i)
    {
        NumericVector el = list[i];
        std::copy(el.begin(), el.end(), output.begin() + index);
        // Update the index
        index += el.size();
    }
    return output;
}

NumericVector transhumance(NumericVector t, NumericVector p, double val, bool trans)
{
    NumericVector l_m(t.size());
    for(int it = 0; it != l_m.size(); ++it) {
        l_m[it]=((trans && fmod(t[it],360.0)>=(p[0]-0.4) && fmod(t[it],360.0)<=(p[0]+0.5))?p[1] : val);
    return l_m;
}

NumericVector SinFun(NumericVector t, NumericVector p){
    NumericVector x(t.size());
    for(int i=0;i<t.size();i++) x[i]= (0.5 * cos(4*atan(1)*(t[i] - p[0])/p[1]) + 0.5);
    return(x);
}

List ODE(NumericVector t, NumericVector state, NumericVector param){
    NumericVector ts, t_al;
    long double b_wl = param[2];
    long double max_rate = param[0], flood_prop = param[1], O_alt = param[3], d1 = param[4], d2 = param[5], d3 = param[6], d4 = param[7], d5 = param[8], d6 = param[9],
    year = param[10], c0 = param[11], mmm = param[12], ds = param[13], nPeak = param[14];
    long double seasonHatch = param[15],g_h= param[16], m_h= param[17], x_h= param[18], a_h= param[19], d_h= param[20], p_mh00= param[21], f_mh1= param[22], f_mh2= param[23], f_mh3= param[24], h_h1= param[25], h_h2= param[26], h_h3= param[27];
    long double p_ha= param[28], p_hb= param[29], p_HC= param[30], p_hd= param[31],
    r_h= param[32], l_h12= param[33], l_h13= param[34], l_h21= param[35], l_h23= param[36], l_h31= param[37], l_h32= param[38];
    long double g_m_u= param[39], g_m_i= param[40], m_m= param[41], x_m= param[42],
    a_m= param[43], d_m= param[44], h_m= param[45], p_ma= param[46], p_mb= param[47],
    p_mc= param[48], p_md= param[49], r_m= param[50], k_m1= param[51], k_m2= param[52],
    // 
}
k_m3= param[53];
long double l_m13= param[54], l_m23= param[55], l_m31= param[56], l_m32= param[57], g_a= param[58], z_a= param[59], m_a= param[60], v_a= param[61], e_a= param[62], e_m= param[63], p_h= param[64], p_a= param[65], k_a= param[66], k_a2= param[67], k_a3= param[68];
long double m_aq1= param[69], m_aq2= param[70], m_aq3= param[71], m_ap1= param[72], m_ap2= param[73], m_ap3= param[74], l_a12= param[75], l_a13= param[76], l_a21= param[77], l_a23= param[78], l_a31= param[79], l_a32= param[80];
long double g_b= param[81], z_b= param[82], m_b= param[83], t_b= param[84], v_b= param[85], e_b= param[86], e_m= param[87], p_h= param[88], p_a= param[89], k_b= param[90], k_b2= param[91], k_b3= param[92];
long double m_bq1= param[93], m_bq2= param[94], m_bq3= param[95], m_bp1= param[96], m_bp2= param[97], m_bp3= param[98], l_b12= param[99], l_b13= param[100], l_b21= param[101], l_b23= param[102], l_b31= param[103], l_b32= param[104];
long double g_c= param[105], m_c= param[106], t_c= param[107], v_c= param[108], e_c= param[109], e_cm= param[110], p_h= param[111], p_cm= param[112], k_c= param[113], k_c2= param[114], k_c3= param[115], m_cp1= param[116], m_cp2= param[117], m_cp3= param[118];
long double l_c12= param[119], l_c13= param[120], l_c21= param[121], l_c23= param[122], l_c31= param[123], l_c32= param[124];
long double g_d= param[125], m_d= param[126], t_d= param[127], v_d= param[128], e_d= param[129], e_dm= param[130], p_h= param[131], p_dm= param[132], k_d= param[133], k_d2= param[134], k_d3= param[135], m_dp1= param[136], m_dp2= param[137], m_dp3= param[138];
long double l_d12= param[139], l_d13= param[140], l_d21= param[141], l_d23= param[142], l_d31= param[143], l_d32= param[144];
long double shearBeg = param[146], shearEnd = param[147], shearUp = param[148];
bool shearing= param[145], wetDry= param[149], flood= param[150], elNino= param[151], transHum= param[152];
long double t_a=param[153], l_m21Base=param[154], l_m12Base= param[155];
NumericVector dry2=param[Range(156,(156+year))];
long double HS1 = state[0], HE1 = state[1], H1 = state[2], HR1 = state[3], HS2 = state[4], HE2 = state[5], H2 = state[6], HR2 = state[7], HS3 = state[8], HE3 = state[9], H3 = state[10], HR3 = state[11];
long double MS1 = state[12], ME1 = state[13], M1 = state[14], MR1 = state[15], MS2 = state[16], ME2 = state[17], M2 = state[18], MR2 = state[19], MS3 = state[20], ME3 = state[21], M3 = state[22], MR3 = state[23];
long double AQ1 = state[24], AP1 = state[25], AS1 = state[26], A1 = state[27], AQ2 = state[28], AP2 = state[29], AS2 = state[30], A2 = state[31], AQ3 = state[32], AP3 = state[33], AS3 = state[34], A3 = state[35];
long double BQ1 = state[36], BP1 = state[37], BS1 = state[38], B1 = state[39], BQ2 = state[40], BP2 = state[41], BS2 = state[42], B2 = state[43], BQ3 = state[44], BP3 = state[45], BS3 = state[46], B3 = state[47];
long double CP1 = state[48], CS1 = state[49], C1 = state[50], CP2 = state[51], CS2 = state[52], C2 = state[53], CP3 = state[54], CS3 = state[55], C3 = state[56];
long double DP1 = state[57], DS1 = state[58], D1 = state[59], DP2 = state[60], DS2 = state[61], D2 = state[62], DP3 = state[63], DS3 = state[64], D3 = state[65];
// population sizes per compartment and per species
//----------------  human
long double NH1 = HS1 + HE1 + H1 + HR1;
long double NH2 = HS2 + HE2 + H2 + HR2;
long double NH3 = HS3 + HE3 + H3 + HR3;
//----------------  animal host
long double NM1 = MS1 + ME1 + MR1;
long double NM2 = MS2 + ME2 + MR2;
long double NM3 = MS3 + ME3 + MR3;
//----------------  vector A
long double NA1 = AQ1 + AP1 + AS1 + A1;
long double NA2 = AQ2 + AP2 + AS2 + A2;
long double NA3 = AQ3 + AP3 + AS3 + A3;
//----------------  vector B
long double NB1 = BQ1 + BP1 + BS1 + B1;
long double NB2 = BQ2 + BP2 + BS2 + B2;
long double NB3 = BQ3 + BP3 + BS3 + B3;
//----------------  vector C
long double NC1 = CP1 + CS1 + C1;
long double NC2 = CP2 + CS2 + CI2;
long double NC3 = CP3 + CS3 + CI3;

