Aus dem Institut für Parasitologie und Tropenveterinärmedizin
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin
und dem
International Livestock Research Institute

Impact of irrigation expansion on the inter-epidemic and between-season transmission of Rift Valley fever in Bura Sub-County, Tana River County, Kenya

Inaugural-Dissertation
zur Erlangung des Grades eines
PhD in Biomedical Sciences
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vorgelegt von
Deborah R. Nyakwea Mbotha
aus Nairobi, Kenia
Master of Science in Veterinary Epidemiology
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Dedicated to
my beloved little girl, Keanna Nwoke (Kikki)

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List of abbreviations

AIC	Akaike Information Criterion
BLAST	Basic Local Alignment Search Tool
BP	Base Pair
CI	Confidence interval
CO_2	Carbon Dioxide
cRNA	complementary RNA
DAAD	Deutscher Akademischer Austauschdienst
	(German Academic Exchange Service)
DNA	Deoxyribonucleic Acid
DVS	State Department for Veterinary Services, Kabete, Kenya
EIP	Extrinsic Incubation Period
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
IEP	Inter-Epidemic Period
IFAD	International Fund for Agricultural Development of the United Nations
ILRI	International Livestock Research Institute
KNBS	Kenya National Bureau of Statistics
NCBI	National Center for Biotechnology Information
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
VC	Vectorial capacity
WHO	World Health Organization

Preface

The world around us is changing. There are more humans on the planet now than at any other point in human history, with the global population having grown from 1 billion in 1800 to over 7.5 billion in 2018. As the human population continues to grow, urbanization, immigration, trade and travel become inevitable. Modern theorists and philosophers have touted the impressive technological innovations and advances as the panacea to the resource conflicts and tensions brought about by the unprecedented growth, but as these advances continue to shape our human experience and make our lives easier, another type of change, a more surreptitious one is taking place. Environmental changes, which can be local, regional or global, are fast outpacing our ability to innovate and provide sustainable and lasting solutions. Local or regional changes include land degradation, forest depletion, urbanization, stresses on food-producing systems, land-use changes, irrigation establishment or expansion, changes in hydrological systems and supplies of freshwater, changes in ecosystems due to modifications or loss of biodiversity, local energy resource use and depletion, among others. Global environmental changes occur as cumulative effects of local and regional changes, and these include climate change and climate variability, stratospheric ozone depletion and the rise of atmospheric carbon dioxide, nitrous oxide and methane levels, global surface temperature anomalies, ocean acidification among others.

The consequences and impacts of these changes are varied in time and space, but the effects on emerging infectious diseases, and in particular vector-borne diseases, cannot be denied. Recent studies have shown the growing risks associated with land-use changes and climate variability on infectious disease such as Rift Valley fever, malaria, dengue fever, infections by other arboviruses, schistosomiasis, trypanosomiasis, onchocerciasis, leishmaniasis, among others. While global environmental changes pose unknown future risks to humans and natural ecosystems, local environmental changes are occurring more rapidly and are having significant effects on local populations and communities, impacting their daily survival and sustainability. Therefore, the principal aim of this thesis was to evaluate the effects of land-use changes on the occurrence and transmission of Rift Valley fever in Bura Sub-County, Tana River County, Kenya.

The cumulative thesis is structured into chapters as follows:

The first chapter intends to provide general background on land-use and factors precipitating changes in Bura Sub-County, the problem statement and justification of this thesis' research. Further, this chapter also highlights the scope and objectives of the thesis.

The second chapter provides a review on the current state of knowledge of Rift Valley fever, the disease under investigation. In addition, the etiology, life cycle, epidemiology and transmission,

vectors and hosts, and the risk factors precipitating the emergence or re-emergence of the disease are reviewed in this chapter. The chapter also briefly addresses mosquito surveillance, types of surveillance and the role played by mosquito monitoring in the control and elimination of mosquito-borne diseases.

The third chapter lays the foundation of the comparative study, by describing how baseline seroprevalence of RVF virus was determined within the three study sites and how sentinel herds were chosen and followed up over a period of 10 months to determine and compare the rates of seroconversion between seasons and between the study sites. This study was used as a direct measure of RVF risk within the local livestock population.

The fourth chapter describes how the study evaluated the differential impact of irrigation expansion in Bura, Tana River County, on local mosquito ecology, which would in turn influence the emergence and maintenance of RVF virus. Specifically, it assessed the seasonal variation in mosquito abundance, the relative differences in mosquito density and diversity of species between the three study sites and identified the host sources of blood meals in mosquito field samples to determine the host spectrum.

The fifth chapter presents an overarching discussion drawn from all previous chapters of the thesis and corroborated with information and references from existing scholarly works. Further, a summary of the thesis is also given while the strengths and limitations of the study are considered, accompanied by recommendations for further research. This chapter concludes with suggestions on how the findings can be utilized with integrated vector surveillance and control measures, in an effort to mitigate mosquito-borne disease outbreaks in increasingly changing local ecosystems.

1.0 CHAPTER 1: Introduction

1.1 Background of the thesis

Kenya's population has experienced rapid growth over the last few decades; from 16.2 million in 1979 to 39.4 million in 2009 (Kenya National Bureau of Statistics, 2014) averaging an increase of more than 3% per annum. Although slowing down over the past decade, this rapid increase, coupled with other challenges such as frequent droughts and insecurity, continue to exert tremendous pressure on existing land and food sources (Nkonya *et al.*, 2008; Food and Agriculture Organization of the United Nations, 2010). Underperformance in the agricultural sector has been blamed for the current state of affairs, leading to an increase in investments in agriculture, with the hope that it will reduce poverty directly through increased food production, and indirectly by offering employment and livelihood opportunities (Thirtle *et al.*, 2001; International Fund for Agricultural Development of the United Nations, 2011). Among the many interventions in addressing these challenges is the construction of dams, establishment of new or expansion of existing irrigation schemes along major rivers and tributaries for maize, wheat, rice and sugar production. The creation of these new schemes and permanent water masses has the potential to modify local ecological conditions and processes, altering biodiversity and upsetting the vector-host-environment equilibrium. The nature and frequency of contacts and interactions between hosts and vectors in these sites could also be changed, as a result of increased human settlement, increased livestock and wildlife movement, resulting in increases in disease prevalence (Lambin *et al.*, 2010; Richards *et al.*, 2010; Sarkar *et al.*, 2012; Lacroix *et al.*, 2014).

Many factors are involved in determining the range of arthropod diseases, as well as changes in the spatial heterogeneity of their vectors within endemic regions. Land-use and land-cover changes have been directly implicated in the emergence or spread of infectious diseases (Vittor *et al.*, 2006; Gottdenker *et al.*, 2011) especially arthropod-borne diseases (Patz *et al.*, 2000; Johnson *et al.*, 2008) such as West Nile fever, malaria, Rift Valley fever and Lyme disease (Barbour & Fish, 1993; Conn *et al.*, 2002; Bowden *et al.*, 2011; Iacono *et al.*, 2018). Most studies investigate various patterns of spatial association, either singly or in combination with other environmental variables, using qualitative or quantitative spatial parameters. Agriculture, urbanization and human encroachment into previously undisturbed ecosystems have also been proposed as catalysts to changes in vector and disease emergence, distribution and behavior (Leisnham *et al.*, 2005).

Mosquito vectors seem particularly sensitive to these changes, which significantly alter their population dynamics, species composition and competence (Dorvillé, 1996; Jones *et al.*, 2004; Derraik & Slaney, 2007; Johnson *et al.*, 2008). In Africa, irrigation and other agricultural production systems have been associated with the establishment and spread of malaria, bilharzia and leishmaniasis (Surtees, 1970; Jacob *et al.*, 2006; Kibret *et al.*, 2014; Barhoumi *et al.*, 2015). Apart from vector density and species composition, land-use or other environmental changes may also alter intrinsic vector traits such as competence, infection rates and lifespan which may in turn affect overall vector capacity for disease transmission (Ciota & Kramer, 2013). Knowledge of the mechanisms by which these changes modify local micro-climatic and environmental parameters, coupled with the ability of arboviruses, such as the Rift Valley fever virus, to adapt to these changes, both within the vectors and hosts, leading to the emergence and maintenance of disease in previously naïve ecosystems is critical in the formation and implementation of surveillance and control efforts for both vectors and diseases. The emergence and geographical spread of RVF into new counties and regions within Kenya presents a unique opportunity to examine whether anthropogenic transformations in these localities might be impacting on the local ecosystem services provision, affecting intrinsic disease regulatory mechanisms and inadvertently providing the prerequisite environment for emergence and prevalence of RFV.

1.2 Scope and objectives of the thesis

The general objective of the study was to evaluate the effects of land-use changes on the occurrence and transmission of RVF in Bura Sub-County, Tana River County, Kenya. The objective was premised on the hypotheses that:

1. The creation of new, or expansion of existing permanent water masses through irrigation alters vector biodiversity and abundance, and modifies the make-up of populations of wildlife, livestock and humans. This potentially increases the frequency of interactions between hosts and vectors, thereby driving RVF emergence and transmission.
2. The occurrence of RVF in these novel, relatively intensive agricultural systems has impacts on the health and wellbeing of local communities and their economies, which differ quantitatively and qualitatively from impacts in minimally altered ecosystems.

A comparative study in three ecologically distinct sites was conducted to determine whether and how these environmental changes influence ecological conditions and processes such as changes in host or vector biodiversity, that in turn affect the emergence and maintenance of Rift Valley fever virus. The specific objectives were:

1. Investigate the relative differences between the sites, and any seasonal changes in vector abundance, species composition and diversity especially with regards to the ‘reservoir’ vectors and other ‘amplifying’ vectors.
2. Identify host sources of blood meal in mosquito field catches to determine host spectrum.
3. Determine the baseline seroprevalence of RVF and evaluate regional and seasonal differences in RVF seroconversion rates in livestock between the three sites, as a direct measure of risk.

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2.0 CHAPTER 2: Literature review

2.1 Rift Valley fever

Rift Valley fever is a mosquito-borne viral zoonosis that causes episodic epidemics in sub-Saharan Africa and the Arabian Peninsula. It is a World Animal Health Organization notifiable disease and mainly affects sheep, cattle, goats, camels and wild ruminants. Other mammalian species can also be affected especially in laboratory settings (World Organisation for Animal Health (OIE), 2009), while humans are affected as spill-over hosts during large epidemics (Nguku *et al*., 2010; Harmon *et al*., 2012). The disease is caused by a virus in the family Bunyaviridae, genus *Phlebovirus* and mosquitoes of the genus *Aedes* are known to be the reservoir vectors (Linthicum *et al*, 1985). However, during outbreaks several genera of mosquito as well as other biting insects can serve as mechanical or even amplifying vectors, transmitting the virus between livestock (Fontenille *et al*., 1998).

Rift Valley fever was first reported among livestock in Kenya in the early 20th century. In 1930, the virus was isolated near Lake Naivasha in the Rift Valley province of Kenya (Daubney *et al*., 1931) and thereafter was rarely reported until 1951 when an epidemic occurred in cattle and sheep with extensive cases in humans in South Africa (Woods *et al.,* 2002). For the next decades, outbreaks were recorded in Egypt, Sudan, South Africa, Madagascar, northern Senegal and southern Mauritania (Clements *et al*., 2007). In these countries, high seroprevalence was observed in the dry arid or savannah regions while low seroprevalence was reported in the wet highlands or tropical regions. RVF epidemics were relatively restricted to sub-Saharan Africa until September 2000, when an outbreak was reported in the Kingdom of Saudi Arabia and Yemen (Balkhy & Memish, 2003), which was later related to importation of infected animals from Africa. This was the first documented outbreak of the disease outside of Africa. Prior to this, serological surveys conducted indicated that outbreaks had continued to spread progressively in previously unaffected regions within Africa from the 1950s to the 1990s, raising concerns that the geographical range of RVF was indeed expanding (Clements *et al*., 2007). RVF epidemics occur in 5-15 year cycles in medium to low rainfall savannah grasslands and are often associated with heavy rainfall and flooding (Daubney *et al*., 1931; Davies, 1975). In forested zones of Africa, the disease occurs endemically following the onset of the rainy season while large epidemics are observed in dry arid and semi-arid zones such as the Horn of Africa (Anyamba *et al*., 2002).

2.1.1 RVF transmission and disease

The RVF virus is believed to be maintained throughout inter-epidemic periods via transovarial transmission in mosquito vectors of the genus *Aedes* that breed in temporary ground pools called *dambos* that form in savannah grasslands (Linthicum *et al.*, 1985). Persistent, above average rainfall raises the ground-water table to a level where the breeding sites of these mosquitos become flooded thereby inducing hatching of the eggs and subsequent emergence of large numbers of RVF infected adult mosquitos, that then feed on hosts (Davies *et al*, 1985). Consequently, virus multiplication occurs in these vertebrate hosts, leading to further infection of other vector species such as those of the genus *Culex* capable of transmitting the virus. The humid and cool conditions present during these periods also enable a greater proportion of adult mosquitoes to survive longer, thus going through more feeding and oviposition cycles.

The life cycle of RVF virus has distinct endemic and epidemic phases (Anyamba *et al.*, 2010). In the endemic cycle, the virus is maintained during the dry inter-epidemic periods via transovarial virus transmission in *Aedes* mosquito eggs (Linthicum *et al.*, 1985; Gargan *et al.*, 1988). In this cycle, the virus circulates at very low incidence without noticeable clinical manifestation in animal and human hosts (Bird *et al.*, 2008). Seasonal rainfall and flooding stimulates the hatching of some eggs and subsequent rapid multiplication of virus resulting in low-level transmission and amplification of the virus (Linthicum *et al.*, 1999). Occasionally, with prolonged and persistent rainfall, coupled with the availability of large numbers of susceptible hosts, mosquito habitats remain flooded long enough for the emergence of large numbers of mosquito vectors and subsequently setting the stage for an epidemic cycle. The importance of each mode of transmission varies according to the phase of the disease. In the inter-epidemic phase, it is believed that vertical (transovarial) transmission plays the most significant role while during the epidemic phase, bites of infected mosquitoes become the principal mode of transmission in the initial stage, whereas direct contact of animals or humans with infected animals, tissues or objects becomes predominant in the amplification and later stages of the epidemic (Chengula *et al.*, 2013).

The RVF transmission cycle involves the virus, mosquito vectors and ruminant or other mammalian hosts. There are three main modes of virus transmission; vectorial transmission that involves the transfer of virus from the vector to vertebrate hosts, direct transfer of virus between vertebrates, vertical transmission through transovarial infection of *Aedes* mosquito eggs, or the transfer of virus from a vertebrate host to its young through suckling (Lumley *et al.*, 2017).

RVF manifests itself as a subclinical disease with mild symptoms such as fever and loss of appetite in older animals, while pregnant animals experience abortions, commonly referred to as 'abortion storms' in massive outbreaks, regardless of the stage of pregnancy. In younger animals, the disease is acute and progresses rapidly, resulting in high morbidity and mortality (Pepin *et al*, 2010; Sumaye *et al.*, 2013). Direct contact with infected animals or animal products, discharges or fluids results in human infection (Laughlin *et al.*, 1979; Davies, 2010). The disease in humans is usually mild and subclinical with fever, headaches and general weakness, with a few cases of acute hemorrhagic fevers. Occasional chronic cases however manifest with encephalitis, retinitis or other ocular disorders (Al-Hazmi *et al.*, 2003; LaBeaud *et al.*, 2011).

2.1.2 RVF epidemiology

2.1.2.1 Geographical spread

The geographic distribution of RVF spanned throughout the African continent following the initial identification of the virus in 1930 in the Rift Valley province of Kenya. After that, outbreaks were reported in South Africa, Namibia, Botswana, Zimbabwe and Uganda from the early sixties to the late seventies (Henderson *et al.*, 1972; Coetzer, 1982). The virus also migrated northwards with initial outbreaks being reported in Egypt and The Sudan in the early seventies (Meegan, 1979). In West Africa, the earliest outbreaks were reported in The Gambia, Senegal, Mauritania and Nigeria in the mid to late eighties (Clements *et al,* 2007). In September 2000 and for the first time, a RVF outbreak was documented in the Middle East, in the Kingdom of Saudi Arabia and Yemen (Balkhy & Memish, 2003). This outbreak was postulated to have been caused by the importation of infected animals from the Horn of Africa. In August 2007, several cases of RVF were documented in humans and cattle in the Indian Ocean island of Grande Comore, Republic of Comoros (Sissoko *et al.*, 2009). This outbreak occurred soon after the large 2006-2007 outbreak in East Africa involving Kenya, Tanzania, Somalia and The Sudan, and hence was thought to be an expansion of the same due to the presence of suitable environmental and climatic conditions (World Health Organisation, 2007; Himeidan *et al.*, 2014). Most recently, endemic RVF has been reported in Angola, Nigeria and the French island of Mayotte (Lernout *et al.*, 2013). Greater geographical expansion of the virus is expected in the future due to the presence of high numbers of competent vector species in currently RVF-free regions as well as globalization and increase in international trade and movement (Gale *et al.*, 2010; Rolin *et al.*, 2013).

