ECOLOGICAL FACTORS ASSOCIATED WITH RIFT VALLEY FEVER DURING INTER-EPIDEMIC PERIOD IN TANZANIA

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EXTENDED ABSTRACT

Rift Valley Fever (RVF) is climate-related arboviral disease of livestock and humans. Rift Valley Fever epidemics are associated with dynamics of mosquito abundance. Studies during inter-epidemic periods (IEP) where there is very little or no virus activity pose challenges including where and how to effectively sample vectors and when should the next outbreaks are expected. Entomological surveys were conducted on abundance and distribution of potential RVF vectors in Ngorongoro district of northern Tanzania. Mosquito sampling techniques and timing were also compared to effectively trap vectors. Mosquitoes were sampled both outdoor and indoor using the CDC light traps and Mosquito Magnets. Outdoor traps were placed in proximity with breeding sites and under canopy in banana plantations in proximity to animals sleeping areas. After every three hours, inspection was done on each trap to recover any trapped mosquito. Traps were set repeatedly in each area for three consecutive days and nights during the study period. All mosquitoes collected were sorted according to site of collection, type of trap and time of collection. Mosquito species were identified morphologically using specific keys. After morphological identification, mosquitoes were kept on ice during transportation to laboratory. Data from this study was used in ecological niche modelling experiment using maximum entropy (MaxEnt) to predict distributions of vectors (*Aedes aegypti* and *Culex pipiens* complex) in relation to disease epidemics for the current and future climate scenarios. A simulation model for mosquito vector population dynamics was developed based on time-varying distributed delays (TVDD) and multi-way functional response equations implemented in C++ programming language. These equations were implemented to simulate mosquito vectors and hosts developmental stages and also to establish interactions between stages and phases of mosquito vectors in relation to host for infection introduction in compartmental phases. An open-source modelling platforms,

Universal Simulator and Qt integrated development environment were used to develop models in C++ programming language. Developed models include source codes for mosquito fecundity, host fecundity, water level, mosquito infection, host infection, interactions, and egg time. Extensible Mark-up Language (XML) files were used as recipes to integrate source codes in Qt creator with Universal Simulator plug-in. A total of 1823 mosquitoes were collected, of which 87.11% were *Culex pipiens* complex, 12.40% *Aedes aegypti* and 0.49% *Anopheles* species. About 36.4% of mosquitoes were collected outdoors using Mosquito Magnets baited with Octenol as an attractant followed by indoor trapping using unbaited CDC light traps (29.60%). Three-hour mosquito collections showed differing patterns in activity, most *Ae. aegypti* species were collected primarily during the first and last quarters of the day. *Cx pipiens* complex was active throughout the night, early evening and early morning then decreased markedly during the daytime. Ecological niche models predicted potential suitable areas with high success rates for both species in the current and future climate scenarios. Model performance was statistically significantly better than random for both species. Most suitable sites for the two vectors were predicted in central and north-western Tanzania with records of previous disease epidemics. Other important predicted risk areas include western Lake Victoria, northern parts of Lake Nyasa, and the Rift Valley region in Kenya. During simulation modelling, floodwater Aedines and Culicine populations fluctuated with temperature and water level over simulation period. Simulated mosquito population showed sudden increase between December 1997 and January 1998, a similar period when RVF outbreak occurred in Ngorongoro district. Results provide insights into mosquito abundances and distribution in the district while emphasizing the possibility of using Mosquito Magnets traps for efficient sampling of day biting mosquitoes. Predicted distributions of vectors provide guidance for selection of sampling areas for RVF vectors during IEP. Simulation model results provide new opportunities for climate-driven RVF epidemic modelling.

DECLARATION

I, CLEMENT NYAMUNURA MWEYA, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my father, Amos Magoti Mweya and my friend, Professor Richard Hill of Michigan State University USA.

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DECLARATION

I, CLEMENT NYAMUNURA MWEYA, do hereby declare to the Senate of Sokoine University of Agriculture, that the listed papers make this thesis summarize my independent work efforts, it is my original work and will not be part of another thesis in the "Published Paper" format in any other University.

LIST OF ABBREVIATIONS AND SYMBOLS

AUC	Area under the Curve
BHK	Baby Hamster Kidney
CCD	Cold Cloud Density
CDC	Centre for Disease Control and Prevention
DLFO	District Livestock Field Officer
DED	District Executive Director
DHF	Dengue Haemorrhagic Fever
DIVA	Differentiation of Infected and Vaccinated Animals
DMO	District Medical Officer
ELISA	Enzyme-linked Immunosorbent Assay
ENMs	Ecological Niche Models
GARP	Genetic Algorithm for Rule-set Prediction
GSST	Graduate School of Science and Technology
HRUTF	Health Research User's Trust Fund
ICIPE	International Centre for Insect Physiology and Ecology
MAXENT	Maximum Entropy Species Distribution
MM	Mosquito Magnet
NDVI	Normalised Differentiated Vegetation Index
NIMR	National Institute for Medical Research
PCR	Polymerase chain reaction
RNA	Ribonucleic Acid
ROC	Receiver Operating Curves
RSSD	Remote Sensing Satellite Imagery
RT-PCR	Real Time Polymerase Chain Reaction
RVF	Rift Valley Fever

RVFV	Rift Valley Fever Virus
SAR	Synthetic Aperture Radar
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TVDD	Time-Varying Distribution Delays equations
XML	Extensible Mark-up Language

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background Information

Rift Valley fever (RVF) is a mosquito-borne disease caused by a single-stranded RNA virus of the family Bunyaviridae within the genus *Phlebovirus* (Daubney *et al.*, 1931). The virus was first described in details by Daubney in sheep in 1930s in the Rift Valley area of Kenya, although it may have occurred earlier (Daubney *et al.*, 1931; Davies, 1975). Traditionally, virus isolation is done through primary inoculation and identification in tissue culture, with baby hamster kidney (BHK), Vero or *Aedes pseudoscutellaris* cell lines is a sensitive, rapid and safe procedure which is recommended for RVF diagnosis. Specific identification of the isolate to distinguish it from other viruses can be made in a virus serum neutralization test by a micro plate or plaque assay (Meegan *et al.*, 1980; Anderson *et al.*, 1989; Paweska *et al.*, 1995). Presence of Rift Valley Fever virus (RVFV) infection elicits antibodies, which are primarily directed against the viral glycoproteins, G_n and G_c, is also accompanied by the production of IgM and IgG antibodies raised against the nucleoprotein, N, and the non-structural protein, NSs. The measurement of anti-N and anti-NSs antibodies can allow a differentiation of infected and vaccinated animals (DIVA) test to be developed. The titration of IgM antibodies is critical for detection of acute infections and can be coupled with the results of RT-PCR and IgG detection assays to accurately stage the time since infection as IgM antibodies do not persist beyond the 50th day in the majority of cases after infection. However, important individual variation in IgM persistence has been demonstrated in cattle where the antibodies may persist for 5 months (Meegan *et al.*, 1980; Anderson, Jr. *et al.*, 1989; Paweska *et al.*, 1995).

1.2 Epidemiology

Rif Valley Fever virus infects both humans and livestock (Bishop and Beaty, 1988). Humans, other primates, cattle, sheep, goats, camels, wild and domestic buffaloes have been shown experimentally to replicate RVFV to a level where infection to feeding mosquitoes would be likely to occur. However, there is considerable variation in the response to infection with RVFV in most animal species. Some breeds and strains are much more susceptible than others. Sheep appear to be more susceptible than cattle or camels (Davies *et al.*, 1992; Adam and Karsany, 2008). Age has also been shown to be a significant factor in the animal's susceptibility to the severe form of the disease: over 90% of lambs infected with RVFV die, whereas mortality among adult sheep can be as low as 10%. Horses, rodents and bats have shown to become infected with RVF in serological investigations (Davies *et al.*, 1992; Adam and Karsany, 2008). Rift Valley Fever virus regularly circulates in endemic areas between wild ruminants and haematophagous mosquitoes. Certain *Aedes* species act as reservoirs for RVFV during inter-epidemic periods and increased precipitation in dry areas leads to an explosive hatching of mosquito eggs; many of which harbour the virus (Gad *et al.*, 1987; Gargan *et al.*, 1988; Jupp and Cornel, 1988). Transmission of the virus to humans occur either via direct contact with the blood of a viraemic animal (e.g. in slaughterhouses, farmers, butchers, ranchers, veterinary surgeons and herdsmen), possibly via the milk of an infected animal (Adam and Karsany, 2008; Anyangu *et al.*, 2010) or via bites of infected mosquitoes, most commonly the *Aedes* mosquito during the primary wave of epidemic. Other vectors are responsible in the secondary and tertiary wave amplification. Transmission of RVFV by hematophagous (blood-feeding) flies is also possible (Jupp and Cornel, 1988). There is some evidence that humans may also become infected with RVF by ingesting the unpasteurized or uncooked milk of infected animals. Major risk factor in human is an association with domestic animals. Farmers, shepherds, veterinarians and abattoir workers

are more at risk than other members of society (Swai and Schoonman, 2009; Youssef, 2009).