vector D

long double ND1 = DP1 + DS1 + DI1;
long double ND2 = DP2 + DS2 + DI2;
long double ND3 = DP3 + DS3 + DI3;

// increased susceptibility of sheep to mosquito bites because of shearing
long double shear = 1.0;
long double sB=(shearBeg-0.4), sE=(shearEnd+0.5);
bool a=(shearing && fmod(t[0],360.0)>sB && fmod(t[0],360.0)<=sE);

shear = (a? shearUp : 1.0);

// transhumance
NumericVector x=NumericVector::create(d1,max_rate);
NumericVector l_m21 = transhumance(t,x,l_m21Base,transHum);
x[0]= d2;
NumericVector l_m22 = transhumance(t,x,l_m22Base,transHum);

// wet and dry years
int year_i= t[0]/360;
long double dry =(({dryWet && dry2[year_i] < c0)? mmm: 1.0});
x[0] = ds, x[1]=nPeak;
NumericVector x0=NumericVector::create(d1,max_rate);
th = dry * (seasonHatch==1? test0: rep_N(1.0,t.size()));
// seasonal and El Nino hatching of Ae. mcintoshi eggs
// annual flooding in march, el nino in december
NumericVector flooded(t.size());
NumericVector elNinoed(t.size());
for(int i=0;i<19;i++){
  flooded[i]=(flood && fmod(t[i],360.0)>=(d3-0.4) && fmod(t[i],360.0)<=(d4+0.5)?
  1.0 : 0.0);
  elNinoed[i]=(elNino && fmod(t[i],3600.0)>=(d5-0.4) && fmod(t[i],3600.0)<=
  (d6+0.5)? 1.0 : 0.0);
}