2.1.2.2 Social and economic impact of RVF

RVF has serious economic, social and health consequences. The most direct economic impact of the disease is loss of both human and animal lives and productivity, and the ensuing income losses for farmers. Previous outbreaks in South Africa, East Africa and the Middle East have resulted in the loss of human lives, while the health and wellbeing of livestock keepers, consumers and other various stakeholders along the market chain are threatened during outbreaks (Rich & Miller, 2005). The World Bank classifies these effects as; a) direct effects that cause direct impact in loss of productivity resulting in income losses at the household level, b) ripple effects that impact on various players along the livestock value chain such as markets, slaughterhouses and traders, c) spillover effects experienced in other sectors including public health systems, availability and quality of nutrition and food security, d) long-term effects that result in loss of access to regional and international markets as a consequence of loss of confidence in the public health or veterinary services of a country and lastly, e) remote effects experienced cumulatively on a global scale as a result of networking of global markets, forces of demand and supply as well as presence of other concurrent animal disease challenges (World Bank, 2010). Country and regional-level economic impacts in Africa, such as loss of trade due to bans on animal movement have been studied, but there is not much data on the socioeconomic impacts on the mostly rural poor livestock producers. In East Africa, RVF has been reported in Kenya, Uganda, Tanzania, Somalia, Sudan and South Sudan. However, no comprehensive studies have been implemented to study its socioeconomic impacts in Somalia, Sudan and South Sudan.

Losses accrued due to RVF outbreaks were documented in Kenya as early as the first outbreak in July 1930. In this outbreak, deaths as well as extensive abortions were observed among ewes of flocks of sheep in the Rift Valley province and extremely high mortality among young lambs. All the four scientists investigating the outbreak as well as several livestock herders were reported to have suffered a malaria-like fever (Daubney *et al.*, 1931). In subsequent years, there have been very few studies conducted to examine the impact of the disease at the local household or societal level, with most studies focusing on epidemiological and macro-economic impacts. Reports from the state Department for Veterinary Services (DVS) in Kabete, Nairobi indicate that from 4th December 2006 to 21st June 2007, 35 out of 47 districts, in all provinces except Nyanza and Western, had reported cases of RVF. In total there were 717 human and 8,252 animal cases. Human cases were reported in 12 of the 35 infected districts with 210 being confirmed in the laboratory and 162 (including one veterinary officer) succumbing to the disease. The North

Eastern region was the hardest hit as it was the region with the highest livestock population and with large communities practicing pastoralism (Murithi *et al*., 2011).

In one comprehensive study conducted in Kenya to document the impact to society and larger economy after the 2006-2007 outbreak, the effects were classified into impacts on the livestock value chain and impacts to the overall Kenyan economy (Rich & Wanyoike, 2010). Value chain players included producers that experienced losses due to deaths and abortions in animals, loss of milk production, falling of animal prices and absolute reduction in future flock sizes. In Garissa County, it is estimated that nearly 2.3 million liters valued at US$77,000 in potential milk production was lost, while livestock mortality accounted for a loss of over US$9.3 million. Slaughterhouses located outside of quarantined areas such as in Nairobi or Thika Counties experienced sharp declines in animal turnover rates due to a decline in the number of animals arriving from source markets. Those located inside quarantined areas remained closed for more than 3 months during the outbreak. Slaughterhouses in Garissa and Mwingi Counties experienced losses of up to US$2,917 and US$812 per month, respectively. Individuals whose livelihoods directly depended on these slaughterhouses were severely affected as were those indirectly dependent on them, such as surrounding cafes, vendors and others providing auxiliary services. Traders incurred losses as a result of animal movement bans imposed to contain the outbreak. They also bore the cost of maintaining animals purchased prior to the outbreak as well as the sharp decline in prices. For those whose animals had died, they had to restart their businesses after the outbreak had subsided. At the consumer end of the market chain, butchers suffered heavy losses due to the fall in demand for red meat. In Thika and Nairobi, sales fell by over 95% on average from 70kg-140kg per day to 2kg-5kg. Like traders, majority of butchers could not resume normal business soon after the outbreak had subsided. At the national level, the value of cattle at market prices declined by 2.3% while goat and sheep production declined by 1% and a further decline of 1% of the value of meat. The value of the total livestock supply fell by US$32 million while the value of related crops fell by over 0.5%. Other sectors such as tourism, transport, and sales of agricultural chemicals and medicines also experienced declines. Nationally, the impact of the 2006- 2007 RVF outbreak was greater on households with higher annual incomes in rural areas than in middle-income households in urban areas (Rich & Wanyoike, 2010; Peyre *et al*., 2014).

2.1.3 The virus

The RVF virus is a mosquito borne Phlebovirus in the *Bunyaviridae* family. It is a RNA virus composed of three negatively polarized fragments L, M and S, enveloped in a lipid bilayer

composed of glycoproteins (Pepin *et al.*, 2010). Virus replication occurs in the cytoplasm of infected cells and subsequent virions mature by budding in the Golgi compartment (Matthews, 1979). As with other negative-stranded RNA viruses during the replication cycle, each segment is transcribed into mRNA and thereafter replicated through the synthesis of an exact copy of the genome, called antigenome or cRNA (Elliott, 1990; Elliott, 1997)). The lipid bilayer plays an essential role during virus penetration into various cell types through specific receptors, as well as in the viral replication cycle. The glycoproteins are recognized by the immune system of the host, thereby inducing the production of neutralizing antibodies, which play an important role in protection.

New and advancing techniques in molecular epidemiology and genetic analysis have demonstrated the plausibility of the RVF virus to have originated from an ancestor that existed in the late 19th century. The predominant hypothesis for the origin of RVF outbreaks is that they occurred as a result of introduction of naïve and highly susceptible European breeds of livestock into East Africa and the subsequent intensification of various agricultural production systems leading to amplification and establishment of the virus (Dar *et al.*, 2013). Molecular studies done during the 2006/2007 outbreak in East Africa demonstrated the presence of two main circulating RVF virus lineages, Kenya-1 and Kenya-2. This was in contrast to previous findings during epidemics in Egypt, Mauritania and South Africa in the 1970s and 1980s, where only single lineages were present (Bird *et al.,* 2008). Additionally, there was evidence of recent RNA segment reassortment between these two lineages, with S and L segments placed within Kenya-1 lineage in one isolate, while the M segment firmly placed within the Kenya-2 lineage. The progenitor virus of these two lineages was present during the 1997/1998 Kenyan outbreak and had been isolated 25 years earlier from an *Aedes mcintoshi* mosquito in Kenya in 1983. These two lineages were also seen to be more closely related to the 1997/1998 Kenyan outbreak prototype than they were to each other. There was also strong evidence of an increase in virus population size and spatial distribution of the S, M, and L segments of lineage Kenya-1. These results show that the presence of multiple circulating virus genotypes during epidemics give ample opportunity for genetic shift or reassortment (Bird *et al.*, 2007; Bird *et al.*, 2008).

2.1.4 The hosts

The initial outbreak of RVF was first described in sheep in Kenya's Rift Valley province in 1930. It was characterized by heavy mortality among lambs and abortion storms in pregnant ewes, while humans were incidental hosts who got infected after handling the sick animals (Daubney *et al.*, 1931). Over the years, other livestock species have been affected, including cattle, goats, camels,

wild ruminants and rodents. Other mammalian species can also be infected especially within laboratory or experimental settings (World Organisation for Animal Health, 2009). Ruminants are considered the primary hosts of RVF, although different species have varying levels of susceptibility to the RVF virus. The species-specific mechanism of susceptibility to RVF virus infection remains unknown (Ikegami & Makino, 2011). Furthermore, despite the ever growing list of hosts, so far, the maintenance or reservoir host for the RVF virus remains unknown (Gubler, 2002; Rosta *et al.*, 2017).

The RVF virus is injected into the host together with mosquito saliva, which contains anti-inflammatory molecules that makes the environment more permissive for the virus to replicate and thrive (Le Coupanec *et al.*, 2013; Briant *et al.*, 2014). It then encounters macrophages and other phagocytes while still replicating both inside and outside the cells (Morrill *et al.*, 2010; Gommet *et al.*, 2011; Nfon *et al.*, 2012). In the next viremic febrile phase, the RVF virus enters the bloodstream and continues replicating in various organs and tissues such as the spleen, pancreas, kidneys and lungs, and especially the liver, being the primary target (Shieh *et al.*, 2010; Smith *et al.*, 2010). Susceptible animals develop fever, general weakness and loss of appetite, which progresses to hemorrhagic diarrhea, conjunctival hemorrhage, widespread petechiae, edema and hemorrhage of the lungs, with nasal and ocular discharges in mature animals (Pepin *et al.*, 2010; Ikegami & Makino, 2011). Hyperacute cases in sheep, goats and cattle may experience 80-100 percent abortion during the early stages of intense RVF virus transmission, regardless of the stage of pregnancy. Mortality rates of 70-100 percent in lambs, kids and calves under ten days of age may also be experienced (Food and Agriculture Organization of the United Nations, 2003; World Organisation for Animal Health, 2009). Older sheep and goats may show some or all of the signs while the infection might be inapparent in older cattle with a mild fever and a fall in milk production. The disease in wild ruminants is also inapparent with subsequent development of antibodies, although pregnant animals may abort (LaBeaud *et al.*, 2011).

Humans are considered ‘incidental’ or ‘dead-end hosts’, because even though they produce a significant viral load, they do not contribute to the natural ecology and transmission cycle of the virus (de St. Maurice *et al.*, 2018). Humans are mainly infected through the bite of an infected mosquito, drinking unpasteurized milk or handling of infected animals or tissues (Helmy *et al.*, 2017). Various players involved in the livestock production chain such as herders, farmers, butchers and animal health specialists, who inadvertently handle infected animals, carcasses or body fluids may become infected as spill-over hosts during large epidemics (Nguku *et al.*, 2010). However, no human-to-human transmission of RVF virus has been documented so far.

Natural disease in humans follows the same pattern as in animals, with most infections being asymptomatic. However, some infected individuals present with a nondescript influenza-like illness with fever, that may progress into hepatitis, retinitis, encephalitis, and, in severe hyperacute cases, hemorrhagic disease (Pepin *et al.*, 2010). Case fatality ratio is estimated at 0.5% - 2% (Magurano & Nicoletti, 1999; Al-Hazmi *et al.*, 2003). Cases that develop clinical symptoms may experience long-lasting sequelae such as ophthalmic disease with vision loss, convulsions, confusion and decreased mental capacity, among other neurologic disorders (van Velden *et al.*, 1977; Alrajhi *et al.*, 2004; LaBeaud *et al.*, 2007).

2.1.5 The vectors

Due to the abrupt and explosive nature of RVF outbreaks after prolonged rainfall and flooding followed by emergence of large numbers of mosquitoes, the current hypothesis is that infected floodwater *Aedes* mosquitoes act as RVF virus reservoirs that maintain the virus through transovarial transmission via eggs during long IEPs. These eggs then hatch after heavy rainfall and flooding, and subsequently transmit the virus when taking their first blood meal. Virus amplification is initiated with onward transmission in the presence of susceptible vertebrate hosts (Linthicum *et al.*, 1985; Mbotha *et al.*, 2017). The primary *Aedes* species implicated in vertical transovarial transmission include *Ae. mcintoshi*, *Ae. ochraceus*, *Ae. sudanensis*, *Ae. dentatus* (Sang *et al.*, 2017). The exact mechanism by which latent infection is maintained in infected eggs for long IEPs and subsequent viral replication after hatching remains unknown. However, it is thought that fragments of viral RNA genome are incorporated into the cells of vectors during development, to form DNA fragments of the same, reducing the vector's susceptibility to potential infection and subsequent pathogenesis (Nag *et al.*, 2016; Goic *et al.*, 2016).

After the onset of an outbreak, the RVF virus is transmitted by many different species of mosquito vectors that feed on infected mammalian hosts. These species can be described as 'amplifying' vectors that allow short-term virus replication and forward transmission into naïve hosts. These include various *Culex* species including *Cx. poicilipes, Cx. bitaeniorhynchus, Cx. univittatus, Cx. pipiens sl*. Other species such as *Mansonia africana, Ma. uniformis* and *Anopheles squamosus* have been known to transmit the virus as well.

A key factor for the emergence or re-emergence of RVF is the presence and number of competent vectors of RVF virus. Vector competence refers to the ability of a vector species to acquire and transmit a virus, while vectorial capacity comprises several parameters that influence the force or intensity of transmission of the virus, at the vector population level. These parameters include the population density of the vector, the vector competence, daily probability of taking a bloodmeal,

daily survival rate, the EIP, and the probability of the vector surviving the EIP (Smith *et al.*, 2012; Kramer & Ciota, 2015; Rückert & Ebel, 2018). Outside of known vectors, RVF virus has been shown to have the ability to infect a wide range of other vectors including *Aedes lineatopennis, Ae. cumminsii, Ae. albopictus, Anopheles christyi, An. pharoensis, An. Coustani*, *Culex antennatus, Cx. zombaensis, and Cx. quinquefasciatus* (Linthicum *et al.*, 1985; Logan, 1991; Sang *et al.*, 2010; Pepin *et al.*, 2010). However, vector competence alone is not enough to cause an outbreak. Other factors such as the abundance, lifespan, distribution and blood-seeking behavior all form important epidemiological components that coalesce together to cause RVF outbreaks. Despite numerous studies on the competence of various mosquito vectors to transmit the RVF virus, the vectorial capacity of these vectors, and the various components that comprise it remains under-studied. Further research is needed to understand the potential of these vectors and their role in the emergence and expansion of RVF into new geographical areas to become important public and veterinary health problems.

2.1.6 Risk factors for the emergence and re-emergence of RVF

Stable ecosystems regulate infectious disease transmission cycles by modifying habitat and vector dynamics, as well as reservoir and susceptible host characteristics. However, due to increasing human population, movement, encroachment and settlement, not only in Kenya but globally, anthropogenic changes continue to exert pressure on the current limited land and water resources everywhere on the planet (Vitousek *et al.*, 1997). It has long been hypothesized that these changes are surreptitiously altering the vector-host-environment equilibrium in previously undisturbed ecosystems, subsequently increasing the risk of infectious diseases (Myers & Patz, 2009). These changes include land alterations or transformations through clearing or reforestation, land division and fragmentation for small scale farming, grazing and intensification of agriculture for food production and mining or fracking for mineral extraction (Skole & Tucker, 1993; Leemans & Zuidema, 1995). Other changes result in destabilization and loss of biodiversity as a result of invasion and diversion of marine and wetland ecosystems for human activities like irrigation, fishing, hunting, hiking and touring. Human activities such as combustion of fossil fuels and burning of forests have also contributed to the alteration of atmospheric cycles of nitrogen, carbon, water and other chemicals and elements (Galloway *et al.*, 1995; Brown *et al.*, 2009).

Further literature suggests that other geographic and geologic factors such as soil types and textures, elevation, temperature and climate fluctuations may also impact on the likelihood of vector-borne disease outbreaks (Hightower *et al.*, 2012). RVF incidence has been shown to be higher in areas with clay or heavy clayey soil types while those areas with slow to extremely slow

natural drainage have an increased risk of having RVF outbreaks. This is due to their ability to retain water and moisture better and longer, plausibly aiding in rehydration of desiccated mosquito eggs in normally arid areas (Nguku *et al*., 2010). Increased risk of RVF was also found in areas with dense shrubs, marshes or bushes and most outbreaks have been observed to occur at elevations below 500 meters above sea level. It is postulated that altitude limits the geographical range of the vectors. These low-lying plains also allow water to pool easily thereby providing suitable habitats for larval activity as opposed to hilly areas. Studies further predict that the gradual increase in global temperatures over the last century has the potential to expand the spatial range of vectors as well as accelerate both the rate of larval development and pathogen replication within the vectors, consequently shortening their incubation period (Bett *et al*., 2017). Increased rainfall or availability of ground water also promotes vegetative growth, thus providing mosquito vectors with resting places and shade, enabling longer lifespans (Anyamba *et al*., 2002).

The risks to humans posed by these changes therefore require a comprehensive understanding and appreciation of the role played by local ecosystems and habitats. Any emerging trends that influence vector abundance and survival, as well as availability of naïve hosts destabilizes the vector-carrying capacity and may lead to spill over of infections into human populations (Molyneux, 1997; Mandal, 2012). In many cases, most of these environmental alterations are gradual and their effects may not immediately be seen or felt, often complicating initial assessment and prediction of their impacts on land development. Further, due to stochasticity and circumstance, the same changes may be associated with decreased risks of disease or other benefits in another locality or time (Patz *et al*., 2000; Lindahl & Grace, 2015). Additionally, these changes may directly impact on disease transmission, or may interact indirectly or diffusely with other environmental or social drivers within the causal pathway to create trade-offs that may result in increases in the incidence, frequency or severity of disease outbreaks (Halstead, 1988). Worse still, studies and techniques conducted to analyze the impacts of these changes on risk of disease are costly and time consuming, limited in scope and are usually unable to predict events of low probability but of high devastating consequences (Myers & Patz, 2009).