The occurrence of sporadic clinical cases in humans and virus isolations from mosquitoes in forest situations have been consistent with the hypothesis that high rainfall is associated with emergence of RVF-infected *Aedes* mosquitoes especially in riverine flood water plains, dambo formations or water pans, whenever flooded for prolonged periods (Gerdes, 2004; Cetre-Sossah and Albina, 2009; Anyamba *et al.*, 2010). In the Sahelian zones of Mauritania and Senegal, some RVF emergence may occur each year if the water pans become flooded (Anyamba *et al.*, 2010). In areas with relatively high rainfall, which have been excised from forest or are adjacent, some RVFV activity may occur every 2-3 years in both human and animal populations. In the drier bushed and wooded grasslands, where most domestic animals are found, the epidemic occur in 5 to 15 year cycles and little or no RVFV activity may be detected between epidemics. In the arid and semi-arid zones, a similar scenario exists, with little or no virus activity between epidemics (Davies *et al.*, 1985; Bicout and Sabatier, 2004; Mondet *et al.*, 2005).

1.3 Pathogenesis

Massive replication of the virus occurs in the nasal and gut epithelia, which are the primary site of infection before reaching the spleen, brain and liver where secondary replication takes place with a resulting viraemia. The lytic effect of the virus causes extensive necrotic hepatitis. The virus is also thought to cause impairment of coagulation resulting in a haemorrhagic syndrome, with widespread haemorrhages seen throughout the body. Abortion in ruminants is an almost invariable outcome mainly as a result of the fatal necrotic hepatitis in the foetus. Foetuses of all ages are affected, so abortion can occur at any stage or pregnancy (Van der Lugt *et al.*, 1996; Popova *et al.*, 2010).

1.4 Clinical Findings

Clinical disease occurs in animals of all ages but is most severe in the young ones. The disease has a very short incubation period in young lambs and kids less than 2 weeks old (Daubney *et al.*, 1931; Davies, 1975). The incubation period is 12 hours and within 36 hours mortality can reach 95% in lambs and 70% in calves. Abortions storms occur in cattle, sheep and goats at any stage of pregnancy. Sheep are particularly susceptible with abortion rates of 40-100% while cattle are somewhat less affected with rates of 15-40% reported (Daubney *et al.*, 1931; Davies, 1975). They show a high fever, listlessness and are unwilling to move. Signs of abdominal pain and rapid respiration may be seen. These animals die within 24-36 hours and are often just found dead without any symptoms being noticed. There is 90% mortality in animals of age less than two weeks (Daubney *et al.*, 1931; Davies, 1975). Animals over two weeks of age and adults show high fever, listlessness, anorexia and weakness. Diarrhoea or melaena may be seen. In humans, there is a severe influenza-like illness that lasts for up to a week. Most people either develop unapparent infections or moderate to severe, non-fatal flu-like symptoms characterised by fever, headache, light-sensitivity, muscle and joint pains. Complete recovery usually occurs within two weeks. A smaller percentage of those affected will develop ocular lesions resulting in blurred vision which may resolve with time, but detachment of the retina may occur in a few cases causing permanent blindness. Less than 1% of people affected develop a severe haemorrhagic syndrome or encephalitis that may be fatal (Joubert *et al.*, 1985).

1.5 Necropsy

Animals dying from RVF will show some degree of liver enlargement, inflammation and necrosis. This may be engorged with many foci of necrosis, 0.5 to 2.0 mm in diameter, bronzed with necrotic and engorged liver tissues that are jaundiced, then mottled yellow-

brown or become completely yellow as jaundice becomes severe. The necrosis of the liver is not restricted to centric- or peri-lobular areas but is a pan-necrosis, which affects all zones in a focal or a wider area (Coetzer, 1977; Abdel-Wahab *et al.*, 1978; Shieh *et al.*, 2010).

1.6 Diagnosis

The disease may be suspected if large numbers of young lambs and goats die, with or without epidemic abortion among the animals and when at the same time multiple human cases with fever and haemorrhagic/neurological symptoms occur. Rift Valley Fever can be diagnosed clinically or using laboratory techniques such as virus neutralization, enzyme-linked immunosorbent assay (ELISA), hemagglutination inhibition, immunofluorescence, complement fixation and immunodiffusion assays. Cross-reactions may occur with other Phleboviruses (Linthicum *et al.*, 1985; Youssef, 2001; Sang *et al.*, 2010). Rapid tests may need to be confirmed by virus isolation. Virus antigens can also be detected by immunofluorescent staining of the liver, spleen or brain. Enzyme immunoassays and immunodiffusion tests can identify virus in the blood (Linthicum *et al.*, 1985; Youssef, 2001).

1.7 Treatment and Control

There is no treatment for the disease in animals or human (Sidwell *et al.*, 1992). Humans contracting RVF can be treated with the antiviral agent ribavirin though may not be effective and there is insufficient data about its use. Bed rest, plenty of fluids and a healthy diet is important but if the symptoms are persisting and the condition worsening, emergency medical treatment may be necessary (Linthicum *et al.*, 1988). Effective control measures for RVF involve sustained vaccination of cattle, sheep and goats especially during wet seasonal cycles. It is usually difficult to control the insects'

breeding sites during epidemics when different vectors are involved. Despite that in epidemics large-scale application of larvicide is required. Larviciding can provide a most promising control of RVF epizootic where *Aedes* mosquitoes breeding sites can be clearly defined with limited area of distribution (Linthicum *et al.*, 1988).

1.8 Aspect of RVF Modelling

Advances in Remote Sensing Satellite Imagery (RSSD) derived from Landsat, SPOT and later Synthetic Aperture Radar (SAR) allowed the use of more sophisticated tools to predict RVF potential epizootic situations over much wider areas. This advanced technology is accompanied by use of pre-epizootic conditions determined by Cold Cloud Density (CCD) and the Normalised Differentiated Vegetation Index (NDVI) (Anyamba *et al.*, 2006; Anyamba *et al.*, 2010), using regular and significant precipitation. However, timely and response to disease outbreak remains a challenge (Metras *et al.*, 2011). Few mathematical models have been developed for RVF epidemics to complement these weather-only-dependent models. In 2007, an epidemiological model of RVF focusing mainly on animals and vectors population dynamics with hypothetical consideration of infection dynamics was developed (Gaff *et al.*, 2007). The role of changing weather conditions such as temperature and rainfall were not directly considered. Further development of the model incorporated the role of vaccination and vector control to describe epidemiology of RVF in areas of intense transmission (Gaff and Schaefer, 2009; Gaff *et al.*, 2011). Many developments in mathematical modelling of RVF epidemics are based on principles previously developed (Gaff *et al.*, 2007) but with fewer modifications (Mpeshe *et al.*, 2011; Niu *et al.*, 2012; Xue *et al.*, 2012). This limits their further applicability in predictive epidemiology due to insufficient incorporation of weather data and on-the-ground biological processes related to RVF disease. In this study a simulation modelling approach that incorporate weather data to simulate on-ground entomological

data on mosquito abundances in relation to their hosts as previously recommended (Jost *et al.*, 2010) was applied.

Modelling potential distributions of RVF vector is useful in providing more understanding about the disease ecology. Distribution of RVF vectors in un-sampled areas is not known in East Africa despite available records of RVF cases. As sampling of mosquitoes vectors can be very expensive, cost effective approach for resource allocation can focus on sampling most important area first. Species distribution modelling has been successful in Agriculture (predicting crop yields etc.), in hydrological studies, in weather prediction etc. Modelling has not been highly implemented in infectious disease prediction for control purposes. Ecological niche models (ENMs) such as Maximum Entropy Species Distribution (MaxEnt) and Genetic Algorithm for Rule-set Prediction (GARP) are new technologies in infectious diseases studies which can supplement and help improve existing surveillance programs by describing, in a spatially explicitly way, suitable habitats for mosquito species (Peterson *et al.*, 2005; Peterson, 2007; Larson *et al.*, 2010). Although ENM has been effectively used for predicting spatial distributions of mosquito species in other locations, there are no such studies in Tanzania. ENM can guide planning of future disease control and prevention interventions like strategic animal vaccination and vector control (Montesinos-Lopez and Hernandez-Suarez, 2007).

1.9 Problem Statement And Justification of the Study

In Tanzania, similar to many parts of Africa where RVF epidemics occur, there is inadequate information on disease outbreaks in relation to ecological factors such as potential vectors, abundance and distribution as well as its linkages with local environmental and climatic patterns. Few studies have been conducted on RVF transmission in relation to entomological and ecological factors during inter-epidemic

period because of several challenges. These challenges include how to select areas for effective sampling of mosquitoes as well as absence or very low virus activity during this period. Detection of RVFV in mosquitoes requires the presence of huge numbers of vectors. This study adds value to understanding the role of misquotes in maintenance and persistence of the disease during inter-epidemics period in relation with ecologically suitable sites. The relationship between abundances of mosquito vectors specifically across habitat and vegetation types experiencing different levels according to 2006/07 epidemic history was examined. This adds value to field-based studies that focused mainly on role of virus antibody within humans, livestock and wildlife populations (Swai and Schoonman, 2009; Mohamed *et al.*, 2010; Lagerqvist *et al.*, 2013; Sindato *et al.*, 2013; Sumaye *et al.*, 2013; Chengula *et al.*, 2014).