NumericVector p1=NumericVector::create(e_ah,v_a,e_bh,v_b,e_ch,v_c,e_dh,v_d,
h_h1,e_am,e_bm,e_cm,e_dm,shear,h_m,0_alt,p_pa,p_ma,p_hb,p_mb);
// 19 indice 20 elements
NumericVector p2=NumericVector::create(p_hc,p.mc,p.hd,p.md,max_rate,p.ah,
p_bh,p.ch,p.dh,p.mh00,f.mh1,f.am,p.b,a,p.cm,p.dm,0.0);
NumericVector x = NumericVector::create(AS1, AI1, BS1, BI1, CS1, CI1, DS1, DI1,
MI1, NM1, HI1, NH1);

// biting rates, mortality rates, infection rates floodplain
NumericVector X = Rates_Updates(X, p1, p2);
long double o_a1=X[0],o_b1=X[1],o_c1=X[2],o_d1=X[3],o_a2=X[4],
o_b2=X[5],o_c2=X[6],o_d2=X[7],b_a1=X[8],b_b1=X[9],b_c1=X[10],
b_d1=X[11],b_h1=X[12],b_m1=X[13];
// Updates h_h1<- h_h2 and f_mh1 <- f_mh2
p[8]=h_h2; p[10] = f_mh2;
X = NumericVector::create(AS2, AI2, BS2, BI2, CS2, CI2, DS2, DI2, MI2, NM2,
HI2, NH2);

// biting rates, mortality rates, infection rates floodplain
NumericVector X = Rates_Updates(X, p1, p2);
long double o_a2=X[0],o_b2=X[1],o_c2=X[2],o_d2=X[3],o_a2=X[4],
o_b2=X[5],o_c2=X[6],o_d2=X[7],b_a2=X[8],b_b2=X[9],b_c2=X[10],
b_d2=X[11],b_h2=X[12],b_m2=X[13];
// Updates h_h2<- h_h3 and f_mh2 <- f_mh3
X = NumericVector::create(AS3, AI3, BS3, BI3, CS3, CI3, DS3, DI3, MI3, NM3,
HI3, NH3);

// biting rates, mortality rates, infection rates floodplain
NumericVector X = Rates_Updates(X, p1, p2);
long double o_a3=X[0],o_b3=X[1],o_c3=X[2],o_d3=X[3],o_a3=X[4],
o_b3=X[5],o_c3=X[6],o_d3=X[7],b_a3=X[8],b_b3=X[9],b_c3=X[10],
b_d3=X[11],b_h3=X[12],b_m3=X[13];
// differential equations

------------- human

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long double dHS1 = g_h*NH1 + l_h21*HS2 + l_h31*HS3 + r_h*HR1 - (m_h + b_h1 + l_h12 + l_h13)*HS1;
long double dHE1 = b_h1*HS1 + l_h21*HE2 + l_h31*HE3 - (m_h + x_h + l_h12 + l_h13)*HE1;
long double dHI1 = x_h*HE1 + l_h21*HI2 + l_h31*HI3 - (m_h + d_h + a_h + l_h12 + l_h13)*HI1;
long double dHR1 = a_h*HI1 + l_h21*HR2 + l_h31*HR3 - (m_h + r_h + l_h12 + l_h13)*HR1;

long double dHS2 = g_h*NH2 + l_h12*HS1 + l_h32*HS3 + r_h*HR2 - (m_h + b_h2 + l_h21 + l_h23)*HS2;
long double dHE2 = b_h2*HS2 + l_h12*HE1 + l_h32*HE3 - (m_h + x_h + l_h21 + l_h23)*HE2;
long double dHI2 = x_h*HE2 + l_h12*HI1 + l_h32*HI3 - (m_h + d_h + a_h + l_h21 + l_h23)*HI2;
long double dHR2 = a_h*HI2 + l_h12*HR1 + l_h32*HR3 - (m_h + r_h + l_h21 + l_h23)*HR2;