2.1.7 Mosquito surveillance

Mosquito surveillance is the sustained and consistent monitoring of adult and larval mosquito populations over time. This involves understanding the population dynamics and species distribution of mosquitoes in a specific locality, for the purpose of detecting changes that may impact on the potential for disease transmission (Centers for Disease Control and Prevention, 2003; Gu *et al*., 2008). It can be targeted to a specific season in time or to a specific geographical

area in a place. Most arboviruses can be detected in mosquito vectors themselves, even before transmission, which can allow vector control measures to be implemented to reduce the risk of human and animal disease outbreaks (Ndiaye *et al.*, 2018). The establishment of a comprehensive mosquito surveillance program is critical to the control and even elimination of most mosquito-borne diseases (Osório *et al.*, 2014). Mosquito surveillance goals include identifying problem and risk areas, assessing the need for, and timing of interventions and monitoring the effectiveness of interventions. Eventually, having a better understanding of transmission cycles and the role of potential or other invasive vector species may be inculcated into the surveillance system (Centers for Disease Control and Prevention, 2003; Schaffner *et al.*, 2013). Key aspects of a successful mosquito surveillance program include mosquito field collections over a representative geographical area, laboratory support for species and host spectrum identification as well as testing for the presence of infectious agents, and data analysis and management for monitoring trends over time and space. A mosquito surveillance system can also be integrated into a larger and more efficient vector surveillance and control program incorporating ecological surveillance, animal sentinel surveillance and surveillance for human cases at clinics or hospitals, in cases of vector-borne diseases of economic or public health importance (World Health Organisation, 2008; Golding *et al.*, 2015).

2.1.7.1 Adult surveillance

Routine adult mosquito surveillance attempts to understand the ecology and evolution of targeted mosquito vector species with the aim of monitoring population dynamics and trends, identifying the species present as well as detecting circulating or emerging mosquito-borne diseases. This information is critical in determining the control measures to be implemented (Ochieng *et al.*, 2013). Tracking changes in mosquito populations allows comparative analyses to be done with historical, seasonal or regional data, which is essential in identifying spatio-temporal differences in risk, detect changes in risk, as well as help in the mapping and forecasting of new problem areas for targeted control (Agha *et al.*, 2017; Agha *et al.*, 2019). In addition to mosquito ecology, it is important to consider the evolution of mosquitoes in their natural environments, due to their ability to develop resistance to insecticides and other chemicals used for long-term control, as well as resistance to drugs and therapeutics used to treat mosquito-borne diseases in animals and humans (Abdel-Muhsin *et al.*, 2004; Mita & Tanabe, 2012; Liu, 2015; Matiya *et al.*, 2019).

2.1.7.2 Larval surveillance

Larval surveillance involves regular monitoring of mosquito breeding areas and other larval habitats for identification and treatment of these sites, reducing larval numbers or eliminating them before adult mosquitoes develop. Larval surveillance also aids in determining the environmental

conditions that favor mosquito emergence and development, helps to identify the species present as well as map the size of mosquito populations and stages of development, in order to define the potential for disease transmission. Tracking larval habitats also aids in determining the timing and type of control needed, and in the assessment of the effectiveness of controls implemented (Centers for Disease Control and Prevention. 2003; Washington State Department of Health, 2008). Larval monitoring can be particularly helpful when sampling mosquito species rarely attracted to the commonly used adult sampling methods.

2.1.7.3 Mosquito collection and preservation

Collection of adult mosquitoes

Mosquito sampling methods can be grouped into two: methods for collecting flying adults, and those for collecting resting adults. The choice of method to be used depends on the objective of the surveillance program, the targeted species, the environmental conditions at the proposed sampling sites and the availability of human and other resources (Paternina & Rodas, 2018). Sampling flying mosquito populations captures those in search of nectar, those looking for suitable sites to lay eggs or those currently seeking a host. Capturing active mosquitoes favors mostly females at various stages of the gonotrophic cycle, while capturing blood-fed females would be ideal for determining the host spectrum. There are a variety of ways of capturing flying adult mosquitoes including: 1) using humans or animals as baits for landing and biting (Hawkes *et al*., 2017); 2) using CO_2-baited suction traps or using dry ice with a net or hand aspirator to suck mosquitoes attracted to the block of dry ice; 3) using light traps which can also be CO_2-baited to improve on their efficiency (Sriwichai *et al*., 2015); 4) using BG-Sentinel and Biogents Mosquitaire traps specifically for *Ae. aegypti* and *Ae. albopictus* (Li *et al*., 2014); 5) using CO_2-baited mosquito magnet traps that produce carbon dioxide by burning butane and 6) using gravid and sticky traps designed to collect gravid egg-laying females. Depending on the species, resting adult mosquitoes can be captured either indoors on walls and ceilings, furniture and clothing, or outdoors on shrubs and bushes, tree crevices and cracks, or rocks and caves. Ways of collecting indoor resting adults include: 1) using mouth or battery-operated aspirators; 2) using collection tubes; and 3) pyrethroid spray catches (Ndiath *et al*., 2011). Methods of collecting outdoor resting adult mosquitoes include: 1) use of resting boxes 2) use of drop-net cages in the vegetation; 3) use of hand-nets and 4) using motorized aspirators. However, sampling a representative number of outdoor resting adults is a very time- and resource-intensive effort (Silver, 2008; Medlock *et al*., 2018). It is vital that the collection method selected takes into consideration the physiological and behavioral characteristics of the targeted mosquito species, in order to ensure representative sampling of the entire population present (Bidlingmayer, 1985; Brown *et al*., 2018).

Larval collection

Larval collection entails the initial localization, mapping and characterization of breeding sites and other potential larval habitats. Depending on the species, mosquito larvae can be found in a variety of naturally occurring permanent or semi-permanent habitats consisting of either stagnant temporary water bodies such as marshes, floodwaters, small ponds and ditches along small rivers, or consisting of clear running water, including small rivers and streams, ditches and drains. Larval breeding grounds can also be artificial, such as irrigation tunnels and feeder canals, old containers and tires, road trucks, disposal pits, cans and plastics especially in urban areas. Different mosquito species will colonize and lay their eggs in diverse habitats, due to differences between the characteristics of water bodies such as the water quality, size, and presence of vegetation and other debris, and the ecological conditions and human activities surrounding them. Larval sampling will therefore require detailed knowledge of the life cycle and behavioral ecology of the targeted species (Fillinger *et al.*, 2009; Low *et al.*, 2016). Mosquito larvae can be collected by netting using a fine net or sieve, dipping using dippers or trays, or aspirating with tubes or pipettes. Each sampling method will vary depending on the species, water size and depth, presence of vegetation or other matter, and water clarity. Dipping is the most widely used method and various dipping techniques include complete submersion, partial submersion and shallow skim. This method is mostly used to determine the abundance of larvae by taking several dips from a defined area and counting the larvae from each (Washington State Departement of Health, 2008; Medlock *et al.*, 2018). The netting method is however more suitable for larger water bodies, as it is less time and resource intensive. (Brisco *et al.*, 2016). After collection, mosquito samples should be transported in their nets or traps to the laboratory in dry ice. At the laboratory, the samples are sorted, counted, pooled, labeled and stored in vials with 70–80% ethanol for immediate morphological identification, blood meal analysis, pathogen search or insecticide resistance testing (Medlock *et al.*, 2018), or cryopreserved at –80 °C in the medium term (Logan *et al.*, 1991) or indefinitely in liquid nitrogen for future DNA or other analyses (Vyletova *et al.*, 2016; Pathak *et al.*, 2018).

2.1.7.4 Mosquito identification

Mosquito identification entails the description and characterization of the morphological features of mosquito samples for the purpose of assigning the correct genera and species name. This is normally achieved by observation under low power magnification of external body features such as the head, thorax, legs, wings and external genitalia, when distinguishing between the sexes. Mosquito identification is vital for the establishment and assessment of mosquito surveillance and control efforts (Erlank *et al.*, 2018). The genera name assigned represents a group of

morphologically related mosquitoes while the species name represents a more closely related group of mosquitoes, similar in structure and physiology, and capable of interbreeding. Mosquitoes are insects belonging to the phylum *Arthropoda*, class *Insecta* and order *Diptera*. All insects in this order have two well developed wings, but unlike other flies, mosquitoes have scales along the veins of their wings and margins, and on most of their body. They also have long multi-segmented antennae, and the female mosquito mouthparts form a long proboscis that functions by piercing and sucking on hosts or nectar. Males have feathery antennae instead, and their mouthparts are only suitable for sucking nectar. Within the order *Diptera,* mosquitoes constitute the family *Culicidae*, which is composed of two subfamilies: *Anophelinae* and *Culicinae*. The genus *Anopheles* is the most significant mosquito in the subfamily *Anophelinae*, constituting nearly 400 species worldwide. Most mosquitoes belong to the subfamily *Culicinae*, whose main genera includes *Aedes*, *Culex*, *Filcalbia*, *Aedomyia*, *Coquillettidia* and *Mansonia*, among others. These genera are further distinguished into various species (Edwards, 1941; Jupp, 1986). Mosquitoes are most frequently identified using the adults and larvae stages. However, the eggs can also be used in identification.

The palpi of adult female *Anopheles* mosquitoes are as long as the proboscis, unlike all other genera. They also have a rounded scutellum, as opposed to other genera of mosquitoes whose scutellum is trilobed. *Anopheles* mosquitoes rest with the head, thorax, and abdomen in a straight line, while other genera rest with the head at an angle to the rest of the body. *Anopheles* eggs are laid singly on the water surface and coalesce into clusters of stellate and other geometric patterns. *Aedes* mosquitoes are quite distinct due to the black and white markings on their bodies and legs. The abdomen of *Aedes* mosquitoes have pointed tips while those of *Culex*, *Mansonia* and *Coquillettidia* are blunt or rounded. *Culex* mosquitoes are distinguished by their lack of prespiracular bristles. *Aedes* females lay their eggs singly above the water line or in depressions while *Culex* females glue their eggs into a raft on the water surface (Edwards, 1941; Jupp, 1986). New methods of mosquito identification such as MALDI-TOF MS and other molecular assays have recently emerged due to shortage of entomological expertise, loss of distinguishing body features during the collection, transport and storage of mosquito samples and the presence of cross-species, leading to human error (Müller *et al.*, 2013; Yssouf *et al.*, 2016; Jourdain *et al.*, 2018; Mewara *et al.*, 2018).

2.1.7.5 Blood meal identification

Mosquito host spectrum analysis attempts to describe the feeding habits and preferences of mosquito vectors, for the purpose of examining the transmission dynamics of mosquito-borne

diseases. Serological techniques such as the precipitin test and the enzyme-linked immunosorbent assay (ELISA) have been used over the years, but these tests are of low specificity, only distinguishing groups of vertebrates as potential hosts (Weitz, 1956; Gomes *et al.*, 2001; Mucci *et al.*, 2015). Molecular assays based on DNA analysis of ingested blood is currently the only way of identifying mosquito host species (Kirchgatter *et al.*, 2014; Hernández-Triana *et al.*, 2017). DNA-based techniques have more recently gained ground due to the ease in carrying out multiple analyses on extracted DNA samples such as mosquito species confirmation, identification of various pathogens and vector population genetic studies on the same sample. Samples can also be preserved dry and stored indefinitely, or shipped to destinations far from the sampling sites for testing (Hernández-Triana *et al.*, 2017). A PCR reaction, which is a molecular technique of replicating a specific DNA segment into several copies, is first run, targeting and amplifying the mitochondrial cytochrome b protein using vertebrate-specific primers. It is followed by DNA sequencing of the PCR product (Kocher et al., 1989; Kent & Norris, 2005; Santos *et al.*, 2019; Gyawali *et al.*, 2019). However, the quality and quantity of blood contained in engorged mosquitoes, as well as laboratory protocols used for analysis are critical in the identification of host DNA, as decomposed blood reduces efficacy (Martínez-de la Puente *et al.*, 2013). The extent of digestion of blood meals also influence the degree to which they can successfully be profiled, with meals ingested more than 8 hours before sampling having low success rates (Mukabana *et al.*, 2002).

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3.0 CHAPTER 3: Publication 1

This chapter describes how the baseline seroprevalence of RVF was determined in order to examine regional and seasonal differences in RVF risk in livestock between the three study sites.

3.1 Inter-epidemic Rift Valley fever virus seroconversions in an irrigation scheme in Bura, south-east Kenya

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3.1.1 Abstract

Rift Valley fever (RVF) is an acute mosquito-borne viral zoonosis whose outbreaks are often associated with prolonged rainfall and flooding, during which large numbers of vectors emerge. Recent studies into the inter-epidemic maintenance of RVF virus suggest that both vertical transmission in vectors and direct transmission between hosts act in combination with predisposing factors for persistence of the virus. A comparative longitudinal survey was carried out in Tana River County, Kenya, in irrigated, riverine and pastoral ecosystems from September 2014–June 2015. The objectives were to investigate the possibility of low-level RVF virus transmission in these ecosystems during an inter-epidemic period, examine variations in RVF virus seroprevalence in sheep and goats and determine the risk factors for transmission. Three hundred and sixteen small ruminants were selected and tested for immunoglobulin G antibodies against RVF virus nucleoprotein using a competitive ELISA during six visits. Data on potential risk factors were also captured. Inter-epidemic RVF virus transmission was evidenced by 15 seroconversions within the irrigated and riverine villages. The number of seroconversions was not significantly different (OR = 0.66, CI = 0.19–2.17, p = .59) between irrigated and riverine areas. No seroconversions were detected in the pastoral ecosystem. This study highlights the increased risk of inter-epidemic RVF virus transmission posed by irrigation, through provision of necessary environmental conditions that enable vectors access to more breeding grounds, resting places and shade, which favour their breeding and survival.

Keywords: arbovirus, emerging infectious disease, irrigation, land-use change, Rift Valley fever,

3.1.2 Introduction

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis that causes recurrent epidemics in sub-Saharan Africa, with recent incursions into the Arabian Peninsula (Linthicum *et al*. 1985, Balkhy & Memish 2003, Grobbelaar *et al*. 2011). The disease was first described in Kenya in 1931 but has since spread across Africa and the Arabian Peninsula (Nanyingi *et al*. 2015). The RVF virus (RVFV) is a *Phlebovirus* of the family Bunyaviridae, that causes high morbidity and mortality in juvenile animals, frequent abortions in susceptible pregnant animals while older animals may experience mild or subclinical manifestations (Pepin *et al*. 2010; Sumaye *et al*. 2013). Humans acquire the infection mainly by direct contact with sick animals or animal products during large epidemics (Laughlin *et al*. 1979; Davies 2010). The disease usually manifests itself as a mild malaria-like illness with fever, headache and joint pains, with chronic cases experiencing encephalitis, retinitis or ocular disease. Case fatality proportions in acute cases are estimated at 1 - 2% and are usually associated with hemorrhagic manifestations, acute renal and hepatocellular failure (Al-Hazmi *et al*. 2003)

Epidemics cause direct economic losses in both animal and human productivity through death, and indirect losses through the ensuing chronic morbidity in human hosts and loss of replacement animals. This leads to overall losses for farmers and other players along the value chain. The quality of nutrition and food security in affected regions also deteriorates while communities undergo immense psychological distress as a result of bereavement, displacement and loss of livelihoods (Sindato *et al*. 2011). Costs associated with controlling the disease are also incurred by exporting countries while bans imposed on livestock and movement of livestock products by importing countries lead to further loss of revenue (Balkhy & Memish 2003; Rich & Miller 2005; Rich & Wanyoike 2010). In eastern Africa, outbreaks of RVF occur in cycles of 5 to 15 years and are often associated with prolonged and above normal rainfall resulting in the emergence of large numbers of blood-feeding mosquitoes, known to transmit the virus (Davies 1975, Linthicum *et al*. 1985). It is believed that susceptible domestic and wild ruminants then become infected with the virus from bites of infected mosquitoes and in turn amplify the virus, thereby propagating the epidemic by spreading it to other vectors (Evans *et al*. 2008). In eastern Africa, these high precipitation levels are often associated with *El Niño* events and hence RVF epidemics have strongly been linked to *El Niño.* A few epidemics have however been observed during the normal wet periods (Anyamba *et al*. 2010). The mechanisms for persistence of the virus in the inter-epidemic period (IEP) are still poorly understood and its incidence, both in human and animal hosts has not been properly determined (Anderson & Rowe 2010; LaBeaud *et al*. 2011).

It is important to study the ecology and epidemiology of RVFV in order to understand how the disease is maintained and sustained during long inter-epidemic periods (Nicolas *et al*. 2014). Current knowledge suggests that the virus survives in mosquito eggs through transovarial transmission from female mosquitoes of the genus *Aedes* when temporary *dambos* dry up (Linthicum *et al*. 1985; Pepin *et al*. 2010). Even though several studies have detected RVFV activity in humans and animals during long IEP (LaBeaud *et al*. 2008; Sumaye *et a*l. 2013; Lichoti *et al*. 2014; Sumaye *et al*. 2015) earlier studies into the inter-epidemic maintenance of the virus proposed that long-term persistence among mosquito species was more important for the maintenance of RVFV in a closed ecosystem, due to the small period of infectiousness in livestock (Favier *et al*. 2006; Gaff *et al*. 2007; Fischer *et al*. 2013; Pedro *et al*. 2014). More recent models however suggest that both vertical transmission in vectors and direct transmission between hosts, may be responsible for persistence of the virus once introduced into an ecosystem (Manore & Beechler 2015; Nicolas *et al*. 2014). The IEP dynamics may also vary with land-use changes, such as the expansion of agriculture and irrigation with the resultant change in vector activity and distribution that may alter RVFV transmission patterns (Khasnis & Nettleman 2005). This is due to sensitivity of mosquito vectors to alterations in local environmental parameters such as temperature, land cover and precipitation (Russell 1998; Githeko *et al*. 2000).The objectives of this study were to determine whether low-level RVFV transmission was ongoing in small ruminants during an inter-epidemic period in Bura irrigation scheme and evaluate the role of potential risk factors for transmission. This is the first study to compare RVFV seroconversion between different ecosystems incorporating land use.