This study conducted entomological investigations to fill in the gaps previously noted including non-existence of reliable techniques for sampling RVF vectors. Inadequate sampling of potential RVF vectors may lead to under-reporting the role of mosquito in transmission and persistence of RVFV during inter-epidemics period. This study used entomological information, species distribution, ecological niche models and development of mosquito population dynamics model to assess risk for RVF transmission. Findings from this study provided more information on understanding disease processes in endemic areas towards improvement of national surveillance efforts in potential RVF outbreak geographic areas with large populations of human, domestic animal and wildlife.

1.10 Research Questions

1. What are the potential mosquito vectors of RVF and their distribution and RVFV infectivity in Ngorongoro districts?

2. What are the effective sampling techniques for RVF vectors?

3. How are RVFV mosquito vectors ecologically distributed in un-sampled areas during inter-epidemic periods and their potential for RVF outbreak in Tanzania?

4. Is there a pattern in simulated population dynamics of RVF mosquito vectors associated with disease epidemics?

1.11 Objectives of the Study

1.11.1 Main objective

To determine the ecological factors associated with Rift Valley Fever occurrence in Tanzania.

1.11.2 Specific objectives

i)   To carry out entomological surveys to determine mosquito species abundance, composition and their RVFV infectivity

ii)   To compare sampling techniques for potential mosquito vectors of Rift Valley Fever virus

iii)   To identify potential habitat preference of RVF mosquitoes in relation to disease epidemic hotspots for the current and future climate scenarios

iv)   To develop a simulation model for RVF mosquito vectors population dynamics that use daily temperature and rainfall in relation to disease epidemics.

1.12 Organization of the Thesis

This thesis is organised in three chapters preceded by an extended abstract which summarises the objectives, materials and methods, principal research findings and conclusion of this study. Chapter one consists of introduction, problem statement, justification of the study and study objectives. Chapter two presents the results obtained from each specific objective which are synthesized into either published papers (papers I, II, III and V) or written manuscripts (papers IV) submitted for publication in peer reviewed scientific journals. The format and writing style of the individual papers in according to the targeted peer reviewed journal. Chapter three provided the conclusions and recommendations based on the findings of the study.

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CHAPTER TWO

PAPER ONE

Inter-epidemic abundance and distribution of potential mosquito vectors for Rift Valley fever virus in Ngorongoro district, Tanzania

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PAPER ONE HAS 8 PAGES

PAPER TWO

Comparison of sampling techniques for Rift Valley Fever virus potential vectors, *Aedes aegypti* and *Culex pipiens* complex, in Ngorongoro District in northern Tanzania

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PAPER TWO HAS 8 PAGES

PAPER THREE

Predicting distribution of *Aedes aegypti* and *Culex pipiens* complex, potential vectors of Rift Valley fever virus in relation to disease epidemics in East Africa

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PAPER FOUR

Climate change influences potential distribution of *Culex pipiens* complex co-occurrence with Rift Valley Fever epidemics in Tanzania

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Abstract

Climate change influences the distribution of suitable habitats for mosquito vectors responsible for transmission of disease vectors. Rift Valley Fever (RVF) is a climate-related arboviral infection of animals and humans. *Culex pipiens* complex is the only mosquito vector known to be associated with RVF epidemics in Tanzania. Climate change is thought to represent a threat towards emerging risk areas for RVF epidemics. The influence of future climate projections on variability of habitat suitability and shift for RVF vectors was assessed in relation to disease outbreaks risk area in Tanzania. Ecological niche models (ENM) were used to estimate potential distributions of disease risk areas based on *Culex pipiens* complex presence only data approach. Current and future projections climatic variables were used as model inputs. Changes in mosquito vectors habitat suitability under future climatic scenarios were estimated. Partial receiver operating characteristic (ROC) and the area under the curves (AUC) approach was used to evaluate model predictive performance and significance. Predicted habitat suitability for *Culex pipiens* complex indicated broad-scale potential for change and shift in the distribution of the vectors and disease for both 2020 and 2050 climate scenarios. Predicted risk areas indicate more intensification in the areas surrounding Lake Victoria and north-eastern part of the country through 2050 climate scenario. Findings show higher probability of emerging risk areas spreading towards the western parts of Tanzania from north-eastern areas and decrease in the southern part of the country. Results presented here identified future sites for consideration in order to guide surveillance and control interventions to reduce risk of RVF disease epidemics in Tanzania. A collaborative approach is recommended to develop and adapt climate related disease control and prevention strategies.

Introduction

Climate models project future increase in precipitations and intensification of extreme events such as floods and droughts globally (Naicker, 2011). Climate change projections reports in Tanzania indicate future increases in average annual temperatures from 1ºC to 3ºC above the baseline by the 2050s (Watkiss *et al.*, 2011). Climate change is likely to affect human health from food insecurity and malnutrition to potential increase in malaria (Parham and Michael, 2010; Mboera *et al.*, 2011) and some other climate sensitive vector-borne diseases (Colwell *et al.*, 1998; Patz *et al.*, 2000; Martin *et al.*, 2008; Watkiss *et al.*, 2011; Mpeshe *et al.*, 2014). Rift Valley fever (RVF) is a climate-dependent arboviral infection caused by a virus belonging to the *Phlebovirus* genus of the *Bunyaviridae* family (Daubney *et al.*, 1931). Climate change is expected to have impacts on the distribution of suitable conditions for breeding of mosquito vectors responsible for transmission and maintenance of RVFV (Gould and Higgs, 2009; Tabachnick, 2010). Climate change is thought to represent a threat to emergence of new risk areas for RVF epidemics worldwide (Harvell *et al.*, 2002; Gould *et al.*, 2006; Gale *et al.*, 2010). These changes may play a major to catalyse the emergence of RVF outbreaks risk areas (Tourre *et al.*, 2009).

Rif Valley Fever virus uses mosquitoes and other arthropods including phlebotomine sand flies, *Culicoides* midges, and *Amblyomma* tick species for infection transfer to animals and humans (Bishop and Beaty, 1988). *Cx. pipiens* complex was recorded as main vector during epidemics in different parts of Africa such as Egypt (Meegan *et al.*, 1980) and South Africa (Jupp and Cornel, 1988). *Cx. pipiens* complex has been demonstrated in the laboratory to be capable of transmitting the virus. Laboratory-established colonies from

Tahiti exhibited the highest disseminated infection when infected with two strains of RVFV, the virulent ZH548 and the avirulent Clone 13 (Moutailler *et al.*, 2008; Le *et al.*, 2013). Vector competence tests indicate that *Cx. pipiens* complex is an important mosquito species for RVFV transmission (Turell *et al.*, 2008; Golnar *et al.*, 2014).

The virus persists throughout the Inter-Epidemic Period (IEP) in the eggs of the floodwater *Aedes* mosquitoes for several years in dry conditions. This vertical transmission provides an explanation for the persistence of the virus through the IEP. Changes in conditions determine the emergence of the infected mosquito populations and the amplification of the virus (Linthicum *et al.*, 2007; Pin-Diop *et al.*, 2007; Anyamba *et al.*, 2010; Soti *et al.*, 2012). In Tanzania, no records exit that confirms the primary *Aedes* species that is responsible for maintenance of RVFV, whereas in in north-eastern Kenya, *Aedes mcintoshi* has been confirmed as a primary vector of RVFV after artificial flooding experiments during IEP (Linthicum *et al.*, 1985). For this reason, *Cx. pipiens* complex has been used in this study to guide identification of potential risk areas for RVF epidemics in Tanzania.

Ecological Niche Models (ENMs) provide useful tools to assess risk of emergence and re-emergence of potential RVF epidemic hotspots due to climate change. ENMs use combination of environmental-climatic factors such as temperature, precipitation, elevation and derived-normalized difference vegetation index to predict climate change effects on disease vectors distribution (Sallam *et al.*, 2013; Conley *et al.*, 2014). In Tanzania, few studies have already been done to identify potential risk areas in relation to RVF (Mweya *et al.*, 2013a) using ENMs. There is inadequate information on linkages of

local environmental and future climatic conditions in determination of potential epidemic risk areas. Hence, ENMs such as Maximum Entropy Species Distribution Modelling (MaxEnt) (Phillips *et al.*, 2006) can be used to evaluate potential for emerging disease epidemics areas in response to climate change based on modelled future-climate scenarios for 2020 and 2050. Findings provide information to guide future surveillance efforts in geographic areas with high potential for RVF outbreak.