long double dHS3 = g_h*NH3 + l_h13*HS1 + l_h23*HS2 + r_h*HR3 - (m_h + b_h3 + l_h31 + l_h32)*HS3;
long double dHE3 = b_h3*HS3 + l_h13*HE1 + l_h23*HE2 - (m_h + x_h + l_h31 + l_h32)*HE3;
long double dHI3 = x_h*HE3 + l_h13*HI1 + l_h23*HI2 - (m_h + d_h + a_h + l_h31 + l_h32)*HI3;
long double dHR3 = a_h*HI3 + l_h13*HR1 + l_h23*HR2 - (m_h + r_h + l_h31 + l_h32)*HR3;

NumericVector dH = NumericVector::create(dHS1, dHE1, dHI1, dHR1, dHS2, dHE2, dHI2, dHR2, dHS3, dHE3, dHI3, dHR3);

NumericVector dM =  combine(List::create(dMS1, dME1, dMI1, dMR1, dMS2, dME2, dMI2, dMR2, dMS3, dME3, dMI3, dMR3);

long double temp = 1.0 - NA1/k_a1;

NumericVector dAQ1 = o_a1*g_a*Sup(0.0, temp)*z_a*AI1 - (m_aq1 + ts*t_a1) * AQ1;
NumericVector dAP1 = g_a*Sup(0.0, temp)*(o_a1*(1.0-z_a)*AI1+(o_a1+o_a1_2)*AS1) - (m_ap1 + ts*t_a1) * AP1;
NumericVector dAS1 = ts*t_a1*AP1 + l_a21*AS2 + l_a31*AS3 - (m_a + o_a1+b_a1 + l_a12 + l_a13)*AS1;
NumericVector dAI1 = ts*t_a1*AQ1 + o_a1*b_a1*AS1 + l_a21*AI1 + l_a31*AI3 - (m_a + o_a1+b_a1 + l_a12 + l_a13)*AI1;

temp = 1.0 - NA2/k_a2;