3.1.3 Materials and Methods

Study sites

The longitudinal study was conducted between September 2014 and June 2015 in Bura irrigation scheme and two other villages with differing ecosystems within Tana River County (between 1.32278S, 39.9532E and 1.12218S, 39.7046E). Tana River County is located in the southeastern region of Kenya with an area of 35,375.8 square kilometers (Figure. 1). This County is currently undergoing rapid land-use changes as a result of construction of new irrigation schemes as well as expansion of existing ones along the Tana River. Over 90% of Tana River County is arid or semi-arid, and experiences low bimodal and erratic rainfall of between 300 – 800 mm per year with areas adjacent to the coast receiving up to 1,200 mm per year. This county reported the first outbreak of RVF in 1961 and has been involved in 5 out of 11 national epidemics between 1951 and 2007, the last one being in 2007 (Munyua *et al.* 2010, Murithi *et al.* 2011). Three different

sites representing an ecological gradient ranging from an irrigated area, riverine ecosystem and pastoral ecosystem were chosen in the county.

Bura irrigation scheme is situated along the Tana River and near Bura town, the second biggest town in Tana River County. Livestock within the scheme are mostly zero-grazed and fed left-over produce from the farms or grazed on nearby bushes. They also occasionally receive veterinary care from local government officials, or self-administration of veterinary products bought from local markets by the owners. Husingo village representing a riverine ecosystem is in close proximity to the river and is surrounded by forested areas around it. Livestock graze in nearby bushes and forests and no regular veterinary care is provided. Chifiri village representing a dry pastoralist ecosystem is a smaller village with fewer households compared to Husingo and Bura irrigation scheme. However, each household in this village had more people and animals compared to the others. There were also more livestock species in Chifiri which included sheep, goats, cattle, donkeys and camels compared to the other two villages that mainly kept sheep and goats. In Chifiri, livestock leave early in the morning to graze away from home, and sometimes do not return for several days. During the dry season, some households move to greener areas in search of water and pasture.

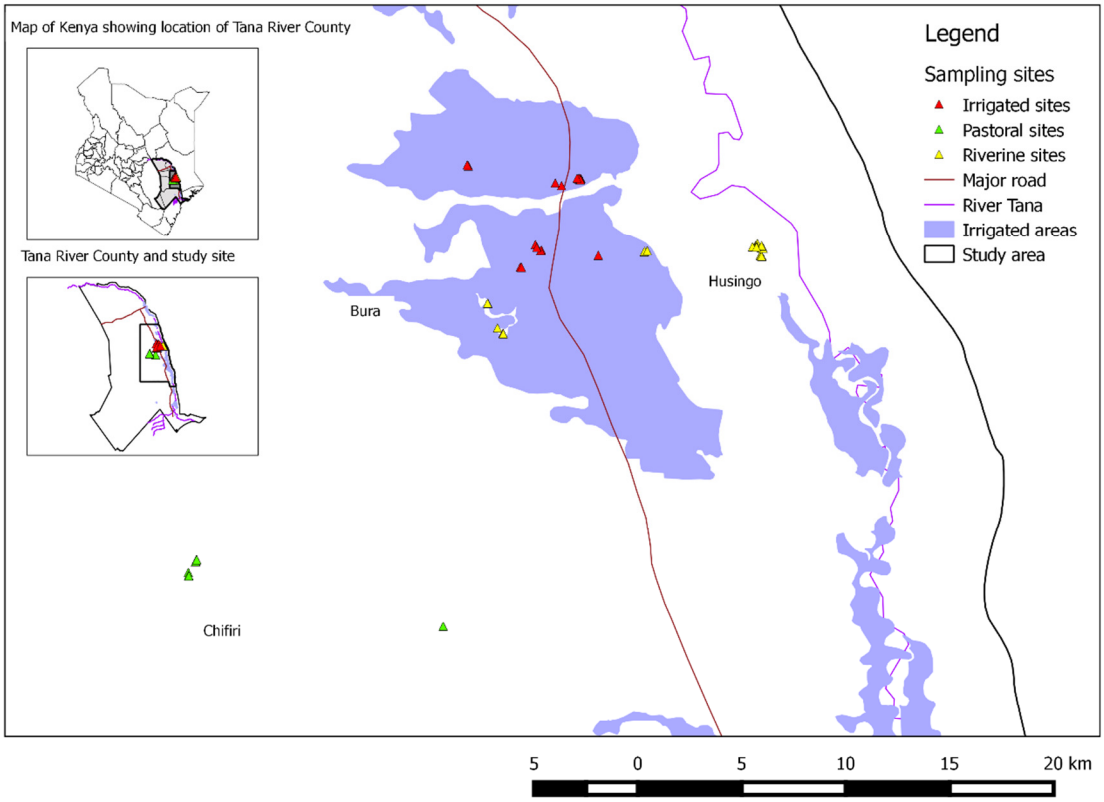

Figure 1. Map of Kenya showing the three study sites; Bura irrigation scheme, Husingo and Chifiri villages. Two households relocated from Husingo village halfway through the study. One settled inside Bura irrigation scheme while the other settled just outside the scheme. One herd in Chifiri village also relocated further into the bush in search of water and pasture also halfway into the study.

Ethical approval

Ethical approval for this project was obtained by the Ethics and Scientific Review Committee (ESRC) of the Africa Medical and Research Foundation (AMREF) number (REF: AMREF-ESRC P65/2013).

Selection of sentinel animals

A meeting with the elders of the villages and local government officials was held at the beginning of the study in which the background and objectives of the study were discussed, and their roles outlined. Oral consent was given by participants who agreed to take part. Sheep and goats of all ages and both sexes were selected and identified using numbered ear tags. Age of animals was estimated using the presence of permanent incisor teeth, and body scores judged by evaluating

the amount of body fat reserves using the standard 5-point scale (Wildman *et al*., 1982). The age categories were kid/lamb (less than 6 months), weaner (6-12 months) and adult (more than 12 months). In total, 21 households were selected for sampling in Bura irrigation scheme and 13 in the riverine Husingo village. These households were randomly selected from a sampling frame of households whose livestock had previously tested seronegative in an earlier randomly selected cross-sectional survey implemented between August – November 2013. Four households were selected randomly from the pastoral Chifiri village, which was not included in the earlier study. The sampling frame was developed by listing all the households with the help of local administrative officers (chiefs and village elders). During subsequent visits, animals lost to the study were replaced with others within the same herd.

Data collection and storage

Data collection tools were designed on Microsoft Excel using the XForm standard (https://www.w3.org/TR/xforms/). The Excel spreadsheets were uploaded to the Azizi Biorepository Open Data Kit's (ODK, https://opendatakit.org/) aggregate server (http://azizi.ilri.org/repository/) and then downloaded to ODK Collect version 1, an android application, which was installed on a Google Nexus 5 phone. For each household, the number of humans and their main occupation, herd size, health and disease history were collected while for each animal, the age, sex, species and body condition of the animal was collected. The data were then uploaded to the ODK aggregate server.

Sampling, transportation and processing

Blood samples were collected every 4-6 weeks between September-December 2014 and 6-8 weeks from January-June 2015. Up to 20 ml of venous blood was obtained from the jugular vein of adults and 10 ml from animals less than 6 months, using uniquely barcoded plain 9 ml vacutainer tubes. In the field laboratory, clotted blood was centrifuged at 3500 rpm for 5 minutes and serum aliquoted into two 2 ml barcoded cryovial tubes. These tubes were then linked to the original vacutainer tube using software for aliquoting samples called Ukasimu version 1 (https://github.com/ilri/ukasimu) pre-installed in a laptop, by scanning the vacutainer tube and its two accompanying aliquots into the system and saving in the database.

Laboratory screening of RVF virus antibodies

Samples from each animal were tested every 4-8 weeks to identify the two testing occasions between which seroconversion had occurred. If animals were positive on the first sampling occasion, they were assumed to be positive for all further samplings, and not tested further. The serum samples were screened for anti-RVF virus IgG antibodies using the commercially available competitive ELISA kit from Innovative Diagnostics (ID Screen® Rift Valley Fever Competition

Multispecies ELISA, ID-Vet, Montpellier, France), following the manufacturer's instructions. In brief, 55 µl of dilution buffer was added to each micro-well of a dilution plate followed by 55 µl of positive control, negative control and test sera. The dilution plate was shaken briefly and 100 µl of the diluted sera were transferred to the recombinant RVF virus-nucleoprotein-coated test micro-plate provided. The plate was incubated for 1 hour at 37°C. The plate was then washed three times with washing solution, dried and 100 µl of Anti-RVF virus-nucleoprotein conjugate solution added before incubating for 30 minutes at room temperature. The plate was then washed three times and dried before 100 µl of substrate solution was added to each well. Incubation of the substrate reaction was then done for 15 minutes at room temperature in the dark. This reaction was stopped by addition of 100 µl of stop solution into each well. The optical densities (OD) of the test plate were read at a wavelength of 450 nm using the Synergy™ HT Multi-detection Micro-plate Reader (Biotek®, Winooski, VT, USA). The optical densities (OD) of the test mirco-plate were exported from the Gen5™ micro-plate software (Biotek®, Winooski, VT, USA) used with the microplate reader into MS Excel files equipped with necessary formulae to automatically validate and interpret the test results. The test was considered valid when the mean OD of the negative control (NC) was greater than 0.7 (ODNC > 0.7) and the mean OD of the positive control (PC) was less than 30% of the mean OD of the negative control (ODPC/ODNC < 0.3). Sample interpretation was determined by calculating the competition percentage (S/N %), by dividing the sample OD with that of the mean negative control and multiplying this with 100 ((OD Sample / ODNC) x100). Samples with S/N% ≤ 40% were considered positive. Those with S/N% > 50% were considered negative and those with 40% < S/N% ≤ 50% were considered doubtful. Samples falling into the latter category were retested, and in case they remained doubtful, would have been excluded from analysis. However, in this testing, no samples were doubtful.

Data analysis

Two sets of analysis were done; the first one examined potential risk factors for seropositivity using all seropositive animals at the end of the study, while the second used seroconversion data of only those found seronegative at the beginning and followed up, to determine the impact of irrigation. Data on the pastoralist Chifiri village were excluded from the first set of analysis due to the differing selection method used. For risk factor analysis, an animal was only included once, and considered positive if being positive at the end of the study. Univariable and multivariable evaluation of animal characteristics was done using a generalized linear mixed-effects model (GLM) with binomial error structure (lme4package) in R 3.2.3 software (R Development Core Team, 2010) (Bates et al., 2014). Variables examined were species, sex, age, breeding status,

body score, vaccination status, total number of susceptible animals in the household, main household livelihood and number of humans in the household.

Variables significant in the univariable analysis, or believed important based on the causal diagram, were included in multivariable analysis. Household was included as a random effect while village was included as a fixed effect to allow comparison between the irrigation and riverine areas. Bidirectional elimination of variables was used, and models compared using the Akaike Information Criterion, corrected for finite sample sizes (AICc). The most parsimonious model with the smallest AICc was selected. Survival analysis was used to examine the time until seroconversion, in which Kaplan-Meier survival curves were utilized to plot the probability of survival within the different sites using the Survival package in R. An animal was considered to have seroconverted when a previously negative ELISA test turned positive in a later visit, during the course of the study. Seroconversions were considered to occur in the middle of two visits while censored animals were considered at risk until the last testing before they were lost to follow up. Cox proportional hazards regression with random effects for household was applied to estimate hazard ratios for the two sites while adjusting for age using the Coxme package in R. Herds that migrated into new areas were captured as time-varying covariates.

3.1.4 Results

Three hundred and sixteen animals were tested for antibodies against RVF virus during six visits (Table 1). Of the 247 animals selected for follow-up at the beginning of the study, 55 were lost to follow-up during the course of the study and were replaced by 69 animals. In total, 228 animals were goats and 88 were sheep, with 78% of animals being female. Most of the animals were adults while 35% were weaners, kids or lambs of between 3 – 12 months old. The pastoral village had larger herd sizes, with an average 130 animals per herd (range 44 - 231), whereas the irrigated villages had 16 and riverine village had 12 animals in average (ranges 2 – 42, 3 – 26 respectively). The average household size in the pastoral village was 13 (range 12 – 16) people while average in the irrigated and riverine villages was 6 and 8 (ranges 2 – 17, 3 – 13) respectively. Majority of households in the irrigated villages reported crop farming as their main livelihood, those in the riverine village reported mixed farming with crop and livestock, while those in the pastoral village reported livestock keeping as their main livelihood.

Table 1: Number of animals sampled and replaced during each visit to Bura irrigation scheme, Husingo and Chifiri villages. The number of RVF virus seropositive animals is shown in brackets.

Site	**Animal species**	**Sampling period**					
		September 2014	November 2014	December 2014	January 2015	March 2015	June 2015
Irrigation scheme (Bura)	Sheep	27(0)	26(0)	29(1)	30(2)	31(2)	30(2)
	Goat	83(3)	77(4)	86(6)	80(5)	83(8)	79(8)
	Number replaced	0	4	6	12	6	0
	Total	110(3)	107(4)	121(7)	122(7)	120(10)	109(10)
Riverine (Husingo)	Sheep	30(0)	28(0)	29(0)	27(3)	25(2)	23(2)
	Goat	59(1)	49(1)	59(1)	49(6)	45(4)	49(5)
	Number replaced	0	11	2	0	4	0
	Total	89(1)	77(1)	90(1)	76(9)	74(6)	72(7)
Pastoralism (Chifiri)	Sheep	9(1)	2(0)	3(0)	6(1)	14(1)	12(1)
	Goat	39(13)	33(16)	35(16)	35(16)	34(16)	34(16)
	Number replaced	0	5	4	6	9	0
	Total	48(14)	40(16)	42(16)	41(17)	57(17)	46(17)
Total tested (month)		247(18)	224(21)	253(24)	245(33)	251(33)	227(34)

Seropositivity

Animals within the pastoralist village had an initial seropositivity of 29% (14/48) which stayed about the same during the course of the study. On the other hand, seropositivity steadily increased from 2.7% in the irrigated area during the first visit in September 2014 to 9.2% at the last visit in June 2015, while it increased from 1.1% to 9.7% in the riverine area (Figure2).

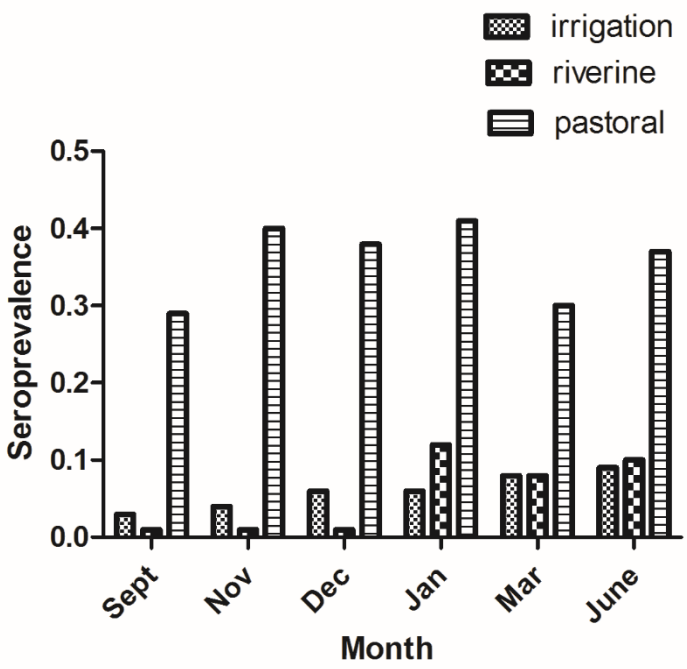

Figure 2. Site specific proportions of seropositive animals for Rift Valley fever virus during each visit in Bura (irrigation), Husingo (riverine) and Chifiri (pastoral) villages between September 2014 and June 2015.

Female animals in Bura irrigation scheme and Husingo village had an overall seropositivity of 9.8% while that of male animals was 3.7%. There was no significant difference in seropositivity between sheep and goats as well as between male and female animals. Seropositivity varied with age, with younger animals less likely to be seropositive than adults. There were no seropositive animals among the young kids and lambs of less than 6 months (Table 2).

Table 2: Results of a multivariable model for Rift Valley fever seropositivity in small ruminants in Bura irrigation scheme and Husingo village in Tana River County, Kenya, during September 2014 – June 2015. The herd size included all susceptible domestic animals kept by the household.