Methods

Data on mosquito vectors occurrence

Data related to the occurrence of *Cx. pipiens* complex in areas previously recorded RVF epidemic was derived from field entomological study previously conducted in six high risk villages in Ngorongoro district (Mweya *et al.*, 2013b; Mweya *et al.*, 2015). Mosquito sampling was done both outdoor and indoor using CDC light traps baited with CO_2 sachets and Mosquito Magnets (Mosquito Magnet Cordless Liberty Plus) baited with Octenol. Ngorongoro district was purposely selected as the only source of mosquito occurrence data used as input to this modelling experiment due to continuous and confirmed history of RVF outbreaks (Daubney *et al.*, 1931; Mohamed *et al.*, 2010; Sindato *et al.*, 2014). In Tanzania, most severe RVF epidemics were reported in 1997/1998 and 2006/2007 (Clements *et al.*, 2007; Kebede *et al.*, 2010). The 2006/2007 outbreak was the most widespread affecting livestock in eleven regions in Tanzania (Anyangu *et al.*, 2010; Aradaib *et al.*, 2013). A total of 16,973 cattle, 20,193 goats and 12,124 sheep died of the disease, with spontaneous abortions reported in 15,726 cattle, 19,199 goats, and 11,085 sheep (Jost *et al.*, 2010; Dar *et al.*, 2013). Ngorongoro district is part of the Serengeti-Mara Ecosystem, which represents a unique zone of interaction

between livestock, wildlife, and humans with annual animal migration. According to the 2012 Tanzania National population and housing census, the human population at risk of infection in Ngorongoro district was 174,278 (NBS, 2013).
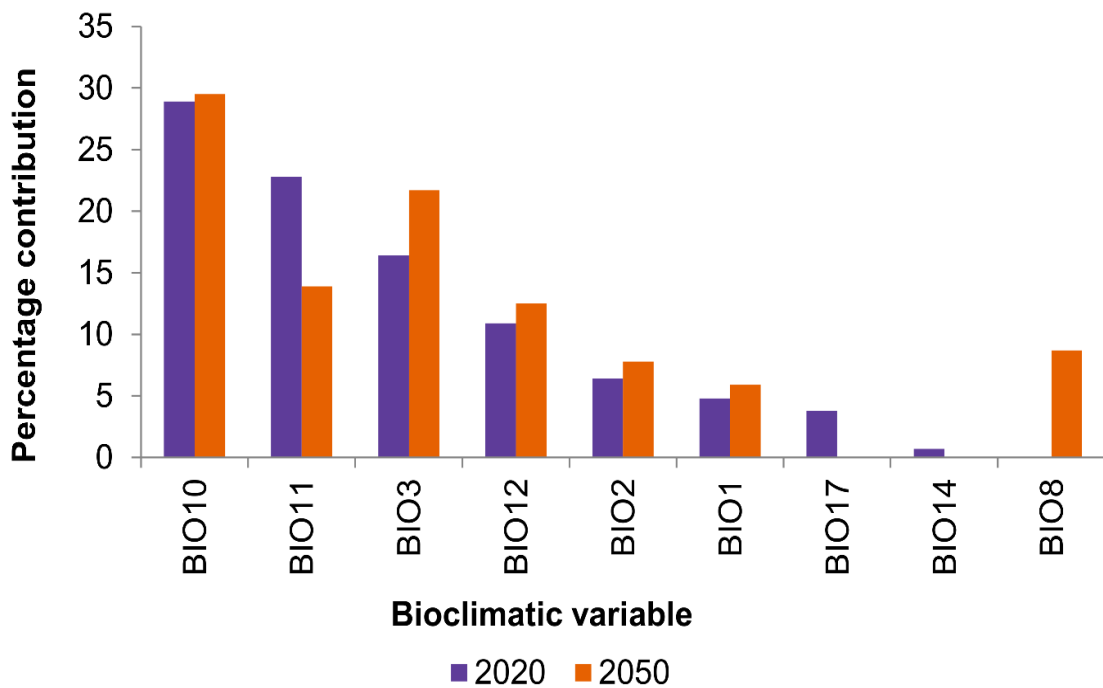
Figure 1: Contribution of bioclimatic variables used in the species ecological niche model.

Key: BIO11=mean temperature of coldest quarter, BIO10=mean temperature of warmest quarter, BIO3=isothermality, BIO12=annual precipitation, BIO2=mean diurnal range, BIO1=annual mean temperature, BIO17=precipitation of driest quarter, BIO14=precipitation of driest month, BIO4=temperature seasonality, BIO8=mean temperature of wettest quarter.

Bioclimatic data

Bioclimatic predictors control the occurrence of mosquito vectors within a habitat. Bioclimatic environmental predictors were used as inputs into the model. Three bioclimatic datasets with a spatial resolution of one square kilometre were downloaded from http://www.worldclim.org; these include the current, 2020 and 2050 scenarios (Hijmans *et al.*, 2005). Nineteen bioclimatic variables were tested, but only 10 were used as predictors for *Cx. pipiens* complex distributions for all climatic scenarios to reduce over-fitting due to many bioclimatic variables during data extrapolation to the whole country. Variables were chosen based on their relevance to mosquito vector distributions after several jack-knifing procedures (Peterson, 2007; Kija *et al.*, 2013; Mweya *et al.*, 2013a). Contributions of each variable to this prediction results were determined by iteration of the algorithm during regularization procedure and by random permutation (Figure 1).

Ecological niche modelling

Based on the assumption that presence of suitable habitats for *Cx. pipiens* complex in areas previously recorded RVF epidemics would contribute significantly to future risk areas, MaxEnt (version 3.3.1) was used to develop ecological niche models for mosquito species in relation to disease. The mosquito occurrence dataset was split in the ratio of 3:1 for the training and testing respectively. Default setting for MaxEnt was used except that the specified random seed of 50% points set aside for model evaluation and the regularization multiplier factor to reduce over-fitting due to many bioclimatic variables used. Modifying the regularization multiplier helped to generate risk maps that can be extrapolated to a larger countrywide scale. Because presence-only originated from

Ngorongoro district where RVF has previously been reported, the regularization multiplier was adjusted such that predicted models results could be extrapolated to the whole country to identify other high-risk un-sampled area as to the main purpose of ENMs. Minimum training threshold was used to convert raw model outputs into actual distributional estimates. The predicted distribution of risk areas is assessed by estimating the probability at maximum entropy based on assumption of uniform probability (Peterson, 2003; Phillips *et al.*, 2006). Predicted areas were identified as risky due to probability estimates on potential occurrence of *Cx. pipiens* complex in the area.

Emerging epidemic risk areas

Generated data from different climate scenarios for *Cx. pipiens* complex were processed to produce difference between present and future raster files. In order to show where areas become newly suitable and areas where conditions stop being suitable, raster map calculator tool in QGIS version 2.6 was used to subtract one from the other scenario. Raster map calculator allows performing manipulations, analysis and maps generation on basis of existing raster pixel values written to a new raster layer with a supported format.

Model performance evaluation

Partial ROC/AUC software was used to evaluate model predictive performance (Peterson *et al.*, 2008). Choice for this approach to model evaluation was based on no absence data to characterize commission errors. Many previous models used ROC approaches which require both absence and presence data (Phillips *et al.*, 2006), and which present numerous other problems (Lobo *et al.*, 2008). In light of lack of absence data, partial ROC/AUC provides the best alternative approach to jack-knifing which also does not

require absence data but when small sample of presence data is used (Pearson *et al.*, 2007). AUC ratios were determined using proportion area predicted present in the x-axis and sensitivity (commission error) in the y-axis. AUC ratios were calculated AUC from observed data and AUC from random prediction. Testing of model performance was done by bootstrapping methods that involved plotting the AUC ratios replicates that would indicate a normal distribution for significance difference from random prediction.
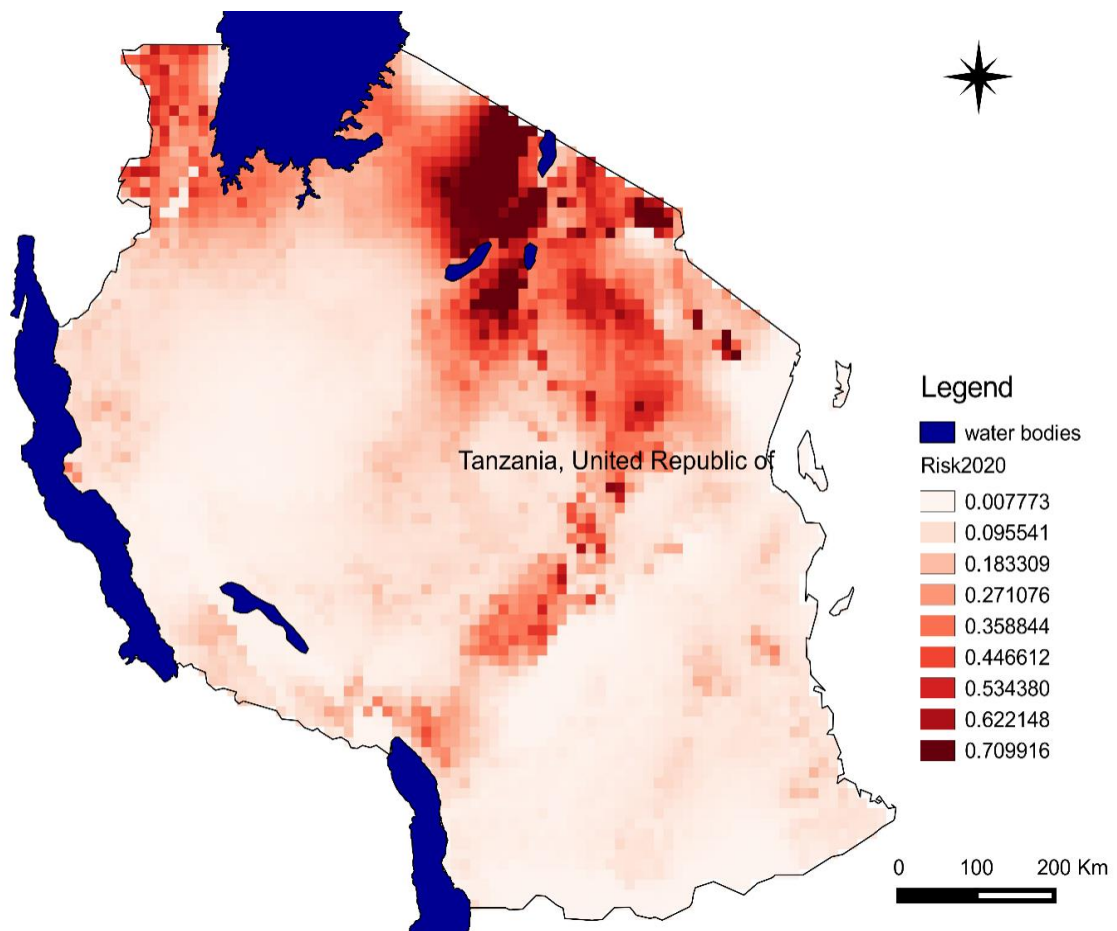
Results