NumericVector dAQ2 = o_a2*g_a*Sup(0.0, temp)*z_a*AI2 - (m_aq2 + ts*t_a2) * AQ2;
NumericVector dAP2 = g_a*Sup(0.0, temp)*(o_a2*(1.0-z_a)*AI2+(o_a2+o_a2_2)*AS2) - (m_ap2 + ts*t_a2) * AP2;
NumericVector dAS2 = ts*t_a2*AP2 + l_a22*AS3 + l_a32*AS4 - (m_a + o_a2+b_a2 + l_a12 + l_a13)*AS2;
NumericVector dAI2 = ts*t_a2*AQ2 + o_a2*b_a2*AS2 + l_a22*AI2 + l_a32*AI4 - (m_a + o_a2+b_a2 + l_a12 + l_a13)*AI2;
\[
\begin{align*}
(m_{aq2} + ts* t_a) & \cdot AQ2; \\
\text{NumericVector } dAP2 &= g_a*Sup(0.0, temp)*(o_{a2}*(1.0-z_a)*AI2 + (o_{a2} + o_{a2_2})*AS2) - \\
(m_{ap2} + ts* t_a) & \cdot AP2; \\
\text{NumericVector } dAS2 &= ts*t_a*AP2 + l_{a21} * AS1 + l_{a23} * AS3 - \\
\text{NumericVector } dAI2 &= ts*t_a*AQ2 + o_{a2} * b_{a2} * AS2 + l_{a12} * AI1 + l_{a32} * AI3 - \\
\text{temp} &= 1.0 - NA3/k_a3; \\
\text{NumericVector } dAQ3 &= o_{a3} * g_a * Sup(0.0, temp) * z_a * AI3 - \\
(m_{aq3} + ts* t_a) & \cdot AQ3; \\
\text{NumericVector } dAP3 &= g_a * Sup(0.0, temp) *(o_{a3}*(1-z_a)*AI3 + (o_{a3} + o_{a3_2})*AS3) - \\
(m_{ap3} + ts* t_a) & \cdot AP3; \\
\text{NumericVector } dAS3 &= ts*t_a*AP3 + l_{a13} * AS1 + l_{a32} * AS3 - \\
\text{NumericVector } dAI3 &= ts*t_a*AQ3 + o_{a3} * b_{a3} * AS3 + l_{a13} * AI1 + l_{a23} * AI2 - \\
(m_{a} + l_{a31} + l_{a32}) & \cdot AI3; \\
\text{NumericVector } xtemp &= ts*t_a1*AP1; \\
\text{NumericVector } xtemp &= ts*t_a1*AP1; \\
\text{NumericVector } dA &= \text{combine(List::create(dAQ1, dAP1, dAS1, dAI1, dAQ2, dAP2, dAS2, dAI2, dAQ3, dAP3, dAS3, dAI3))}; \\
\text{//---------------- vector B} \\
\text{NumericVector } dBQ1 &= o_{b1} * g_b * Sup(0.0, 1.0- NB1/k_b1) * z_b * BI1 - \\
(m_{bq1} + ts* t_b) & \cdot BQ1; \\
\text{NumericVector } dBP1 &= g_b * Sup(0.0, 1.0- NB1/k_b1) *(o_{b1}*(1.0-z_b)*BI1 + (o_{b1} + o_{b1_2})*BS1) - \\
(m_{bp1} + ts* t_b) & \cdot BP1; \\
\text{NumericVector } dBS1 &= ts*t_b*BP1 + l_{b21} * BS2 + l_{b31} * BS3 - \\
(m_{b} + o_{b1} * b_{b1} + l_{b12} + l_{b13}) & \cdot BS1; \\
\text{NumericVector } dB1I &= ts*t_b*BQ1 + o_{b1} * b_{b1} * BS1 + l_{b21} * BI2 + l_{b31} * BI3 - \\
(m_{b} + l_{b12} + l_{b13}) & \cdot BI1; \\
\text{NumericVector } dBQ2 &= o_{b2} * g_b * Sup(0.0, 1.0- NB2/k_b2) * z_b * BI2 - \\
(m_{bq2} + ts* t_b) & \cdot BQ2; \\
\text{NumericVector } dBP2 &= g_b * Sup(0.0, 1.0- NB2/k_b2) *(o_{b2}*(1.0-z_b)*BI2 + (o_{b2} + o_{b2_2})*BS2) - \\
(m_{bp2} + ts* t_b) & \cdot BP2; \\
\text{NumericVector } dBS2 &= ts*t_b*BP2 + l_{b21} * BS1 + l_{b32} * BS3 - \\
(m_{b} + o_{b2} * b_{b2} + l_{b21} + l_{b23}) & \cdot BS2; \\
\text{NumericVector } dB2I &= ts*t_b*BQ2 + o_{b2} * b_{b2} * BS1 + l_{b12} * BI1 + l_{b32} * BI3 - \\
(m_{b} + l_{b21} + l_{b23}) & \cdot BI2; \\
\text{NumericVector } dBQ3 &= o_{b3} * g_b * Sup(0.0, 1.0- NB3/k_b3) * z_b * BI3 - \\
(m_{bq3} + ts* t_b) & \cdot BQ3; \\
\text{NumericVector } dBP3 &= g_b * Sup(0.0, 1.0- NB3/k_b3) *(o_{b3}*(1.0-z_b)*BI3 + (o_{b3} + o_{b3_2})*BS3) - \\