		Animals	**Total seropositive**	**Odds ratio**	**Confidence Intervals**	**P-value**
Species	Goat	182	16			
	Sheep	65	5	0.70	0.21-1.96	0.52
Sex	Female	193	19			
	Male	54	2	0.60	0.09-2.51	0.53
Age	Adult	166	19			
	Young	81	2	0.15	0.03-0.89	0.02
Site	Irrigation (Bura)	139	12			
	Riverine (Husingo)	108	9	0.90	0.32-2.61	0.82
Herd size	<5 animals	7	2			
	6-15 animals	19	10	0.82	0.19-5.70	0.82
	>15 animals	8	9	1.50	0.33-10.62	0.64

Seroconversion

At the beginning of the study, 229 animals out of 247 were seronegative and an additional 65 seronegative animals out of 69 were recruited during the study. Of the 15 animals that seroconverted during the study period, 7 occurred in the irrigation village and 8 in the riverine village. There were no seroconversions observed in the pastoral village. Using the Fisher's exact test, the proportions of seroconversions was not significantly different (OR=0.66, CI=0.19-2.17, p=0.59) between irrigated and riverine areas. Seroconversions in the irrigation villages were spread over 4 months with 3 occurring in December 2014. All 8 in the riverine area occurred in January 2015 (Figure 3). Observed seroconversions were significantly higher in the wet season between November 2014-January 2015 than in the rest of the dry season (OR=71.22, CI=13.54-752.15, p=<0.001)

After adjustment of RVFV seroconversion hazard for age using Cox proportional hazards technique, seroconversion rates were not significantly different between the irrigation and riverine sites (Figure 4). The hazard ratio for RVF virus seroconversion was 0.71 (CI=0.26-1.91, p=0.11).

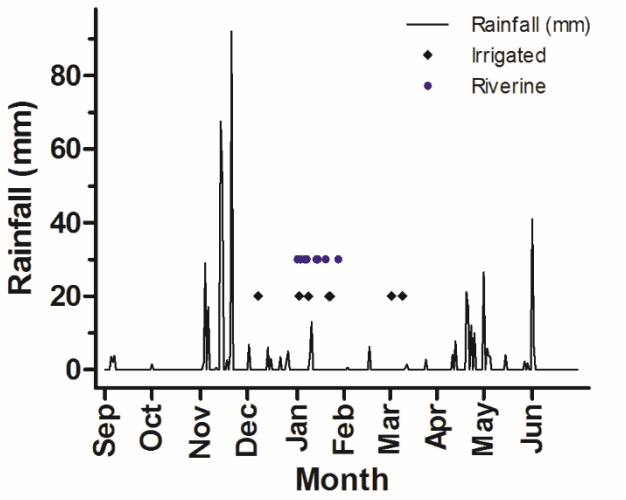

Figure 3. Rainfall received in the study site during September 2014 – June 2015. Circles and squares indicate the time of seroconversion of 15 animals sampled during this period (Dec 2014 – Mar 2015).

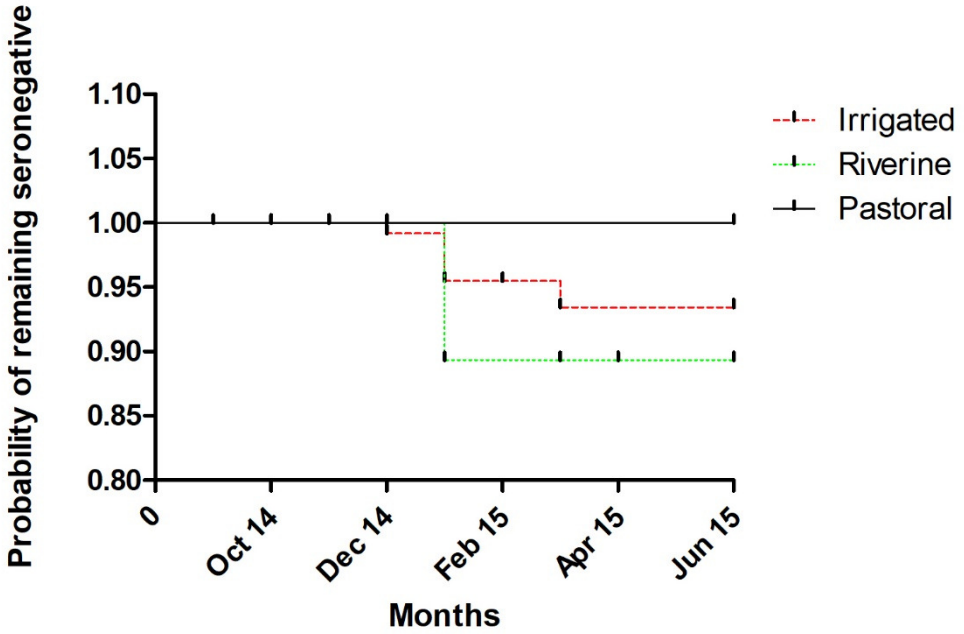

Figure 4. Kaplan-Meier survival plots of seronegative sheep and goats in the 3 study sites. Lines indicate percent survival in months of 294 animals sampled in Bura (irrigation), Husingo (riverine) and Chifiri (pastoral) villages between September 2014 and June 2015.

3.1.5 Discussion

This study investigated RVF seroconversions among sheep and goats during an inter-epidemic period between September 2014 and June 2015 in Bura, Tana River County. The presence of antibodies against RVF among these animals confirms the existence of RVF in this part of Kenya. Detected seroconversions also suggest the presence of low-level RVF virus transmission during this time. Further evidence was provided by the presence of RVF virus antibodies among two young animals of between 6-12 months old in Bura irrigation scheme. Previous studies have detected RVF virus seropositivity among domestic and wild animals during long inter-epidemic periods in southern Africa and other parts of east Africa, with seroprevalences ranging between 15% - 23% (Evans *et al.* 2008; Anderson & Rowe 2010; Sumaye *et al.* 2013; Lichoti *et al.* 2014). Similarly, two studies on human hosts in East Africa detected RVF seroprevalences of between 11% - 13% (A Desiree LaBeaud et al., 2008; Robert David Sumaye et al., 2015). Most of these earlier studies were seroprevalence studies carried out within one local ecosystem and did not actually observe seroconversions. In our study, which was carried out in three differing ecosystems, there was no significant difference found in seropositivity between animal species

or between sexes. Increasing seroprevalence with age, as found here, was also consistently reported in these studies.

There were no seroconversions detected among animals sampled in the much drier pastoral Chifiri village located further into the hinterland. There were also no antibodies detected among young animals aged below 12 months in this village. This suggests extremely low or non-existent transmission in this ecosystem, despite high RVF seropositivity among adult animals. The higher seropositivity in Chifiri village was due to biased selection of households, whereby participating households were selected randomly in the pastoral area while only seronegative households from a previous cross-sectional study in the irrigated and riverine area were included in the study. The lack of seroconversions in this village might be explained by a lack of suitable vectors, owing to the harsh and dry climatic conditions in this ecosystem, despite the larger herd sizes and hence higher numbers of susceptible animals. High RVF seropositivity among adult animals could be as a result of seroconversions during an earlier short wet season in this ecosystem or government sponsored vaccination campaigns, the last of which was carried out in March 2012 (pers. comm., Dr Anderson Thuo Njau, DVO Bura). On the other hand, there was high turn-over of animals in the irrigation scheme and Husingo households in which animals would be sold, gifted or slaughtered in order to fulfil household necessities. There were also more mosquitoes and other insects observed in these ecosystems owing to cooler conditions, availability of water and vegetation which would potentially provide shade (data not shown). Transmission of RVF was therefore probably due to the presence of both vectors and naïve hosts leading to persistence of the virus in these habitats. The smaller number of hosts was however likely not enough to cause an epidemic.

In an attempt to understand how RVF is sustained between epidemics, many studies have tried to look at the wildlife-domestic animals interface, noting that wild animals could serve as potential reservoirs (Michel & Bengis, 2012; Olive et al., 2012). Several studies have indeed reported anti-RVF antibodies among different wildlife (Evans *et al.* 2008; LaBeaud *et al.* 2011) but one study that surveyed the seroprevalence in cattle and several wildlife species from numerous locations in Kenya over a 7-year IEP found no seropositive cattle that were in contact with wildlife (Lwande et al., 2015)

Other studies have suggested that low-level transmission during inter-epidemic periods of average rainfall may be significant in the maintenance of RVF virus in natural habitats by providing the primary cases and ideal conditions for rapid virus amplification and spread to other hosts during periods of prolonged rainfall (A Desirée LaBeaud et al., 2011). These riverine areas and forest edges appear to have both the vectors and hosts required to maintain inter-epidemic

transmission in domestic animals without necessarily requiring wildlife or transovarial transmission via dormant mosquito eggs.

Effective contact between infected vectors and naïve hosts is vital for successful RVF virus transmission (Weaver & Barrett, 2004). While there were no seroconversions detected in the pastoralist ecosystem, it is interesting to note that there was no significant difference between the RVF seroconversion rate in irrigated and riverine ecosystems, considering that riverine ecosystems are natural habitats while irrigation schemes are man-made. It is also intriguing to note the steady increase in seroprevalence within the irrigation scheme versus the sharp increase in the riverine ecosystem, which was primarily precipitated by rainfall. Creation and expansion of irrigation schemes in this region might consequently have the effect of establishing more habitats that contribute to the endemic transmission of vector-borne diseases that naturally occur in other similar ecosystems.

In summary, serological testing for anti- RVF virus antibodies in a longitudinal study of sheep and goats in Bura irrigation scheme, Husingo, and Chifiri villages representing irrigation, riverine and pastoral ecosystems between September 2014 and June 2015 demonstrated that spatial inter-epidemic transmission of RVF virus is highly heterogeneous in these habitats, as a result of diverse ecological drivers of the virus. It is also evident that low-level transmission continues on a regular basis in the irrigated and riverine areas, highlighting the increased risk of local RVF virus endemicity that is associated with the establishment or expansion of these schemes.

Irrigation schemes seem to be able to support inter-epidemic RVF transmission as well as riverine areas. These findings will provide policy makers with a better understanding for vector and RVF prevention and control within this changing environment. Future longer-term studies that track ongoing land-use, human and animal movements and settlements with the consequent micro-climatic changes in relation to vector and RVF virus transmission will greatly aid in targeting surveillance in order to combat the disease as well as prevent possible human exposure.

3.1.6 Acknowledgement

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4.0 CHAPTER 4: Publication 2

This chapter describes how the study evaluated the impact of irrigation expansion in Bura, Tana River County, on local mosquito ecology, which would in turn influence the emergence and maintenance of RVF virus. Specifically, it assessed the seasonal variation in mosquito abundance, the relative differences in abundance of mosquitoes and diversity of species between the three study sites and identified the host sources of blood meal in mosquito field samples to determine the host spectrum.

4.1 Relative distribution, diversity, and bloodmeal sources of mosquitoes and known vectors of Rift Valley fever Phlebovirus in three differing ecosystems in Bura, Tana River County, Kenya

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4.1.1 Abstract

Environmental modifications disturb the equilibrium of mosquito populations, altering the risk of mosquito-borne diseases. Mosquito distribution, diversity and blood-meal sources were examined to compare Rift Valley Fever (RVF) risk between irrigated, riverine and pastoral ecosystems in Bura, Tana River County, Kenya, between September 2014 and June 2015. Thirty-eight households and 21 irrigation fields were selected for the study. Mosquitoes were trapped with carbon-dioxide-impregnated CDC traps, one trap per household and three traps per irrigated field, and morphologically identified using taxonomic keys. Host DNA was extracted from engorged females and cytochrome b genes amplified by PCR to identify sources of blood meals. A total of 21,015 mosquitoes were collected: 5,742 within households in the three ecosystems and 15,273 within irrigated fields. Mosquitoes collected within irrigated fields belonged to eight genera and 37 species, while those from households within the irrigation scheme belonged to six genera and 29 species. Collections from riverine and pastoral households belonged to five and four genera, respectively. The most abundant genera in the irrigated fields were *Aedes* (21%) and *Mansonia* (22%), while *Anopheles* (43%) was the most abundant within households. Most mosquitoes in riverine and pastoral households belonged to *Anopheles* (76%) and *Aedes* (65%) genera respectively. Seasonal variation driven by rainfall was evidenced by spikes in mosquito numbers within irrigated and riverine ecosystems. Host species identification revealed goats and humans were the main sources of blood meal. There was an overall increase in mosquito abundance and diversity as a result of the presence of the irrigated ecosystem in this county, and an increased availability of highly RVF-susceptible hosts as a result of the establishment and concentration of residential areas, promoting potential vector-host contacts. These results highlight the impact of anthropogenic changes on mosquito ecology, potentially heightening the risk of transmission and maintenance of RVF in this region

Keywords: Rift Valley fever, mosquitoes, irrigation, species diversity, species distribution, arbovirus

4.1.2 Introduction

Land-use and land cover changes have been directly implicated in the emergence or spread of arthropod-borne diseases (Patz *et al*., 2000) such as West Nile fever, malaria and lyme disease. Mosquitoes seem particularly vulnerable to these changes, which significantly alter their population dynamics, species composition and competence (Johnson *et al*., 2008). Known mosquito vectors of RVF virus are broadly grouped into two. 'Reservoirs' are mostly *Aedes* species believed to maintain the virus through trans-ovarial transmission, and subsequently lay their infected eggs in temporary 'dambos' or semi-permanent pools of water that form in low-lying plains after heavy rains. 'Amplifying' vectors consist of mostly *Culex* species found within more permanent water bodies. These vectors become infected and amplify the virus, resulting in the transmission of disease to more hosts thereby propagating the epidemic (Sang *et al*., 2010; Marcantonio *et al*., 2015).

Recent studies in Kenya have attempted to associate the abundance and geographic distribution of vectors of RVF virus with spatio-temporal differences in RVF risk. In Sang *et al*., 2017, the abundance and diversity of known vectors of RVF virus varied significantly between different ecological sites.

This study aimed to examine and compare the abundance, seasonal variation and species distribution of mosquitoes and vectors of RVF virus in three differing ecosystems, within the context of irrigation expansion efforts in Tana River County and determine their vertebrate sources of blood meal. Other variables such as animal herd sizes and human hosts were also investigated. The results will help determine the likelihood of changes in RVF risk in this region because of changes in mosquito population dynamics, and in formulating comprehensive strategies for disease control. This study was part of a larger one whose objective was to evaluate and compare the inter-epidemic transmission of RVF virus in sheep and goats in differing ecosystems, and the risk factors precipitating these differences (Mbotha *et al*., 2017).

4.1.3 Materials and Methods

Study sites

Mosquitoes were captured in three ecologically distinct sites within Bura, Tana River County between September 2014 and June 2015 (between 1.32278S, 39.9532E and 1.12218S, 39.7046E, Figure 1). These were Bura irrigation scheme, Husingo and Chifiri villages. Bura irrigation scheme, situated along Tana River near Bura town, constitutes large tracks of land under irrigated cultivation. Households farming these lands congregate adjacent to the fields. They also keep small herds of sheep and goats for household use. Husingo, also located near

the river, represents a riverine locale. It is surrounded by forested areas, bushes and shrubs and occasionally attracts wildlife. Inhabitants practice seasonal farming on small tracks of land that flood during the rainy season and keep small herds of sheep and goats. Chifiri is located deeper in the drier hinterland and represents a pastoral ecology. Inhabitants keep large herds of diverse livestock including sheep, goats, cattle, camels and donkeys for commercial purposes. There is very scarce vegetation in Chifiri.

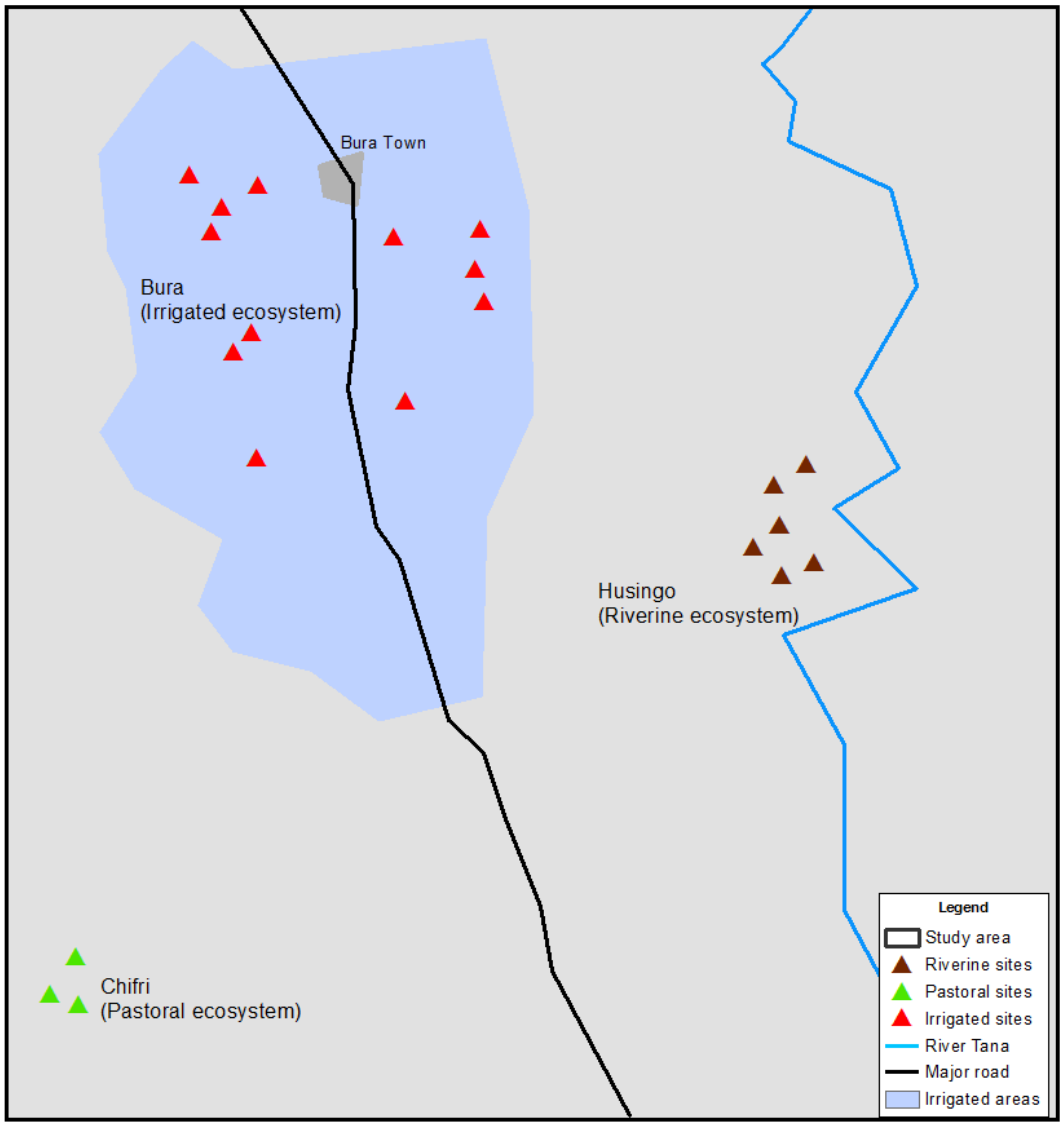

Figure 1: Schematic representation of the three study sites in Bura, Tana River County, sampled between September 2014 and June 2015; Bura irrigation scheme, Husingo and Chifiri villages. The map is not drawn to scale. Original map published in Mbotha et al., 2017

Selection of participating households

The selection of households has been described previously (Mbotha *et al.*, 2017). Briefly, 21 households in Bura irrigation scheme and 13 in Husingo village with RVF-sero-negative animals were selected for sampling for the previous study, and consequently used for this study. An additional four households were randomly selected from a list of all households in Chifiri village, which was not included in the previously mentioned study. Household characteristics such as size, animal herd size, source of livelihood and insecticide treated bed-net use were collected.