Bioclimatic variable importance

Jack-knife approach to determine variables of importance and their percentage contributions show that mean temperature of warmest quarter (BIO-10), mean temperature of coldest quarter (BIO-11), isothermality (BIO-3) and annual precipitation (BIO-12) were the most important for all climate scenarios (Figure 1). Relative contributions of each variable to prediction results by iteration of the algorithm during regularization and by random permutation in the jack-knifing procedure showed that the BIO-14 and BIO-7, contributed more to the model output for the current scenario whereas BIO-18 and BIO-14 had higher permutation significance for the current scenario. For 2020 climate scenario, BIO-14 and BIO-2 contributed more whereas BIO-14 and BIO-18 had higher percentage on permutation importance. For 2050 climate scenario, BIO-14 and BIO-2 contributed more whereas permutation importance was only contributed by BIO-14, BIO-19 and BIO-15 only. BIO-7, BIO-10 and BIO-4 did not indicate any contribution to model output in 2050 climate scenario. Of the nineteen bioclimatic variables tested, only nine generally contributed importantly to the best model and were used as predictors for *Ae. aegypti* distributions for all climatic scenarios (Figure 1).

Model performance

Partial ROC/AUC programme show that model predictive performance was statistically significantly better than random prediction for both species ($p < 0.05$) in all climate scenarios. One thousand subsets of points were generated to specify the number of iterations during partial ROC-AUC ratios calculations. The program generated the partial ROC/AUC ratios ranging from 1.003 to 1.256 at the given 1- omission threshold of 0.95

using accepted omission error of 5% to the AUC at 50% for random prediction to specify the percentage of testing points which included in each of the random subsets. The predicted risk distribution maps show that the probability of the presence of *Cx. pipiens* complex in relation to disease appears to be high to medium across large areas of the country.

Figure 2: Predicted potential distributions of disease outbreak risk areas based on distribution of *Cx. pipiens* complex in Tanzania for 2020 climate scenario.

Predicted habitat suitability for *Cx. pipiens* complex

The results show that the habitat suitability for *Cx. pipiens* complex for 2020 scenario indicated to be apparently spreading. Anticipated high risk areas are distributed towards the southern parts of Lake Victoria and north-eastern parts of Tanzania indicating more

intensification of risks in Tanga regions as well as some areas sounding Lake Tanganyika and Lake Nyasa (Figure 2). During this 2020 years climate scenario, risks seem to disappear in western part of Lake Victoria and central parts of Tanzania (Figure 2). For 2050 climate scenario, predicted habitat suitability for *Cx. pipiens* complex indicated slightly similar potential for change as for 2020 but increased intensification and slight shift in the risk distribution towards the western areas of Lake Victoria and with higher risk in the central and north-eastern parts (Figure 3). This scenario indicated areas around Ngorongoro district and Arusha region will continue to remain at relatively high risk through 2050 (Figure 3) with less emerging new risks (Figure 5). Predicted results of this modelling approach show relatively high association with areas previously recorded with RVF outbreaks for both humans and animals.
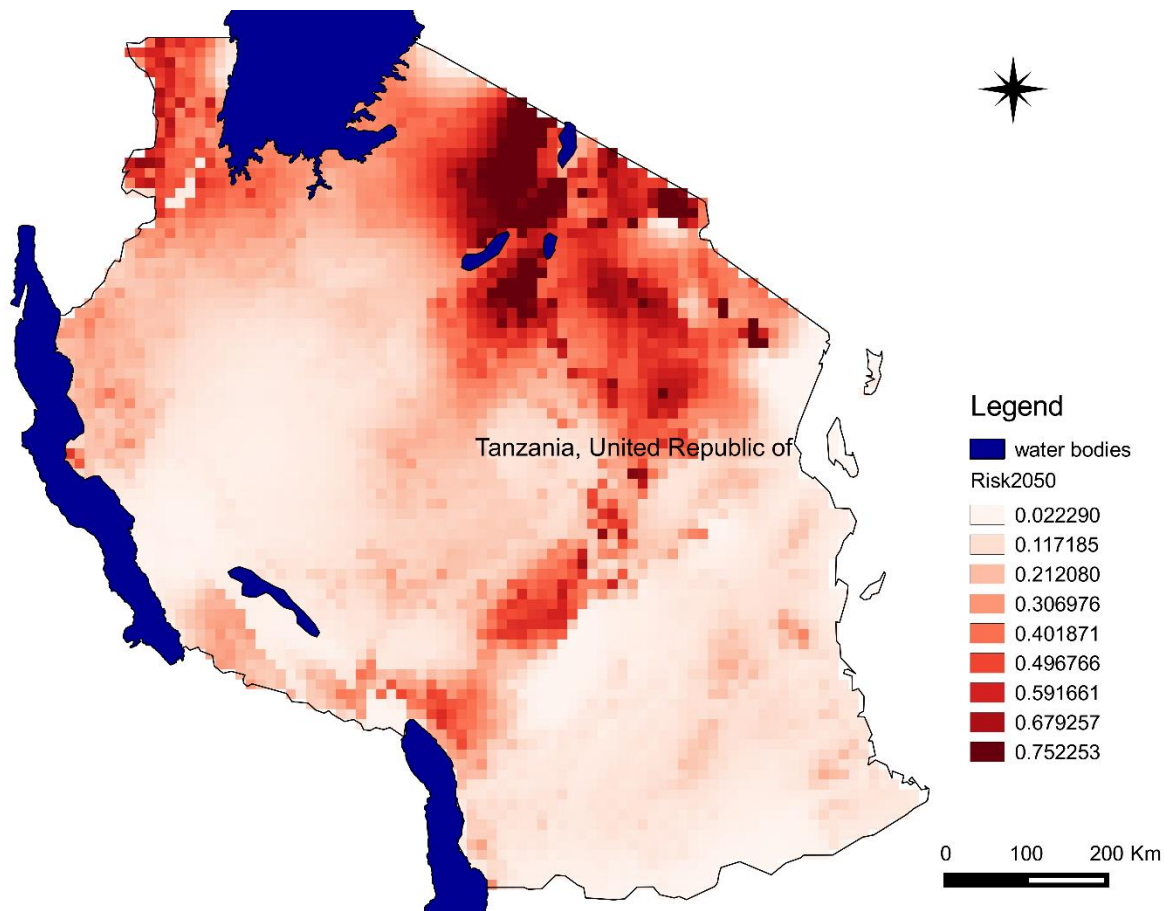
Figure 3: Predicted potential distributions of disease outbreak risk areas based on distribution of *Cx. pipiens* complex in Tanzania for 2050 climate scenario.