(m_{bp3} + ts* t_b) & \cdot BP3; \\
\text{NumericVector } dBS3 &= ts*t_b*BP3 + l_{b13} * BS1 + l_{b23} * BS2 - \\
(m_{b} + o_{b3} * b_{b3} + l_{b31} + l_{b32}) & \cdot BS3; \\
\text{NumericVector } dB3I &= ts*t_b*BQ3 + o_{b3} * b_{b3} * BS3 + l_{b13} * BI1 + l_{b23} * BI2 - \\
(m_{b} + l_{b31} + l_{b32}) & \cdot BI3; \\
\text{NumericVector } dB &= \text{combine(List::create(dBQ1, dBP1, dBS1, dBI1, dBQ2, dBP2, dBS2, dBI2, dBQ3, dBP3, dBS3, dBI3))}; \\
\text{//---------------- vector C} \\
\text{NumericVector } dCP1 &= g_c * Sup(0.0, 1.0- NC1/k_c1) *(o_{c1} * CI1 + (o_{c1} + o_{c1_2}) * CS1) - \\
(m_{cp1} + ts* t_c) & \cdot CP1; \\
\text{NumericVector } dCS1 &= ts*t_c*CP1 + l_{c21} * CS2 + l_{c31} * CS3 - \\
(m_{c} + o_{c1} * b_{c1} + l_{c12} + l_{c13}) & \cdot CS1; \\
\text{long double } dCI1 &= o_{c1} * b_{c1} * CI1 + l_{c21} * CI2 + l_{c31} * CI3 - \\
(m_{c} + l_{c12} + l_{c13}) & \cdot CI1; \\
\text{NumericVector } dCP2 &= g_c * Sup(0.0, 1.0- NC2/k_c2) *(o_{c2} * CI2 + (o_{c2} + o_{c2_2}) * CS2) - \\
(m_{cp2} + ts* t_c) & \cdot CP2; \\
\text{NumericVector } dCS2 &= ts*t_c*CP2 + l_{c12} * CS1 + l_{c32} * CS3 - \\
(m_{c} + o_{c2} * b_{c2} + l_{c12} + l_{c23}) & \cdot CS2; \\
\text{long double } dCI2 &= o_{c2} * b_{c2} * CI2 + l_{c12} * CI1 + l_{c32} * CI3 - \\
(m_{c} + l_{c12} + l_{c23}) & \cdot CI2; \\
\text{NumericVector } dCP3 &= g_c * Sup(0.0, 1.0- NC3/k_c3) *(o_{c3} * CI3 + (o_{c3} + o_{c3_2}) * CS3) - \\
(m_{cp3} + ts* t_c) & \cdot CP3; \\
\text{NumericVector } dCS3 &= ts*t_c*CP3 + l_{c13} * CS1 + l_{c23} * CS2 - \\
(m_{c} + o_{c3} * b_{c3} + l_{c13} + l_{c32}) & \cdot CS3; \\
\text{long double } dCI3 &= o_{c3} * b_{c3} * CI3 + l_{c13} * CI1 + l_{c23} * CI2 - \\
(m_{c} + l_{c13} + l_{c32}) & \cdot CI3; \\
\text{NumericVector } dC &= \text{combine(List::create(dCP1, dCS1, dCI1, dCP2, dCS2, dCI2, dCP3, dCS3, dCI3));}
\end{align*}
\]
//------------------ vector D
NumericVector dDP1 = g_d*Sup(0.0,1.0-ND1/k_d1)*(o_d1*DI1+(o_d1+o_d1_2)*DS1) - (m_dp1 + ts*t_d)*DP1;
NumericVector dDS1 = ts*t_d*DP1 + l_d21*DS2 + l_d31*DS3 - (m_d + o_d1*b_d1 + l_d12 + l_d13)*DS1;
long double dDI1 = o_d1*b_d1*DS1 + l_d21*DI2 + l_d31*DI3 - (m_d + l_d12 + l_d13)*DI1;
NumericVector dDP2 = g_d*Sup(0.0,1.0-ND2/k_d2)*(o_d2*DI2+(o_d2+o_d2_2)*DS2) - (m_dp2 + ts*t_d)*DP2;
NumericVector dDS2 = ts*t_d*DP2 + l_d12*DS1 + l_d32*DS3 - (m_d + o_d2*b_d2 + l_d21 + l_d23)*DS2;
long double dDI2 = o_d2*b_d2*DS2 + l_d12*DI1 + l_d32*DI3 - (m_d + l_d21 + l_d23)*DI2;
NumericVector dDP3 = g_d*Sup(0.0,1.0-ND3/k_d3)*(o_d3*DI3+(o_d3+o_d3_2)*DS3) - (m_dp3 + ts*t_d)*DP3;
NumericVector dDS3 = ts*t_d*DP3 + l_d13*DS1 + l_d23*DS2 - (m_d + o_d3*b_d3 + l_d31 + l_d32)*DS3;
long double dDI3 = o_d3*b_d3*DS3 + l_d13*DI1 + l_d23*DI2 - (m_d + l_d31 + l_d32)*DI3;
NumericVector dD = combine(List::create(dDP1,dDS1,dDI1,dDP2,dDS2,dDI2,dDP3,dDS3,dDI3));
List ret=List::create(dH,dM,dA, dB, dC,dD);
NumericVector xw = combine(ret);
return  List::create(xw);