Mosquito collections and handling

Study sites were visited in the years 2014 (September and November) and 2015 (January, March and June), respectively. Carbon dioxide-baited CDC light traps (John W. Hock, Gainesville, Florida, USA) were placed within compounds of selected households, between the house and the livestock night shed adjoining it, from 5PM to 6AM for one night per visit. They were placed about 5 feet from the ground. In the irrigation scheme, three traps were set inside selected fields. Traps set in fields were placed in different locations around flooded areas or other potential larval breeding sites. Trapped mosquitoes were transferred to a field laboratory and immobilized using 99.5% triethylamine (Sigma-Aldrich, St. Louis, Missouri, USA), sorted, counted, labeled as the total number per household or field per trap, and stored in liquid nitrogen. The presence and number of larval breeding grounds was noted while immature stages of mosquitoes were collected from irrigation feeder canals, block feeders, unit drains and stagnant or marshy water between crops. They were left to mature in the field laboratory and included with the rest. All collections were transported to the Kenya Medical Research Institute's (KEMRI) laboratory and identified using the keys of Edwards (1941) and Jupp (1986) and pooled in groups of up to 25 by collection date, species, sex, and site. Blood-fed mosquitoes were preserved individually.

Identification of source of blood meal

Extraction of genomic DNA from blood fed mosquitoes was done using QIAGEN DNeasy blood and tissue kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's protocol and samples stored at -20^{0}C. Extracted DNA was used as template in PCR amplification of the targeted mitochondrial cytochrome b (cyt b) 358-bp region using primers L14841 (forward 5'-CCATCCAACATCTCAGCATGATGAAA-3') and H151494 (reverse 5'-GCCCCTCAGAATGATATTTGTCCTCA-3') (Kocher *et al.*, 1989). Purified amplicons were sequenced in forward direction (Molecular Biology GmbH, Germany) and sequences edited in BioEdit (Hall, 1999). Edited sequences were compared to nucleotide entries at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov/) via BLASTN®

(https://blast.ncbi.nlm.nih.gov/Blast.cgi, Altschul *et al.*, 1990). The cut-off for identity was set at 99%.

Data management and analysis

Regression analysis was done on mosquitoes collected within households in Bura and Husingo for comparison, while those from Chifiri were left out due to selection bias, as this village had not been included in the previous study (Mbotha *et al.*, 2017). Multivariable evaluation of counts of mosquitoes collected per trap during each visit were compared using a negative binomial model (MASSpackage) in R 3.3.3 software (R Development Core Team, 2010; Hilbe, 2011; Bates *et al.*, 2014). Two analyses were done; the first included mosquitoes from households in the two sites, while the second was limited to known vectors of RVF virus namely *Aedes mcintoshi, Ae. tricholabis, Ae. ochraceus, Anopheles squamosus, Culex poicilipes, Cx. bitaeniorhynchus, Cx. univittatus, Cx. pipiens sl, Mansonia africana,* and *Ma. uniformis*. Independent variables examined included rainfall, month of visit, number of humans and animals in a household and household livelihood. Household was incorporated as a random effect while village as a fixed effect. Bidirectional elimination of variables was used, and models compared using the Akaike Information Criterion (AIC).

Ethical Approval

Ethical approval was obtained from the Ethics and Scientific Review Committee (ESRC) of the Africa Medical and Research Foundation (AMREF) number (REF: AMREF-ESRC P65/2013).

4.1.4 Results

Relative abundance and species composition

A total of 21,015 mosquitoes and larvae were collected and examined. Of these 5,742 were from all households (Table 1), 14,498 were from irrigated fields in Bura (Table 2) and 775 were immature stages from breeding habitats within irrigated fields in Bura (Table 3). Over half (11,539) of all mosquitoes were known vectors of RVF virus (Table 4).

Table 1: Number of mosquitoes collected and identified from selected households during 5 visits to Bura irrigation scheme, Husingo and Chifiri villages between September 2014 and June 2015

	***Aedes* spp.**	***Aedomyia* spp.**	***Anopheles* spp.**	***Culex* spp.**	***Ficalbia* spp.**	***Mansonia* spp.**	**Total**
Bura (irrigated) total	868	308	2187	1602	1	93	**5059**
Sept 2014	11	108	77	328	0	2	**526**
Nov 2014	202	31	25	299	0	2	**559**
Jan 2015	90	20	995	368	1	38	**1512**
Mar 2015	27	111	177	292	0	41	**648**
Jun 2015	538	38	913	315	0	10	**1814**
Chifiri (pastoral) total	26	0	2	11	0	1	**40**
Sept 2014	0	0	0	0	0	0	**0**
Nov 2014	26	0	0	1	0	0	**27**
Jan 2015	0	0	0	4	0	1	**5**
Mar 2015	0	0	0	3	0	0	**3**
Jun 2015	0	0	2	3	0	0	**5**
Husingo (Riverine) total	11	2	492	135	0	3	**643**
Sept 2014	0	0	1	14	0	0	**15**
Nov 2014	5	1	1	6	0	0	**13**
Jan 2015	0	0	393	82	0	1	**476**
Mar 2015	0	0	1	18	0	1	**20**
Jun 2015	6	1	96	15	0	1	**119**
Total	**905 (15.8%)**	**310 (5.4%)**	**2681 (46.7%)**	**1748 (30.4%)**	**1 (0.0%)**	**97 (1.7%)**	**5742**

Mosquitoes collected in all households in the three ecosystems consisted of 29 species of six genera namely *Aedes* (15.76%), *Aedomyia* (5.40%), *Anopheles* (46.69%), *Culex* (30.44%), *Ficalbia* (0.02%) and *Mansonia* (1.69%, Figure 1). Members of *Aedes* comprised of *Ae. aegypti*, *Ae. mcintoshi, Ae. ochraceus, Ae. simpsoni, Ae. stegomyia* spp., *Ae. sudanensis and Ae. tricholabis. Aedeomyia* genus consisted of *Ad. furfurea* and other unidentified *Aedomyia* spp. *Anopheles* genus consisted of *An. constani, An. funestus, An. gambiae, An. pharoensis, An. squamosus* and unidentified *Anopheles* spp. *Culex* genus included *Cx. annulioris, Cx. bitaeniorhynchus, Cx. ethiopicus, Cx. pipiens, Cx. poicilipes, Cx. tigripes, Cx. univittatus, Cx. vansomereni* and other unidentified *Culex* spp. One mosquito was identified as belonging to *Ficalbia* genus while members of *Mansonia* were *Ma. africana, Ma. Uniformis.* Majority of mosquitoes (88.1%) from households were from the irrigated ecosystem. It was also the most diverse, with six genera being recorded. The riverine ecosystem had five genera, while the pastoral had four. Overall, majority of mosquitoes captured from households belonged to *Anopheles* and *Culex* genera. Univariable analysis revealed no significant difference between the number of mosquitoes from the irrigated and riverine households (IRR = 1.02, CI = 0.73–1.35, p = .92).

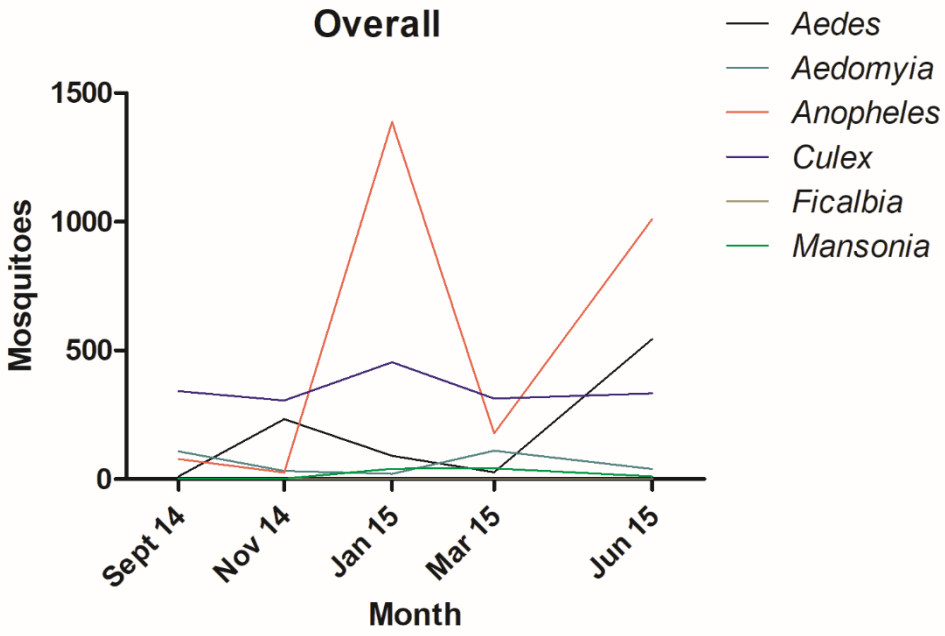

Figure 2: Distribution of mosquito genera collected from all households in Bura irrigated scheme, the riverine Husingo village and the pastoral Chifiri village during 5 visits between September 2014 – June 2015.

Table 2: Number of mosquitoes collected and identified from traps set within irrigated fields during 5 visits to Bura irrigation scheme, between September 2014 and June 2015

	***Aedes* spp.**	***Aedomyia* spp.**	***Anopheles* spp.**	***Coquillettidia* spp.**	***Culex* spp.**	***Ficalbia* spp.**	***Mansonia* spp.**	**Total**
Sept 2014	81	461	31	1	614	0	2	**1190**
Nov 2014	871	560	48	0	734	0	26	**2239**
Jan 2015	1249	550	1409	1	1080	26	1830	**6145**
Mar 2015	183	436	218	0	720	0	736	**2293**
Jun 2015	707	368	310	0	601	0	645	**2631**
Total	**3091 (21.3%)**	**2375 (16.4%)**	**2016 (13.9%)**	**2 (0.01%)**	**3749 (25.9%)**	**26 (0.18%)**	**3239 (22.3%)**	**14498**

Mosquitoes captured within irrigated fields in Bura exhibited the highest diversity in species composition, registering 35 species belonging to seven genera. These were *Ae. aegypti*, *Ae. funestus, Ae. mcintoshi, Ae. ochraceus, Ae. simpsoni, Ae. Stegomyia*spp*, Ae. sudanensis, Ae. tricholabis*, *Ae. poicilipes, Ae. tarsalis* and other unidentified members. *Aedomyia* genus included *Ad. furfurea* and unidentified members. Members of *Anopheles* genus included *An. constani, An. funestus, An. gambiae, An. pharoensis, An. squamosus, An. sudanensis* and other unidentified species. Two *Coquillettidia aurites* mosquitoes, belonging to genus *Coquillettidia* were also identified. Members of *Culex* genus included *Cx. annulioris, Cx. antennatus, Cx. bitaeniorhynchus, Cx. ethiopicus, Cx. pipiens, Cx. poicilipes, Cx. uniformis, Cx. univittatus, Cx. vansomereni, Cx. zombaensis* and other unidentified members. *Filcalbia* genus members were *Fi. splendens* and other unidentified species. Members of *Mansonia* genus were *Mn. africana, Mn. uniformis* and other unidentified species.

Majority of known vectors of RVF virus were captured within the irrigated ecosystem (98.8%). All ecosystems however registered at least one member of each of the ten species of known vectors. Immature stages collected from breeding habitats within irrigated fields consisted of 15 species belonging to five genera namely *Ae. mcintoshi, Ad. furfurea, An. funestus, An. gambiae, An. squamosus* and unidentified *Anopheles* spp*, Cx. annulioris, Cx. bitaeniorhynchus, Cx. ethiopicus, Cx. pipiens, Cx. poicilipes, Cx. univittatus, Cx. vansomereni* and other unidentified *Culex* spp, and three members belonging to *Uranotaenia* genus.

In addition to increased mosquito density after the rains, there were notable differences in spatio-temporal distribution of species in all ecological sites. *Anopheles* species was the most abundant genus and had the highest peak in the irrigated and riverine ecosystems after the rains, while *Aedes* species had the highest peak in the pastoral Chifiri village. *Culex* and *Mansonia* species were collected in all sites and maintained their relative abundance throughout the study period. The most abundant genus within households in Bura and Husingo was *Anopheles* whereas collections from irrigated fields in Bura mirrored the dry pastoralist ones with the abundant genera being *Aedes* and *Mansonia*.

Table 3: Number of immature mosquito stages collected from their breeding habitats within the irrigated fields during 5 visits to Bura irrigation scheme, between September 2014 and June 2015.

	Arrow root holes	**Block feeder**	**Drain feeder**	**Farm furrow**	**Feeder canal**	**Stagnant water**	**Unit canal**	**Unit drain**	**Total**
***Aedes* (5.2%)**	0	0	9	0	1	18	1	11	**40**
***Aedomyia* (1.4%)**	0	0	0	0	11	0	0	0	**11**
***Anopheles* (20.3%)**	1	2	42	0	26	63	9	14	**157**
***Culex* (72.8%)**	21	1	182	4	128	153	14	61	**564**
***Uranotaenia* (0.39%)**	0	0	0	0	3	0	0	0	**3**
Total	**22**	**3**	**233**	**4**	**169**	**234**	**24**	**86**	**775**

Seasonal variation in mosquito abundance

Anopheles genus had the highest spike after the rains in the irrigated and riverine ecosystems. *Culex* was the only genus present in all three study sites during all visits except September 2014, and its numbers remained relatively constant within each ecosystem (Figure 3). The first visit in September 2014 accounted for the least number of mosquitoes collected overall (9.42%). Numbers increased slightly in November 2014 (10.43%) then spiked in January 2015 (34.71%), mainly caused by an increase of *Anopheles* spp. The March 2015 visit registered a significant dip (11.69%) that bounced back in the final June 2015 visit (33.75%). This pattern was however not seen in Chifiri, as there were no peaks in mosquito numbers in January and June 2015. Seasonal variation among mosquitoes from irrigated fields in Bura and known vectors of RVF virus followed the same pattern as those from households, with majority captured after the rains in January 2015. Most larvae and pupae of mosquitoes were collected in November 2014 (54.8%) and June 2015 (26.7%).

Table 4: Total number of known mosquito vectors of RVF virus collected and identified from households and irrigated farms during 5 visits to Bura irrigation scheme, Husingo and Chifiri villages between September 2014 and June 2015.