Emerging habitat suitability for *Cx. pipiens* complex

Predicted emerging habitat suitability for *Cx. pipiens* complex and disease risk indicated broad-scale potential for change and shift in the distribution of the vectors and disease for both 2020 and 2050 projections (Figure 3 and 4). Findings show anticipated emerging risk areas southern parts of Lake Victoria spreading to eastern parts of Lake Tanganyika while leaving higher emerging risks in many parts of Tanga region. Some possible expansion may be expected in parts of Rukwa and Mbeya regions also indicated potential risk areas. Predicted suitability probability of emerging hotspots for 2050 indicated risk intensification in nearly all parts of the country (Figure 3 and 4). The anticipated risk

seems to disappear only in a few areas in the western parts of Lake Victoria and the whole of southern parts of Tanzania. The phenomenon of a shifting suitability could be visualized from the increased red and green colouration which seems to cluster around the high and least risk areas respectively (Figure 4 and 5).
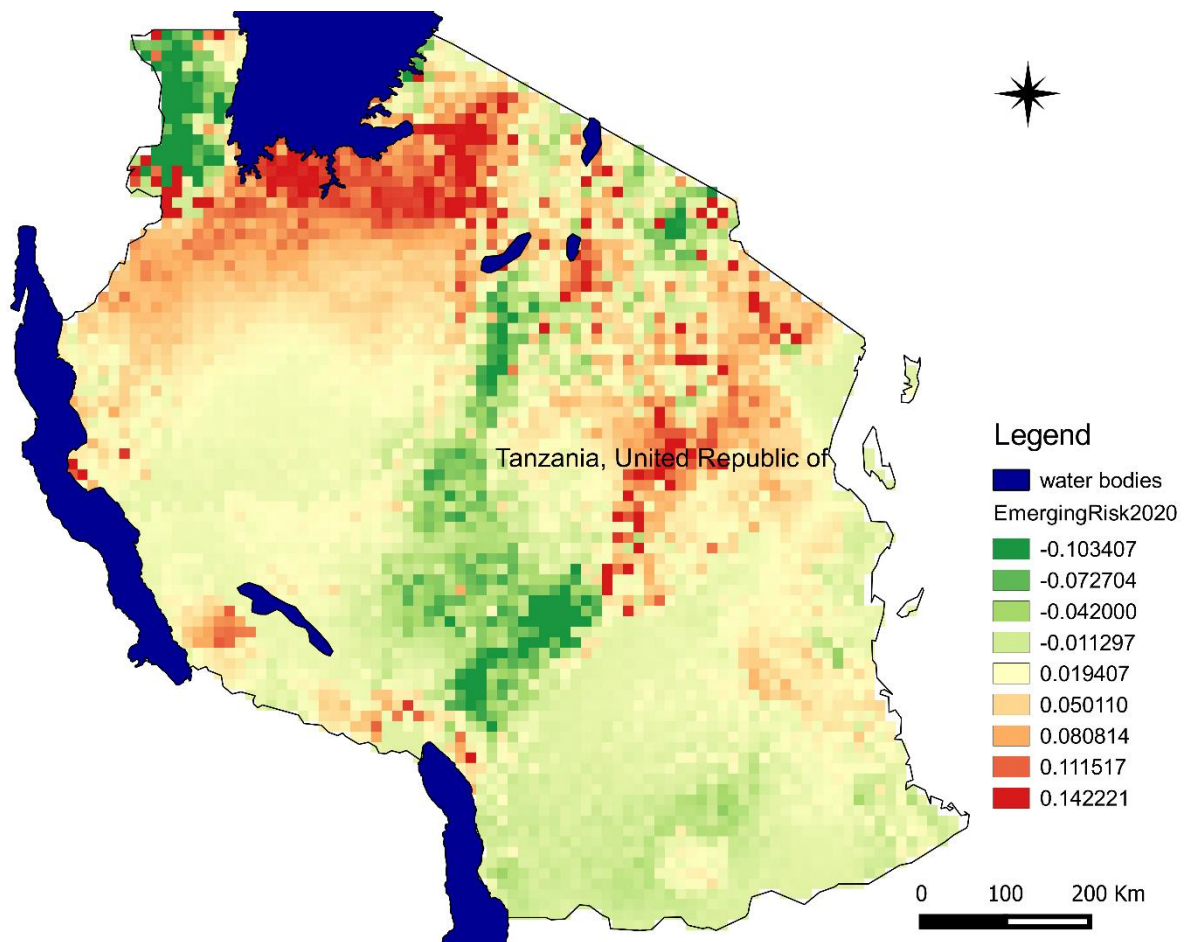
Figure 4: Emerging and disappearing predicted potential suitable distribution areas for *Cx. pipiens* complex in Tanzania for 2020 climate scenario.

Discussion

This study presents the unique application of niche-based climate models for prediction of suitable areas for RVF vectors in Tanzania at different scenarios. Findings reflects the distribution of RVF outbreak history based on habitat suitability for *Cx. pipiens* complex which suggests the continued persistence of RVF outbreak episodes due to changing climate condition. The anticipated potential for distribution risk shift to the eastern parts of Lake Tanganyika and parts of Tanga region for *Cx. pipiens* complex agrees with the actual distribution as previously predicted in the current scenario indicating distribution range limits similar to disease outbreak records (Mweya *et al.*, 2013a). Model projections onto 2050 conditions predict increases in suitable areas in the north-east of the country. Future climate models scenarios show spread of disease risk from previously dry zones to towards wetter zones in the country. This shift in direction to the north-eastern has also been observed to occur in other vector-borne diseases in temperate regions (Asin and Catala, 1995; Wu *et al.*, 2013).
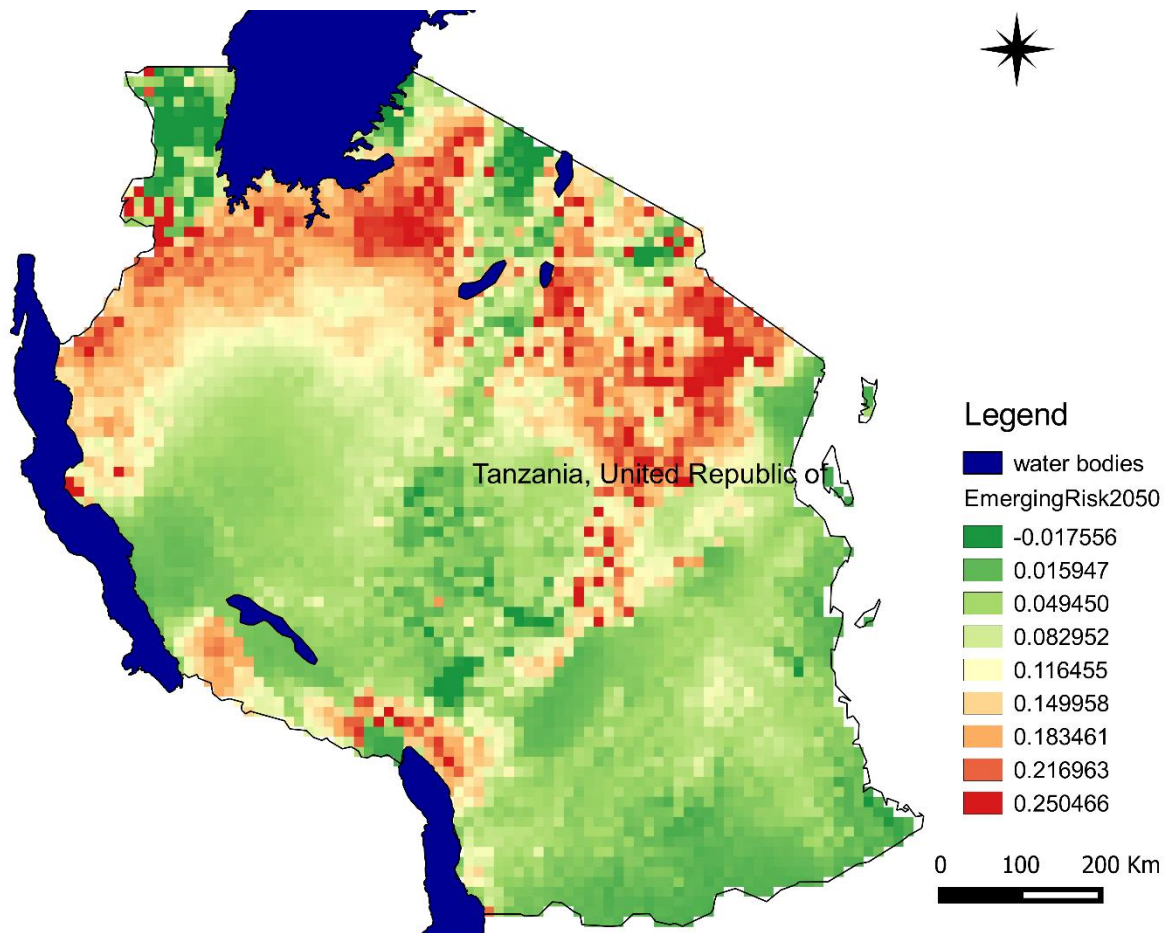
Figure 5: Emerging and disappearing predicted potential suitable distribution areas for *Cx. pipiens* complex in Tanzania for 2050 climate scenario.

This model indicate higher habitat suitability for *Cx. pipiens* surrounding the Great Rift Valley regions, which suggests that climate change could have a significant influence on emergence of disease outbreak risk in areas. Climate change is likely to cause a shift in the habitat suitability for this vector from lower altitudes to higher altitudes in the future. This shift may in turn force the vector to migrate towards previously lower disease risk at higher altitudes making these areas as potential high disease risk areas in the future. It is assumed that the concentration of CO_2 in the atmosphere will double by 2050 leading to an average of 1.5 degrees Celsius increase in temperature. This leads to the general order of the habitat suitability maintenance and a much greater shift as evident from the less and

least suitable area as shown in Figures 4 and 5. Also, the peripheral less suitable areas at much lower altitudes increasingly become least suitable. For instance, the less suitable areas at the southern part of Tanzania in Figures 4 and 5 becomes least suitable under the 2050 climate scenario.

