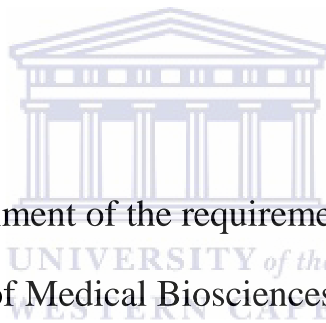
BIONOMICS OF VECTOR-BORNE DISEASES IN SITES ADJACENT TO LAKES VICTORIA AND BARINGO IN KENYA

David Omondi Ouma



A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Medical Biosciences, University of the Western Cape.

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Co-supervisors: Dr Jandouwe Villinger and Dr Daniel K. Masiga

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Keywords

Vectors

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Rickettsiosis

Arboviruses

ABSTRACT

Bionomics of vector-borne pathogens (VBPs) is a complex phenomenon that involves understanding the ecology of arthropod borne pathogens and vertebrate hosts potentially involved in their transmission cycles. Investigations into the bionomics of viral and bacterial VBPs circulating in Baringo and Homa Bay Counties of Kenya were carried out. Specifically, vertebrate hosts represented in mosquito bloodmeals, presence of arboviruses in blood fed mosquitoes and patients presenting with acute undiagnosed febrile illnesses in rural health facilities, and tick borne pathogens (TBPs) diversity in ticks of animals were identified. Mosquitoes were trapped by BG sentinel and CDC light traps, while ticks were sampled directly from domestic animals and tortoises close to human habitation along the shores and adjacent islands of Lakes Victoria and Baringo in Kenya. Blood and sera were also sampled from patients presenting with acute febrile illnesses visiting four rural health facilities in Homa Bay County. Mosquitoes and ticks were sorted and identified to species using standard morphological taxonomic keys. All the biological samples (blood-fed mosquitoes, ticks and blood/sera) were processed using molecular and culture procedures for detection of VBPs (arboviruses, *Ehrlichia*, *Anaplasma*, *Rickettsia* and protozoa). Among 445 blood-fed *Aedeomyia, Aedes, Anopheles, Culex, Mansonia,* and *Mimomyia* mosquitoes, 33 bloodmeal hosts were identified including humans, eight domestic animal species, six peridomestic animal species and 18 wildlife species. Further detection of Sindbis and Bunyamwera viruses was done on blood-fed mosquito homogenates by Vero cell culture and RT-PCR in *Culex, Aedeomyia*, *Anopheles* and *Mansonia* mosquitoes from Baringo that had fed on humans and livestock. In TBPs assay, 585 tick pools were analysed consisting of 4,126 ticks collected in both study areas. More ticks were sampled in Baringo (80.5%), compared to Homa Bay (19.5%). In Baringo, agents of ehrlichiosis were detected from *Amblyomma* and *Rhipicephalus* ticks including *Ehrlichia ruminantium* (12.3%), *Ehrichia canis* (10.5%) and

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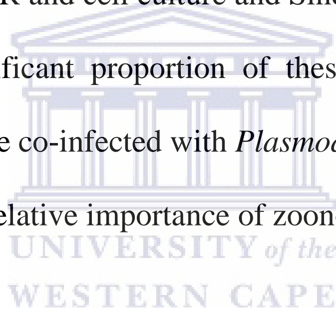
Paracoccus sp. (4.4%). Agents of anaplasmosis included *Anaplasma ovis* (7.2%)*, Anaplasma platys* (4.4%) and *Anaplasma bovis* (4.0%), all from *Hyalomma*, *Amblyomma* and *Rhipicephalus* ticks, as well as agents of rickettsiosis, including *Rickettsia africae, Rickettsia aeschlimannii, Rickettsia rhipicephali, Rickettsia montanensis* and a *Rickettsia* sp. that was not conclusively characterized. *Babesia caballi*, *Theileria* sp. and *Hepatozoon fitzsimonsi* were also detected from both *Rhipicephalus* ticks and *Amblyomma* ticks. In Homa Bay, *Ehrichia ruminantium* (17.5%) and *Ehrichia canis* (9.3%) were isolated from *Amblyomma latum* and *Rhipicephalus pulchellus*, as well as *Anaplasma platys* (14.4%) and *Anaplasma ovis* (14.4%) from *Amblyomma* and *Rhipicephalus* species. In determination of the occurrence of arboviruses among patients presenting with acute febrile illnesses, acute Bunyamwera 3 (0.9%) and Sindbis 2 (0.6%) infections were detected by RT-PCR and cell culture and Sindbis seroprevalence was determined by plaque assay. Though a significant proportion of these patients tested positive for low *Plasmodium* parasitemia, none were co-infected with *Plasmodium* parasites and arboviruses. This study highlights the presence and relative importance of zoonotic VBPs in both study areas.