	Aedes (30.1%) ***-Ae. mcintoshi*** ***-Ae. ochraceus*** ***-Ae. tricholabis***	**Anopheles (1.8%)** ***-An. squamosus***	**Culex (36.8%)** ***-Cx. bitaeniorhynchus*** ***-Cx. pipiens*** ***-Cx. poicilipes*** ***-Cx. Univittatus***	**Mansonia (31.3%)** ***-Mn. africana*** ***-Mn. uniformis***	**Total**
Bura (irrigated) total	3448	198	4156	3602	11404
Sept 2014	110	3	751	5	869
Nov 2014	1092	0	907	28	2027
Jan 2015	1383	147	1272	2266	5068
Mar 2015	207	18	595	650	1470
Jun 2015	656	30	631	653	1970
Chifiri (pastoral) total	21	1	6	1	29
Sept 2014	0	0	0	0	0
Nov 2014	21	0	1	0	22
Jan 2015	0	0	3	1	4
Mar 2015	0	0	1	0	1
Jun 2015	0	1	1	0	2
Husingo (riverine) total	10	5	88	3	106
Sept 2014	0	0	3	0	3
Nov 2014	5	0	4	0	9
Jan 2015	0	2	69	1	72
Mar 2015	0	0	0	1	1
Jun 2015	5	3	12	1	21
Total	**3479**	**204**	**4250**	**3606**	**11539**

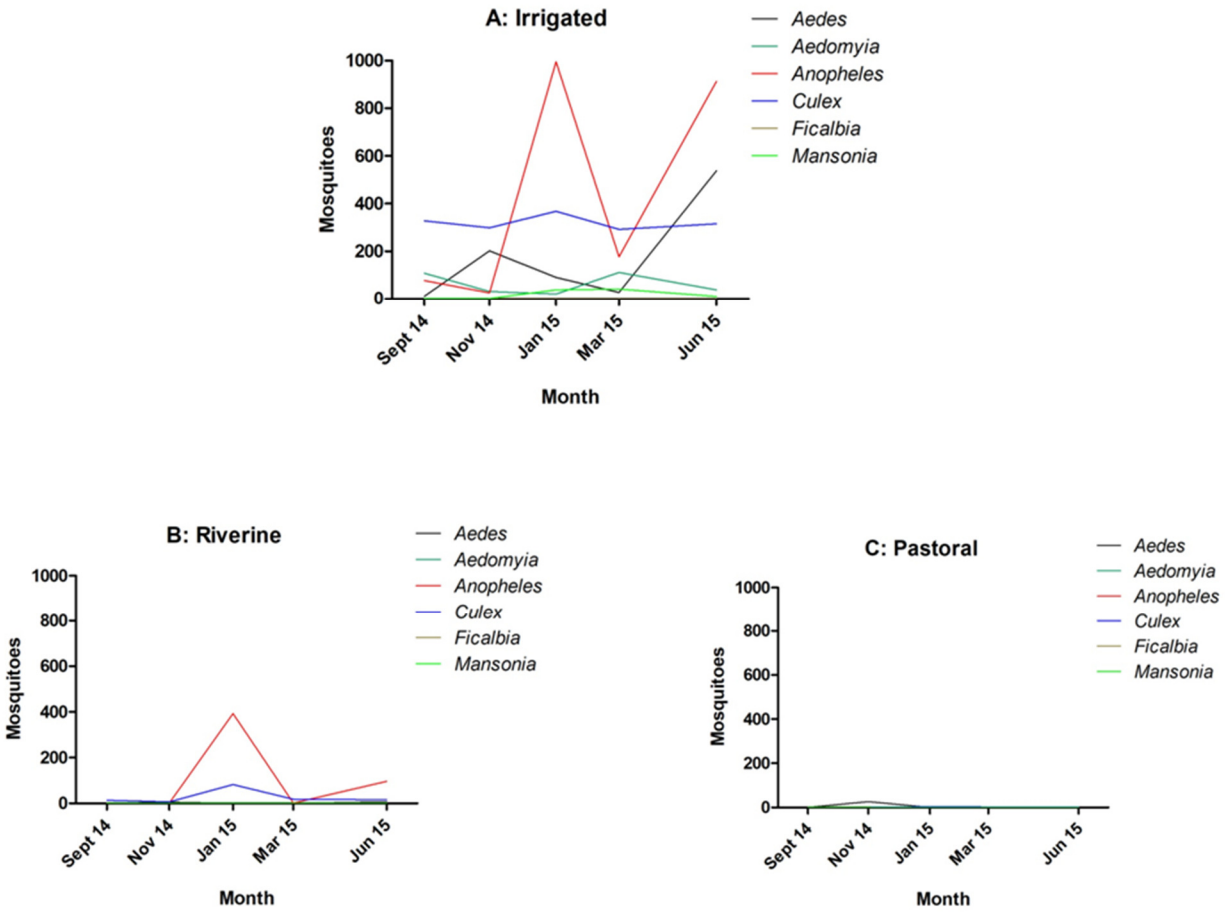

Figure 3: Distribution of mosquito genera collected from households in Bura irrigated scheme (A), the riverine Husingo village (B) and the pastoral Chifiri village (C) during 5 visits between September 2014 – June 2015.

Table 5: Results of a multivariable model for the counts of mosquitoes collected from households in Bura irrigation scheme and Husingo village only in Tana River County, Kenya, during September 2014 – June 2015

			Mosquitoes collected	**Incidence Risk Ratio (IRR)**	**Confidence interval**	**P-value**
Site	Irrigated		5059			
	Riverine		643	0.77	0.56-1.08	0.11
Month	Sept 14		541	0.51	0.37-0.69	<0.001*
	Nov 14		572	0.44	0.32-0.59	<0.001*
	Jan 15		1988			
	Mar 15		668	0.52	0.39-0.71	<0.001*
	Jun 15		1933	1.01	0.77-1.33	0.92
Hosts	Humans in households	1 – 5	2825			
		6 – 10	2457	1.05	0.81-1.36	0.69
		>10	420	0.67	0.48-0.96	0.02*
	Animals in households	1 – 5	1314			
		6 – 15	3074	1.18	0.91-1.53	0.23
		>15	1314	1.06	0.77-1.46	0.71

Results of regression analysis

There was no significant difference in counts of mosquitoes collected between the irrigated and riverine ecosystems for both total mosquitoes and known vectors of RVF virus. There were however significant differences in counts of mosquitoes collected between different months for total mosquitoes (Table 5), while known vectors of RVF virus experienced a significant reduction in numbers captured in June. Larger households tended to have significantly lower mosquito catches. Animal herd size did not have any impact on the number of mosquitoes captured (Table 6).

Table 6: Results of a multivariable model for the counts of known mosquito vectors of RVF virus collected from households in Bura irrigation scheme and Husingo village in Tana River County, Kenya, during September 2014 – June 2015

			Mosquitoes collected	**Incidence Risk Ratio (IRR)**	**Confidence interval**	**P-value**
Site	Irrigated		1456			
	Riverine		124	0.74	0.48-1.15	0.16
Month	Sept 14		191	0.69	0.46-1.04	0.07
	Nov 14		351	0.88	0.61-1.28	0.05
	Jan 15		514			
	Mar 15		283	1.03	0.71-1.53	0.88
	Jun 15		241	0.57	0.40-0.81	0.001*
Hosts	Humans in household	1 – 5	931			
		6 – 10	519	0.72	0.51-0.98	0.04*
		>10	130	0.59	0.38-0.95	0.03*
	Animals in household	1 – 5	393			
		6 – 15	830	1.41	1.00-1.99	0.06
		>15	357	1.27	0.85-1.92	0.23

Host species identification of blood meals

There were 343 engorged females collected overall from the three sites. Majority were from households (301/343) while the rest were from irrigated fields. Most engorged mosquitoes from households originated from Bura (86%), Husingo (12%) and Chifiri (2%). Most were captured a few months after the start of the short rainy season in November 2014 (18%), January 2015 (43%), and the long rainy season in June 2015 (29%). Blood meal analysis revealed 302 samples contained vertebrate DNA (88%), while the rest were unidentified (12%). One third of mosquitoes (n=100) with identified host species DNA were *An. funestus*, while the rest were *Ae. mcintoshi* (n=46, 15%), *Cx. annulioris* (n=40, 13%), *Cx. univittatus* (n=30, 10%) and other species. Of these, 126 (42%) were known vectors of RVF virus. Majority (78%) of samples contained DNA originating from goats while 47 (16%) were of human origin (Table 7). Majority of engorged females containing unidentified host DNA were *An. funestus* (n=28, 68%), *Ae. mcintoshi* (n=6, 15%), *An. gambiae* (n=3, 7%), two unidentified *Aedes* and *Anopheles* species, one *An. squamosus* and one Cx. *vansomereni.*

Table 7: Vertebrate sources of blood meals identified from female fed mosquitoes collected from Bura irrigation scheme households and fields, Husingo and Chifiri villages between September 2014 – June 2015.

*Represents known vectors of RVF virus.

	Antelope	**Bird**	**Cow**	**Donkey**	**Goat**	**Human**	**Mouse**	**Sheep**	**Total**
Ae.mcintoshi*	1	0	6	0	29	8	1	1	**46**
Ae.ochraceus*	0	0	0	0	4	0	0	0	**4**
Ae.sudanensis	0	0	0	0	0	2	0	0	**2**
An.funestus	0	0	3	0	88	8	0	1	**100**
An.gambiae	0	0	0	0	10	6	0	0	**16**
An.squamosus*	0	0	0	0	3	0	0	0	**3**
Anopheles spp.	0	0	0	0	3	0	0	0	**3**
Culex spp.	0	1	0	0	7	2	0	0	**10**
Cx.annulioris	0	0	0	1	32	5	0	2	**40**
Cx.pipiens*	0	0	0	0	18	3	0	2	**23**
Cx.poicilipes*	0	0	0	0	4	2	0	0	**6**
Cx.univittatus*	0	0	0	0	23	7	0	0	**30**
Cx.vansomereni	0	1	0	0	2	0	0	0	**3**
Filcalbia spp.	0	0	0	0	0	2	0	0	**2**
Ma.africana*	0	0	0	0	12	2	0	0	**14**
Total	**1**	**2**	**9**	**1**	**235**	**47**	**1**	**6**	**302**

4.1.5 Discussion

This study was informed by the current global increase in the emergence and spatial expansion of infectious diseases either as a result of human actions such as land-use changes, or as a consequence of human behavior such as climate change (Mboera *et al.*, 2011; Lindahl & Grace, 2015). In particular, the spread of vector borne diseases such as RVF has been known to occur in areas favoring the emergence of massive numbers of mosquito vectors as a result of prolonged rainfall or flooding (Linthicum *et al.*, 1985; Hightower *et al.*, 2012). The present study investigated the impact of irrigation on the abundance, distribution and diversity of mosquitoes and potential vectors of RVF virus during an inter-epidemic period between September 2014 and June 2015, in Bura, Tana River County. Multivariable analysis revealed that the irrigated and riverine ecosystems were similar in mosquito abundance and seasonality, despite one being naturally occurring while the other man-made. Further, the irrigated ecosystem was the most diverse, and maintained an abundance of mosquitoes throughout all seasons. The presence of the irrigation scheme at this site led to an overall increase in mosquito population and species diversity in this region. This effect has been demonstrated in previous studies in Kenya and elsewhere, in which mosquito abundance and diversity was highly site-dependent (Lutomiah *et al.*, 2013; Serpa *et al.*, 2013). Imbahale *et al.* (2011) also indicated that most larvae breeding grounds such as feeder and drainage canals, tire tracks and rice paddies were man-made, compared to naturally occurring habitats such as riverine flooded edges and swampy fringes.

Seasonal variation correlating with rainfall, also significantly contributed to increased mosquito numbers, especially in Bura and Husingo. Mosquito numbers peaked in January 2015, approximately six weeks after the start of the short wet season in November 2014, due to the growth of abundant vegetation optimal for the survival of large numbers of emerging mosquitoes (Anyamba *et al.*, 2009). This was also observed in previous studies, in which mosquito numbers correlated with weekly or monthly rainfall intensity (Patz *et al.*, 1998; Bomblies, 2012). Chifiri had virtually no breeding grounds and had significantly reduced vegetation that could support survival of large numbers of emerging mosquitoes. This was probably due to insufficient rainfall.

Although vector density is important in pathogen transmission, species distribution is equally important due to the differing epidemiological roles played by each species, all contributing to the emergence or maintenance of disease. The spatial distribution of mosquitoes in these ecosystems conforms with previous findings that showed *Anopheles* species, being well established in the Tana Delta (Lutomiah *et al.*, 2013), was the most abundant in the irrigated and riverine ecosystems. Species of the flood water *Aedes* genus were predominantly found in the dry and arid Garissa region of north-east Kenya, a pastoral area belonging to the same ecological

group as Chifiri (Arum *et al.*, 2015). Lutomiah *et. al.*, (2013) showed that members of *Mansonia* were mainly collected in large swampy marshes in Baringo, Kisumu, and Budalangi in western Kenya, where they appeared to be well-adapted to the hot and humid ecology with swampy breeding sites. Members of *Culex* have previously been found to be ubiquitous in Kenya, with different regions being inhabited by various predominant species (Sang *et al.*, 2010).

Interestingly, more than half of all mosquitoes captured were known vectors of RVF virus. While rainfall appeared to have an impact on seasonal variation of the overall number of mosquitoes collected within households, regression analysis revealed a significant reduction in the number of known vectors of RVF virus captured in June, perhaps due to the shutting down of secondary and tertiary irrigation channels in most fields at the end of the growing season in April, to allow for maturation and harvesting of crops later in the year. Thus, the rain that fell later may not have been sufficient for flooding and emergence of more floodwater mosquitoes.

Large households with many children were few and tended to have more animals within the homestead. They also contained several treated mosquito nets which could explain the reduced numbers of mosquitoes captured. While it may be impossible to eliminate all stagnant water especially within the irrigated ecosystem, increased distribution of insecticide treated bed nets and use of window and door screens to limit human contact as well as other practical chemical and biological control efforts could be explored.

While most engorged mosquitoes were captured within households in the irrigation scheme, majority of the unfed mosquitoes were collected within irrigated fields. Mosquito flight range, duration and speed has long been known to contribute to overall vector capacity in disease transmission. Host species identification revealed a limited diversity of hosts available in Bura and Husingo households, who mainly kept few goats and sheep for household use. The proportion of human blood meals was higher than one previous study that found it ranging between 5.1% – 5.3% in this region (Lutomiah *et al.*, 2014). These results have significant implications for RVF virus transmission, bearing in mind that goats and sheep are the most susceptible hosts for the virus (Chevalier *et al.*, 2010; Lichoti *et al.*, 2014). Further, they also reveal the potential for human exposure to the virus, as emerging vectors look for alternate sources of blood meal, such as humans (LaBeaud *et al.*, 2008; Mbotha *et al.* 2017).

4.1.6 Conclusion

Mosquitoes collections from Bura irrigation scheme, Husingo, and Chifiri villages representing irrigated, riverine and pastoral ecosystems during 5 visits between September 2014 and June 2015 revealed that the irrigation ecosystem is promoting mosquito abundance and diversity in

this region, because of the availability of breeding grounds and resting places as well as availability of vertebrate hosts for bloodmeals. These sites might enhance RVF virus endemicity through sustained breeding and prolonged lifespans, increasing mosquito abundance including known vectors of RVF. The establishment or expansion of more irrigation schemes may also result in the permanent change of predominant host species available to include highly susceptible sheep and goats, while the settlement and concentration of residential areas will increase mosquito-livestock-human contact, potentially leading to human exposure. These findings highlight the impact of agricultural and other anthropogenic land-use changes on mosquito ecology, within the context of other natural environmental forces.

4.1.7 Acknowledgement

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5.0 CHAPTER 5: Overall discussion and recommendations

This study was focused on understanding and assessing the role of anthropogenic changes on the transmission dynamics of RVF virus. Primarily, it examined irrigation expansion and other land-use changes associated with extensive agriculture for the large-scale production of staples and cash crops such as cotton, maize, wheat, vegetables and fruits, for a rapidly growing population. Although founded in the early 1980s with an irrigatable area of over 12,000 acres, the area under current irrigation is only 3,500 acres. Recent expansion efforts have seen the projected irrigatable area gazetted up to 25,000 acres (National Irrigation Board, 2016). The broad aim of the study was to find out whether and how these changes influence local ecological conditions and processes that might in turn affect the occurrence and maintenance of RVF. This was specifically through the evaluation of any spatio-temporal changes or differences in vector dynamics, determination of their sources of blood meal, as well as assessment of the potential impact these changes have on the relative risk of RVF on the local livestock population.

Even though RVF virus transmission and disease pathology within mammalian hosts has been extensively studied, the impact of anthropogenic changes to vector-virus interactions that contribute to or result in emergence and inter-epidemic maintenance of the virus is yet to be comprehensively understood. It has long been established that mosquitoes, predominantly of the *Aedes* and *Culex* genera, are the principal vectors of transmission (Smithburn *et al.*, 1948). Recent models have additionally demonstrated that any modifications to the environment that would impact on the vector-host-equilibrium balance, such as vegetation, temperature or humidity, would consequently affect the risk of transmission, once the virus is introduced into an ecosystem (Lashari & Zaman, 2011; Mosomtai *et al.*, 2016). Further research has also shown that disease transmission does not occur in single and isolated pathogen-host interactions, but in a complex framework of co-infections of all diseases observed within a diversity of hosts, populations and habitats at a given point in time (Swaddle & Calos, 2008; Johnson & Thieltges, 2010). Environmental changes could be natural, such as prolonged drought in dry and arid regions that results in significant reduction of mosquito numbers or prolonged rainfall and flooding that boosts abundance. Natural ecological changes tend to be seasonal and may only impact on the short-term risk of transmission while anthropogenic changes usually occur as a consequence of well-intentioned pursuit of some ecosystem benefit, product or service. Unfortunately, these changes occur in tandem with and may even compound the effects of the naturally or seasonally occurring changes, leading to the long-term destabilization of the vector-host-equilibrium and

thereby permanently impacting on the risk of transmission, causing endemicity (Khasnis & Nettleman, 2005).