Findings show that the apparent shift in disease outbreak risk areas is due to the likely changes in precipitation levels and increases in temperatures with the changing climate as the global climate gets warmer. In other words, there is an apparent ascend in emerging risk areas from lower altitudes to higher altitudes with respect to climatic conditions of the current and future scenarios respectively. The reason for this observed trend is that the suitability is strongly influenced by altitude that determines the micro-climatic conditions at any given location. The climatic conditions of temperature and precipitation which currently prevail in the suitable habitat range under the current scenario will likely be attainable only at much higher altitudes in the future. Since climate affects both the physiology of the disease vectors and availability of resources; in the future, species currently occupying the suitable habitat are likely to migrate towards favourable areas in order to encounter favourable climatic conditions that will be similar to those prevailing in the suitable habitat range under the current scenario.

Model projections suggest that *Cx. pipiens* complex could find suitable conditions in the areas surrounding Lakes Victoria, Tanganyika and Nyasa. Therefore, the areas mentioned should be monitored for potential outbreaks. RVF outbreaks in areas surrounding Lake Tanganyika in Kigoma region has recently been reported (Kifaro *et al.*, 2014). This could be a consequence of changes in macro and micro-climatic extremes that can directly

impact distribution of RVF vectors. Increases in temperature can contribute to disease incidence by reducing pathogen incubation period, expediting vector generation time, larval survival rate, and overall population growth rate (Patz *et al.*, 2000). Also changes in seasonal precipitation regimes impact vectors life cycles by changing microclimate that provide stable and seasonal humidity at egg-laying, hatchability of infectious eggs and larval development sites (Harvell *et al.*, 2002). In addition to potential climate change, other factors also present potential increase for future disease risk areas such as ecological imbalanced state as a result of habitat fragmentation, urbanization, land-use changes, and human-imposed species disequilibria, making some other areas especially susceptible to the uncertain effects of global change.

In this study, the predicted results under the future climate scenarios likely inherited certain level of uncertainty from the modelled climate dataset that was used since the climatic conditions for the future are themselves predictions from the models. Thus, the performance and accuracy assessments shown cannot be prodigious. The results showed that besides the other environmental variables used in the model, the altitude plays a pivotal role on the habitat suitability for *Cx. pipiens* complex. Also, there is an apparent shift in the suitability from lower altitudes to higher altitudes with the changing climate from current through the 2020 and 2050 scenarios of the future modelled climate. Local human activities are concentrated in the habitat range currently suitable for *Cx. pipiens* complex. This overlap puts the disease vectors future survival in to higher environmental suitability since many human activities creates favourable environmental conditions.

Conclusion

The predicted distribution presented here shows that *Cx. pipiens* complex, the potential vectors for transmission of RVF can widely be a cause of concern among disease ecologists, epidemiologists and vector control professionals in most parts of the country. Climate change conditions show a shift in habitat suitability for vectors while maintaining suitability in areas currently observed as high-risk zones. Under these conditions, it will be necessary to continue studies on the distribution of vectors in the southern part of the country in order to enhance understanding of the risk for RVF transmission. A collaborative approach is recommended to develop and adapt control and prevention strategies that will help manage the risk, and reduce the burden of RVF in animals and human populations living within predicted high-risk zones.

Acknowledgments

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PAPER FIVE

Simulation modelling of population dynamics of mosquito vectors for Rift Valley fever virus in a disease epidemic setting

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PAPER FIVE HAS 11 PAGES

CHAPTER THREE

3.0 CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions

Findings of the studies described in this thesis provide insights into unique ecological and environmental characteristics that relate to vector abundances and distribution in relation to RVF epidemics. This study adds value to strategic disease control measures during inter-epidemic periods.

Results from this study set forth improved knowledge on how to effectively sample RVF vectors. Findings have indicated that Mosquito Magnet trap is an efficient and practical tool for collecting *Ae. aegypti*. These traps have the advantage of collecting day biting outdoor mosquitoes.

Findings from this study provide the first model-based distribution risk maps for *Ae. aegypti* and *Cx. pipiens* complex in East Africa for the current and future climate scenarios. Generated maps show that distribution areas for *Ae. aegypti* and *Cx. pipiens* complex correspond closely to the Great Rift Valley region where RVF outbreaks have occurred. Predicted distribution in the current and future climate scenarios suggests that a more comprehensive assessment of the risk implied to distribution of these vectors for targeted disease control strategies such as vector control and animal vaccination. Predicted distribution provides understanding of transmission of RVFV during inter-epidemic periods in East Africa. This information can help researchers and disease control teams targeting areas for mosquito surveillance and control.

Simulated RVF vectors population dynamics provide new opportunities for weather-driven RVF epidemic simulation modelling. As RVF epidemic prediction is limited by absence of data, this approach can be used as an alternative source for generation of RVF epidemics data in different scenario for use in advanced computational analyses. This is starting point towards use of modelling to predict, prevent and control RVF epidemics. It is my hope that this model provides useful tool for further studies.

3.2 Recommendations

i.   There is need for future studies to investigate long term seasonal vector abundance in locally known disease epidemic sites by taking into consideration the structure of the ecology and habitat distribution. Such studies could add value to strategic disease control measures.

ii.   There is need for joint stakeholder approach involving multidisciplinary teams of scientists similar to one health approach in order to further investigate and develop useful tool for predicting, detection, prevention and control because RVF epidemics is driven by combination of factors from animals, vectors, humans, environment, climate and ecology.

3.3 Future Studies

- Search for RVFV in floodwater *Aedes* mosquitoes using artificial flooding experiments or search for infectious eggs in disease epidemics zones.

- To conduct a laboratory based study on RVFV transovarial transmission in mosquitoes using inactivated viruses.

- Further development of RVF vectors population dynamics model towards a disease epidemics predictive model. This can be made possible by incorporating the diseaase-vectors spatial components, machine learning algolithms and inclusion of automated capture of daily weather data in a web-based platform.

- Use of serological data to develop mathematical model for estimating RVFV transmission at community level by adding the role of host immunity.

APPENDICES

Appendix 1: Data collection form

Collection site information:

Village_____Ward:_____

District_____Region _____

GPS position: **S** [ ][ ]^0 [ ][ ]'[ ][ ][ ]" **E** [ ][ ]^0 [ ][ ]'[ ][ ] . [ ]'

Altitude: _____Cloud Cover information (if available): _____.

Weather today: Temperature _____

    [ ] Sunny

    [ ] Cloudy

    [ ] Raining

    [ ] Windy

Collection method:

CDC Light traps Outdoor catch   [ ]   Mosquito nets Outdoor catches   [ ]

CDC Light Traps Indoor catches   [ ]   Others specify_____

Vegetation type:[ ] Little/ none present [ ] Shrubs/bushes [ ] Trees overhanging

Proximity of vegetation [ ] none [ ] > 10 m away [ ] 5-10 m away [ ] < 5 m away

Vicinity of blood hosts? [ ] Yes [ ] No

If yes what blood hosts (e.g. birds/rabbits/humans etc.)_____

Is the site in [ ] full sunlight [ ] partial shade [ ] full shade

Soil type information (for egg sampling)

Type of Trap	Trap ID	Species	Number Collected	Sample Pool ID

Remarks_____

Appendix 2: Protocol for molecular diagnosis of RVFV in mosquitoes

Scope: RNA extraction and RT-PCR for detection of RVFV in mosquito samples

Responsibilities:

All procedures done by trained experts under supervision of Head of laboratory in collaboration with the PhD student

Record:

All data of RNA extraction, RT-PCR experiments and Gel electrophoresis must be registered in the ongoing report notebook.

Extraction of total RNA with TRI reagent® BD from mosquito samples

1. Introduction

TRIZOL® Reagent is a reagent designed to allow total RNA extraction from cells and /or tissues. The TRIZOL® Reagent, a monophasic solution of phenol and guanidine isothiocyanate is used in an improved RNA extraction procedure. During the lysis process, TRIZOL® Reagent keeps the RNA integrity at the same time that it promotes cellular lysis and solves the cellular components. The addition of chloroform followed by a further centrifugation allows separate to fractions: aqueous and organic phases. The RNA is located exclusively in the aqueous phase. The RNA extraction technique using TRIZOL® Reagent from cells and tissues can be used to process amounts of cells or tissues from $\geq 1g/\geq 10^7$ to less than $50\text{-}100mg/5x10^6$ irrespectively its origin, human, animal, plant origin or bacterial. The simplicity of the methods allows to process may samples at the same time.

2. Range of application

With this extraction procedure an intact RNA can be obtained, with no residual DNA or proteins. So, it can be used for Northern blotting, dot blot hybridization, in vitro transfection, cloning. This procedure has to be performed in the Virology ore Cell Culture Laboratories in Bio containment Unit, and always strictly following the laboratory biosafety rules depending on the nature of the virus with we are working on.

3. Material

Single used sterile plastic ware, RNAses free; 1.5 ml-Eppendorf and filter tips of several volumes (1000 µl, 200 µl, 100 µl and 20 µl); sterile scissors and forceps. The needed reagents are the following ones:

- TRIZOL® Reagent. Invitrogen #15596-018 (room temperature)

- Chloroform. Sigma #C2432 (room temperature)

- Isopropanol. Sigma #I9516 (room temperature)

- 75% Ethanol 75%. => from Absolute Ethanol. Panreac #212801 (room temperature)

- Sterile MilliQ water

- DEPC water. Ambion #9916

4. Equipment

- Biological safety cabinet. CR0245 and/or CR0244

- Centrifuge Eppendorf 5810R. CR0273, and Centrifuge Eppendorf 5417R. CR0457

- MS1 minishaker IKA. CR0501

- Oven Memmert CR0469

- Micropipette finnpipette 1000 µl P235-P351-P354

- Micropipette finnpipette 200 µl P298-P349-P347

- Micropipette finnpipette 50 µl P236-P167-P343

- Micropipette finnpipette 40 µl P237

5. Procedure

Sample preparation

- Add 50-100mg* of tissue in 1ml TRIZOL®. Homogenize. Alternatively add 5-10×10^6 animal or plant cells, or yeasts or 1×10^7 bacterial cells.

- Incubate 5 minutes at room temperature.

- Add 200 µl of chloroform and thoroughly shake for 15 seconds.

- Keep 2-3 minutes at room temperature.

- Centrifuge at 12,000g for 15 minutes at 2-8ºC.

Sample separation

- Pipette the aqueous phase (which accounts around 60 percent of the Eppendorf volume) and transfer to a new eppendorf.

- Add 500 µl of isopropanol, mix by inversion and keep for 30 minutes at -20ºC.

- Centrifuge at 12,000g, for 10 minutes at 2º-8ºC. A *pellet* will be observed.

- Discard with a pipette the supernatant and add 1000 µl of cold 75 percent ethanol.

- Centrifuge at 7,500g for 5 minutes at 2º-8ºC.

RNA precipitation

- Discard the supernatant. Let the pellet dry for 5 to 10 minutes at room temperature (do not let too dry in excess, as later solubilisation can be difficult).

- Add 60 µl of previously warmed DEPC water.

- Incubate at 55-60ºC in an oven for 10 minutes.

- Store the sample at -80ºC until further process.

* For tissue volumes (weights) around 1-10mg, 800 µl of TRIZOL® has to be used, readjusting then all the volumes of the other reagents.

6. Personnel Qualification

Laboratory technician skilled in molecular biology techniques or virology techniques

7. Risk Level

- The risk level is deeply linked to the type of biological sample (viral or bacterial) we are dealing with.

- The general biosafety rules inside Bio-containment Unit will be followed. Moreover, the work will be performed attending the specific biosafety rules of each laboratory, or any other particular or specific coming from the virus or bacteria which it has been used.

8. Wastes

At any times, the waste disposal regulation of Bio-containment Unit has to be followed.

9. Responsibilities

The Laboratory Manager of Bio-containment Unit is the person in charge to inform and train personnel about the requirements of this procedure but also to force its compliance. The laboratory personnel involved in the application of this procedure is responsible of reading, understand and fulfil all procedural requirements.

10. Forms

All activities and reagents used will be written down in the corresponding laboratory notebook.

Molecular diagnosis of Rift Valley Fever virus by one step RT-PCR

Equipment

- RNase free and DNase free tubes
- Filter tips (0.5 µl – 10 µl, 10 µl -100 µl, 100 µl -1000 µl)
- Ice maker
- Safety Cabinet Level 2
- Centrifuge
- Vortex
- Sampler 0.5 µl – 10 µl
- Sampler 10 µl -100 µl
- Sampler 100 µl -1000 µl
- Thermo cycler
- Power supply and gel electrophoresis tank

Procedure

1. Thaw template RNA, primer solutions, dNTP mix, QIAGEN One-step RT-PCR Buffer, dNTP mix, RNase-free water and place on ice. Mix thorough before use.

2. Prepare a reaction mix accordingly. The reaction mix contains all the components except the template RNA. Prepare a volume of reaction mix 10 percent greater than that required for the total number of reactions to be performed. Note: A negative control (without template RNA) should be included in every experiment.

3. Mix the reaction mix gently but thoroughly, for example, by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes.

4. Add template RNA (≤2 μg/reaction) to the individual PCR tubes. The QIAGEN One-step RT-PCR Kit can be used with total RNA, messenger RNA, or viral RNA[U1][U2].

5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in kit table. The protocol includes steps for both reverse transcription and PCR and gives satisfactory results in most cases. For maximum yield and specificity, temperatures and cycling times should be further optimized for each new target and primer pair.

6. Start the RT-PCR program while PCR tubes are still on ice. Place the PCR tubes in the thermal cycler once it has reached 50°C. Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.

7. We have evaluated several specialized protocols and particular hints for the cases. For details, please refer to the indicated appendix in the QIAGEN One-step RT-PCR Kit Handbook

Protocol for agarose gel electrophoresis

1. Prepare gel solution as outlined under Recipes section below.

2. Pour the gel when the agarose has cooled to about 55° C. Insert the proper comb for the particular gel rig. The gel should be allowed to cool until it has set (it will turn whitish and opaque when ready). The amount of agarose depends on the size of the gel rig. Gels should be fairly thin, approximately 1/4 to 1/2 inch.

3. Carefully remove the comb and place the gel in the gel rig with the wells closest to the cathode (black) end. Cover the gel with 1X TAE running buffer.

4. Cut a piece of parafilm and place a 5 µl drop of glycerol loading dye onto the waxy side for each sample to be loaded.

5. Keeping samples on ice, pipette up 5 µl of a sample, wipe the excess oil from the pipette tip with a Kimwipe and add the sample to one of the drops of loading dye.

6. Switch the pipette tip to another pipette set for 10 µl. Mix the sample and loading dye by filling and emptying the pipette a few times then load the mixture into a well.

7. Continue loading the rest of the samples, placing 5 µl of 1 Kb ladder at both ends of the series of samples and between every 10 samples.

8. Place the cover on the gel rig and run the samples towards the anode (red) end. For a small gel, we set the power pack to about 60 ma. For a large gel, we use about 120ma. Milliamperage increases during the run, so check it periodically. Stop the run before the bromophenol blue loading dye front exits the gel.

9. Turn off the power pack, remove the gel and place it in a stain box with 40 µl ethidium bromide: 200 ml 1X TAE for approximately 45 minutes. NOTE: Ethidium bromide is light sensitive and must be stored in darkness.

10. Visualize with U.V. light (take proper precautions!) and photograph with a polaroid Photo documentation camera.

11. Dispose of the gel properly. (Check to see how your facility handles disposal of ethidium bromide).

Recipes section for agarose gel electrophoresis

A. 1.4 percent Agarose gel: Nanopure water.................... 392 ml

50X TAE buffer.................. 8 ml

Electrophoresis grade agarose... 5.5 g

Heat to boiling

Excess gel can be stored at room temperature and re-melted for future use. To expedite gel runs, we make 1.6 l and divide into 200 ml aliquots in covered, 500 ml Erlenmeyer flasks. These can quickly be re-melted in a microwave oven as needed.

B. 0.5 M EDTA (pH 8.0):

mw= 336.2 g (anhydrous), 354.2 g (H_2O), 372.24 g (2 H_2O)

For 500 ml, start with approximately 450 ml water and adjust pH initially with NaOH pellets.

Autoclave.

C. 50X TAE buffer:

Tris-borate................ 242 g

Glacial acetic acid......... 57 ml

0.5 M EDTA, pH 8.0... 100 ml

Dilute to 1 L with nanopure water

D. Running Buffer (1X TAE):

Dilute 50X TAE to 1X and use the amount appropriate for the particular gel apparatus.

E.1 Kb Ladder (1 µg/ml):

Ladder stock.................. 5 µl

1 M NaCl..................... 44 µl

Glycerol loading dye (6X) 7.5 µl

We use Life Technologies 1 Kb Plus DNA Ladder.

F. Glycerol Loading Dye (6X):

bromophenol blue... 0.26 g

Glycerol............... 30 ml

To 100 ml with nanopure water

G. 1 M NaCl

mw= 58.44 g/liter nanopure water

H. Ethidium Bromide:

10 mg/ml, store in a dark bottle at 4˚ C.

CAUTION! Ethidium Bromide is carcinogenic, so wear proper protection when handling.

SAFETY

- Mask must be used.
- Gloves must be used.
- Helmet must be used.
- Gown must be used.
- Goggle must be used.
- All works must be carried out under Biological hood level 3

Appendix 3: Ethical clearance certificate

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TANZANIA

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26th January 2012

Mr Clement Nyamunura Mweya
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CLEARANCE CERTIFICATE FOR CONDUCTING
MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Ecological Factors Associated with Rift Valley Fever in
Tanzania during INTER-Epizoic Period in Ngorongoro, Bahi and Chamwino Districts in Tanzania
(Mweya C N *et al*), has been granted ethics clearance to be conducted in Tanzania.
The Principal Investigator of the study must ensure that the following conditions are fulfilled:
1. Progress report is submitted to the Ministry of Health and the National Institute for Medical
   Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare
   and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of
   an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III
   Section 10(2).
5. Approval is for one year: 26th January 2012 to 25th January 2013.

Name: Dr Mwelecele N Malecela

Signature

CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

CC: RMO
         DMO

Name: Dr Deo M Mtasiwa

Signature

CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL
WELFARE