5.1 Impact of irrigation expansion on mosquito density and diversity in Bura Sub-County, Tana River County.

The results of this study indicate that there was an overall increase in vector abundance as well as in species diversity in Bura Sub-County, as a result of the presence of Bura irrigation scheme in this region. Comparative and multivariable analysis between the irrigated (Bura irrigation scheme), riverine (Husingo village) and pastoral (Chifiri village) ecosystems revealed that the irrigated and riverine ecosystems were similar in terms of mosquito abundance and seasonality, despite one being naturally occurring while the other being man-made. Further, the irrigated ecosystem maintained a constant and minimum presence of mosquitoes throughout all seasons, while the riverine ecosystem exhibited much more sensitivity to rainfall, with mosquito abundance significantly increasing during the wet season. This was mainly due to the availability of permanent breeding sites and larval habitats in both these ecosystems, as well as suitable vegetation that offered shade and resting places for emerging mosquitoes. Additionally, the irrigated ecosystem exhibited the most diversity in species present, providing further evidence of the possible emergence of new mosquito species as a result of these intensive agricultural expansion efforts.

Even though it was not evaluated in this study, recent research has also demonstrated that the presence of irrigation schemes in an area potentially modifies extremes of surface temperature and humidity, making them suitable for mosquito survival and thus prolonging their lifespans (Lobell *et al.*, 2008). It therefore follows that any further expansion efforts would lead to further increases, both in the spatial and temporal range of vectors. This expansion translates to the steady presence of vectors, giving rise to the possibility for constant virus transmission and endemicity. Further, the role of rainfall on the seasonal risk of RVF was revealed by comparing the results from the irrigation scheme with those of the riverine Husingo ecosystem. In both ecosystems, there were significant spikes in mosquito numbers approximately eight weeks after the start of the rains.

The seroconversion study, which was a direct measure of RVF disease risk in sheep and goats, also indicated that most seroconversions occurred during the rainy season especially within the riverine ecosystem, while those within the irrigated ecosystem were spread out over several months thereafter. These results reveal the potential for the riverine ecosystem initiating transmission during periods of short but intense rainfall, perhaps through contact with a wild

mammalian reservoir, and the neighboring irrigated ecosystem picking and prolonging it in the presence of abundant mosquito vectors and naïve domestic livestock. It also seems plausible that the irrigated ecosystem could be capable of horizontal inter-epidemic maintenance of the virus, without necessarily requiring transovarial transmission via infected eggs of *Aedes* genus mosquitoes. Although regression analysis on mosquito catches from the dry pastoralist Chifiri ecosystem was not possible due to selection bias, there appeared to also be a slight increase in vector numbers initially at the beginning of the rains in November. However, due to the lack of sufficient rains and flooding for the formation of larval habitats and growth of supportive vegetation, there was not a peak two months later as seen in the other two ecosystems. Species composition was also narrow in this ecosystem, while seroconversion tests showed no animals seroconverted in this ecosystem during the duration of the study.

The findings of this study lead credence to results of two similar studies done in the same region in south eastern Kenya that compared species composition, distribution and abundance of RVF vectors and other zoonotic and arboviral pathogens between two ecologically distinct counties (Sang *et al.*, 2017; Bett, 2017). Clear differences attributable to local ecological and environmental conditions in the two localities were demonstrated, in which the high-risk Tana River County exhibited overall higher diversity and abundance of species compared to the low risk Garissa and Isiolo Counties. These studies further postulated that the sensitivity and specificity of risk assessment and forecasting exercises could be fine-tuned by characterizing the numerous and diverse local habitat factors to augment currently established epidemiological components as well as prevent high human infections through promotion of zooprophylaxis. Other studies in Africa and elsewhere have also reported on the quantitative, speciation and genetic adaptation of various vector species to changing ecosystems as a result of anthropogenic and environmental variability, especially temperature and precipitation, consequently affecting the epidemiology and transmission of vector borne diseases (Coluzzi *et al.*, 1979; Lindsay *et al.*, 1998; Ghebreyesus *et al.*, 1999; Ernould *et al.*, 1999; Sutherst, 2004). These studies also conclude that the relative abundance and species distribution can be used to accurately predict vector-borne disease risk, as well as forecast potential changes associated with these ecological disease drivers.

This study had several limitations that occurred during the design period due to financial and time constraints. Firstly, it was a sub-study of a larger cross-sectional program that focused on dynamic drivers of infectious diseases in the region and the pastoralist Chifiri village was not selected in the initial cross-sectional screening of RVF. Consequently, it was not possible to include this village in any statistical analyses in order to shed more light on findings observed or compared with those of the other two ecosystems. Secondly, the evaluation of the potential role of

zooprophylaxis or mosquito host preference and the conditions needed for the vector-host ratio to influence transmission was not done. This would have enabled accurate assessment of the impact of integrating different livestock species and host densities, as a practical and long-term option in the prevention of human cases of RVF. It would also have been valuable to examine vector lifespan in order to determine whether these ecological changes have any differing impact across the three ecosystems. Measurements of local temperature, precipitation and humidity within each ecosystem would have enabled precise comparison and evaluation of the impact of irrigation expansion on local climatic and meteorological parameters, and its ability to modify extremes of these parameters across different seasons. Finally, the laboratory detection of circulating RVF virus from pools of mosquito catches would have further corroborated our findings.

5.2 Conclusion and recommendations for future study

Vector competence studies for species of mosquitoes that were found in these ecosystems but not yet recognized as vectors of RVF virus, are needed in future in order to further improve on the evaluation of disease risk, surveillance and control, in the presence of irrigation expansion programs. Vectoral capacity, which is a product of several vector traits including biting rate, feeding preference and lifespan, would provide a more accurate assessment of the potential for disease transmission (Liang *et al.*, 2015). Other recommendations include efforts to disrupt vector life cycles by improving the regulation and management of irrigation feeder and drainage systems such as intermittent flooding and increasing velocity of river flow, to reduce unnecessary flooding and pooling of water that leads to creation of larval breeding sites. Community outreach and education on simple environmental management practices such as draining of pools and swamps around residential areas, trimming or clearing of excessive vegetation and bushes, removal and destruction of old tires, containers and other household waste that may serve as alternate breeding sites and proper use and maintenance of pit latrines.

While it might be impractical to completely halt all human activities that result in environmental alterations such as irrigation, it is possible to integrate several vector management strategies that ultimately result in the reduction or complete elimination of the risk of disease transmission. These include targeted vector surveillance programs, efforts to decrease host susceptibility to infection via vaccination of livestock, as well as minimizing human contact by locating residential areas far away from irrigation fields, use of window screens in houses, insecticide treated bed nets, indoor spraying and zooprophylaxis. Testing for mosquito insecticide resistance may however be needed before the use of chemical larvicides, due to the numerous chemicals used in the irrigated

ecosystem for intensive crop production. Biological control of mosquitoes such as the use of some fish and crustacean predators that feed on immature stages, introduction of bacteria, plants and fungi with larvicidal and mosquitocidal properties and the sustained release of sterile males could also be explored. Finally, research has shown that efforts to reduce or eliminate vector-borne disease risk are best realized when integrated with other developmental components that improve the socio-economic and overall well-being of societies. This is because households are in a better position to control and invest in some mitigating aspects such as improved housing conditions, purchase of bed nets, access to information, drugs and vaccinations for their livestock, as well as cooperate and adhere with local government or public health programs for disease control.

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Summary

The government of Kenya has prioritized food production through revitalized and sustained agricultural expansion as a means of achieving food security for the rapidly growing population, uplifting the living standards of rural communities by providing them with livelihood opportunities as well as jumpstarting overall economic growth for employment and wealth creation through the export of excess produce and by products. Key among several policy directives issued in this regard was the harnessing of the country's vast irrigation potential, through the expansion of existing irrigation schemes as well as establishment of new ones along the Tana and Athi river basins as well as along the shoreline of Lake Victoria. The development of these schemes however has the potential to alter local environmental and ecological conditions that may influence the risk of RVF disease transmission. Given that a thorough understanding of the risk factors precipitating the occurrence of any infectious disease is vital for its effective control, this study aimed to investigate whether these land-use changes associated with the development of irrigation schemes had any impact on the transmission dynamics of RVF virus.

This study involved the spatio-temporal evaluation and comparison of the abundance, distribution and species diversity of potential vectors of RVF virus across three villages with differing ecological habitats in Bura, Tana River County, Kenya. These included the irrigated ecosystem represented by Bura irrigation scheme located near Bura township, the riverine ecosystem represented by Husingo village that is adjacent to Tana River and the dry, pastoral ecosystem represented by Chifiri village which is located further inland, away from both the irrigated and riverine ecosystems. A concurrent longitudinal study was also undertaken to measure and compare the risk of RVF on the local population of sheep and goats kept by households, and between the three ecosystems.

Representative samples of mosquitoes were collected from all ecosystems and morphologically identified using taxonomic keys. The sources of blood meals were also examined in order to identify the host spectrum of engorged females while sampling of sentinel animals for RVF antibodies was undertaken as a direct measure of risk. Comparative and multivariable analysis between these ecosystems revealed that the irrigated and riverine ecosystems were similar in terms of mosquito abundance and seasonality, despite one being naturally occurring while the other being man-made. Further, the irrigated ecosystem maintained a constant and minimum presence of mosquitoes throughout all seasons, while the riverine ecosystem exhibited much more sensitivity to rainfall, with mosquito abundance significantly increasing during the wet season. The host spectrum of blood fed females revealed that most blood meals came from easily available and accessible hosts such as goats, sheep and humans. Screening of sheep and goats

for RVFV antibodies detected several seroconversions in the riverine and irrigated ecosystem, with those within the riverine ecosystem all occurring in one month approximately eight weeks after the start of the rainy season while those within the irrigated ecosystem were spread out over several months thereafter. The seroconversion study, which was a direct measure of RVF disease risk, indicated that most seroconversions occurred during the rainy season within the riverine ecosystem, while those within the irrigated ecosystem were spread out over several months. No seroconversions were detected in the much drier pastoralist ecosystem.

The findings of this study suggest that further expansion of the irrigation scheme or establishment of new ones in this region will lead to a gross increase in the abundance and diversity of total mosquitoes, as well as potential vectors of RVF virus. The results further imply that with increased numbers of vectors, in the presence of low numbers of animal hosts may pose an increased risk of spillover infection to humans as opportunistic hosts during large epidemics. In addition to irrigation expansion, rainfall and flooding still remains a significant risk factor for the transmission of RVF virus in this county, especially in the non-irrigated riverine and pastoral ecosystems, and particularly in the presence of large numbers of naïve animal hosts. Practical implications of these findings include targeted vector surveillance especially of known vectors of RVF virus as well as the formulation and implementation of integrated vector and environmental control programs. Vector competence studies of other mosquito species identified that might be potential vectors are recommended in future in order to improve on current outbreak prediction models as well as evaluate the success of potential surveillance and control options.

Zusammenfassung

Auswirkungen des Ausbaus von Bewässerungssystemen auf die Übertragung des Rift Valley-Fiebers zwischen Epidemien und Jahreszeiten im Bura Sub-County, Tana River County, Kenia

Die kenianische Regierung hat die Lebensmittelproduktion zur Priorität gemacht, um die Versorgung mit Lebensmitteln für eine schnell wachsende Bevölkerung sicherzustellen. Dies erfolgte vor allem durch anhaltende Expansion landschaftlicher Nutzflächen in zuvor naturbelassenen Gebieten. Dadurch erhöhte sich der Lebensstandard ländlicher Gebiete, weil sich neue Erwerbsmöglichkeiten bildeten, was die positive wirtschaftliche Entwicklung dieser Gebiete förderte, dabei insbesondere den Export landwirtschaftlicher Produkte.
Ein Schlüsselfaktor dieser Politik war die umfassende Planung neuer aber auch der Ausbau bestehender Bewässerungssysteme entlang der Flussläufe des Tana und Athi, sowie des Ufers des Viktoriasees, um diese fruchtbaren Trockengebiete landwirtschaftlich nutzen zu können.
Diese Entwicklung hat das Potential, das Ökosystem so zu verändern, dass es das Übertragungsrisiko des Rift-Valley-Fieber-Virus (RVFV) beeinflusst.
Diese Studie hatte die Untersuchung der Einflüsse solcher Bewässerungssysteme auf die Übertragungsmechanismen des RVFV zum Ziel. Denn die intensive Auswertung aller bekannter Risikofaktoren einer Infektionskrankheit ist die Grundlage zur Entwicklung effizienter und wirksamer Bekämpfungsstrategien. Diese Studie umfasst eine geographische und zeitliche Auswertung der Fangzahlen, Verbreitung und Diversität potenzieller Vektoren des RVFV in drei Dörfern mit unterschiedlichem Habitat in der Gemeinde Bura, Tana River, Kenia. Hierbei repräsentieren Bura Township und Umgebung eine durch Bewässerungssysteme geprägte Landschaft, während sich das Dorf Husingo durch die natürlichen Nebengewässer des Flusses Tana auszeichnet. Das Dorf Chifiri ist Vertreter eines trockenen, pastoral geprägten Ökosystems. Parallel dazu wurde eine vergleichende Langzeitstudie zur Ermittlung des RVF-Risikos für die Bevölkerung und ihrer Ziegen und Schafe unter Berücksichtigung der Unterschiede zwischen den drei Gemeinden durchgeführt.
Repräsentative Proben von Stechmücken wurden aus allen Ökosystemen gefangen und mit taxonomischen Schlüsseln morphologisch bestimmt. Außerdem wurden die Blutmahlzeiten der Mücken untersucht, um das Wirtsspektrum vollgesogener Weibchen zu ermitteln, während das Beproben von Sentinel-Tieren auf RVFV-Antikörper als direkter Indikator für das Übertragungsrisiko durchgeführt wurde. Vergleichende und multivariable Statistikanalysen ergaben, dass die künstlich bewässerten und natürlichen Fluss-Ökosysteme sich in

Mückenvorkommen und Saisonalität ähnelten. Allerdings zeigte das künstlich bewässerte Ökosystem ein konstantes, wenn auch minimales, Vorkommen von Stechmücken, das nicht mit Jahreszeitenwechseln korrelierte. Das natürliche Flussökosystem hingegen zeichnete eine bedeutende Zunahme der Mückenmenge während der Regenzeit aus.
Das Wirtsspektrum der vollgesogenen Weibchen ergab, dass die meisten Blutmahlzeiten von leicht zugänglichen Wirten wie Ziegen, Schafen und Menschen stammten. Bei der Untersuchung von Schafen und Ziegen auf RVFV-Antikörper wurden mehrere Serokonversionen sowohl im künstlich sowie auch natürlich bewässerten Ökosystem festgestellt. Die Serokonversionsstudie, die ein direktes Maß für das RVF-Übertragungsrisiko darstellte, zeigte, dass die meisten Serokonversionen während der Regenzeit im Gebiet des natürlichen Fluss-Ökosystems auftraten, während die des künstlich bewässerten Ökosystems über mehrere Monate verteilt waren. Im trockenen, pastoralen Ökosystem wurden keine Serokonversionen festgestellt.
Die Ergebnisse dieser Studie deuten darauf hin, dass ein Ausbau bestehender Bewässerungssysteme und auch die Einrichtung neuer Systeme, zu einer generellen Zunahme der Stechmückenzahlen, sowie einer erhöhten Artenvielfalt der Mücken führen kann, einschließlich potenzieller Vektoren des RVFV. Die Ergebnisse legen die Vermutung nahe, dass diese Vektoren, bei weniger werdenden oder gänzlich fehlenden tierischer Wirten, zunehmend Menschen als opportunistische Wirte wählen könnten, was RVFV-Infektionen im Menschen begünstigen würde.
Neben der Expansion von Bewässerungssystemen sind Regenfälle und Überschwemmungen nach wie vor ein wichtiger Risikofaktor für die Übertragung des RVFV in diesem Landkreis. Insbesondere in den natürlich bewässerten Flussökosystemen, aber auch in pastoralen Ökosystemen, vor allem in Gegenwart einer großen Anzahl immunologisch naiver Wirte. Praktische Anwendung dieser Erkenntnisse wären eine routinemäßige Überwachung des Vorkommens und Infektionsstatus bekannter RVFV-Vektoren, sowie die Formulierung und Implementierung integrierter Vektor- und Umweltkontrollprogramme. Vektorkompetenzstudien anderer identifizierter Mückenarten, die potenzielle Vektoren sein könnten, werden empfohlen, um aktuelle Vorhersagemodelle für Ausbrüche in der Zukunft zu verbessern und den Erfolg potenzieller Überwachungs- und Kontrolloptionen zu bewerten.

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Disclosure of contribution to the intellectual content

Dr. Delia Grace, Dr Bernard Bett, Dr Johanna Lindahl and Dr Salome Kairu-Wanyoike jointly conceived the study and were lead applicants on the grant awarded by the Ecosystem Services for Poverty alleviation (ESPA) programme UK, through the Dynamic Drivers of Disease in Africa Consortium (DDDAC) that funded the field work component in Kenya. Prof. Dr. Peter-Henning Clausen, Prof. Dr. Franz J. Conraths, Dr Johanna Lindahl and Antje Hoppenheit, PhD, were responsible for supervision of the study and made significant contributions to the protocol design, collection, analysis and interpretation of data. Deborah Mbotha drafted the study protocol, designed sample collection tools and carried out field sample and data collection with the help of ILRI and KEMRI technicians. She also carried out laboratory sample and statistical data analysis, compiled, analyzed and interpreted the data and drafted both manuscripts. All co-authors contributed intellectually during the revision of the manuscripts and approved the published articles.

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Statement of Authorship

Except where reference is made in the text, this thesis contains no material published elsewhere or extracted in whole or in part from any previous work presented by me for another degree or diploma. No other person's work has been used without due acknowledgement in the main text of the thesis. I have not submitted this thesis at any other institution for the award of any degree or diploma.

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