DECLARATION

I declare that: **'Bionomics of vector-borne diseases in sites adjacent to Lakes Victoria and Baringo in Kenya'** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

David Omondi Ouma. January 2016

Signed

PUBLICATION, MANUSCRIPTS AND CONFERENCES FROM THIS RESEARCH WORK

- **Omondi D**., Masiga D., Ajamma Y.U., Fielding B., Njoroge L. and Villinger J. (2015) Unravelling host–vector–arbovirus interactions by two-gene high resolution melting mosquito bloodmeal analysis in a Kenyan wildlife–livestock interface. PLOS ONE 10(7), e0134375. doi: 0134310.0131371/journal.pone.0134375.
- **Omondi D**., Masiga D.K., Fielding B.C., Kariuki E., Ajamma Y.U., Mwamuye M.M., Ouso D. and Villinger J. Molecular detection of tick-borne pathogens along the shores and adjacent islands of Lakes Baringo and Victoria, Kenya. Submitted to *Tick and Tick-borne pathogen diseases Journal*.
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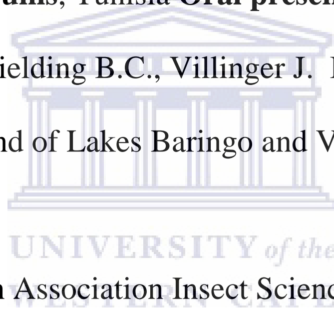

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LIST OF COMMON ABBREVIATIONS

ARPPIS	African Regional Postgraduate Programme in Insect Science
CB&ID	Capacity Building and Institutional Development
CCHF	Crimean Congo Haemorrhagic Fever
CDC	Centre for disease control
CHIKV	Chikungunya virus
Cyt b	Cytochrome b gene fragment
DDSR	Division of Disease Surveillance and Response
DHF	Dengue Haemorrhagic Fever
ECF	East Coast Fever
EIDs	Emerging Infectious Diseases
HRM	High resolution melting analysis
icipe	International Centre of Insect Physiology and Ecology
KEMRI	Kenya Medical Research Institute
MBBU	Molecular Biology and Bioinformatics Unit
NTDS	Neglected tropical diseases
PBS	Phosphate Buffered Saline
PRNT	Plaque reduction and neutralisation assay
RVFV	Rift Valley Fever Virus
SIDA	Swedish International Cooperation Development Agency
SSA	sub-Saharan Africa
TBPs	Tick-borne pathogens
VBPs	Vector-borne pathogens
WNV	West Nile Virus

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 General Introduction

Arthropods are responsible for hundreds of million cases of diseases in humans, livestock and wildlife each year (Gubler, 2001). In the last decade, there has been a global re-emergence of infectious diseases in man and animals, especially vector-borne diseases with increased frequency of epidemic transmission and expanding geographical distribution (Mableson *et al*., 2014). This broad group of infectious vector-borne diseases encompass viral, bacterial and protozoan agents, including mosquito-vectored malaria, Dengue, Chikungunya, West Nile, Rift Valley and Yellow fevers, tick-vectored Tick-borne encephalitis, Crimean-Congo haemorrhagic fever (CCHF) and bacterial pathogens such as *Rickettsia*, *Anaplasma* and *Ehrlichia* (Braks *et al*., 2011). A major problem is that the most of these important vector-borne diseases occur in the tropics, usually resource poor settings where surveillance is scarce (Maudlin *et al*., 2009). However, increase globalization has led to frequent human and animal mobility due to air travel and commerce. These have led to vector-borne pathogen (VBP) problems not only in the tropics, but to the rest of the world (Marks, 1986). This underscores the need for understanding the ecology of VBPs and possible transmission dynamics around humans, domesticated animal hosts and wildlife.

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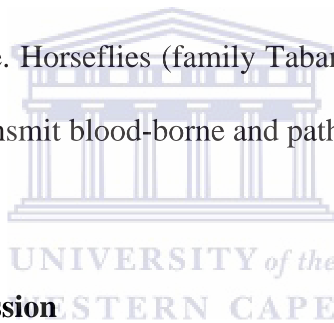
1.1 Vector-Borne Disease transmission

Vector-Borne Pathogens (VBPS) fall into four main taxa of microorganisms: nematodes, protozoa, bacteria (including *Rickettsia* and *Anaplasma* spp.) and viruses. The majority of these are zoonotic and have wildlife hosts serving as primary vertebrate hosts (disease reservoirs)

(Cantas and Suer, 2014). Humans and domestic animals are considered incidental hosts for most VBPs, and they may or may not contribute to the transmission cycle on a temporary basis; as a result, they are not usually required for survival of the pathogen in nature (Medlock and Jameson, 2010). An arthropod may transmit disease agents from one person or animal to another in one or two basic ways, as outlined below.

1.1.1 Mechanical transmission

This consists of a simple transfer of the organism on contaminated mouthparts or other body parts. No multiplication or developmental change of the pathogen on or in the arthropod takes place during this type of transmission (Gray and Banerjee, 1999). Examples of pathogens that are transmitted in this way include various enteric viruses, bacteria, and protozoa that have a direct fecal-oral transmission cycle. Horseflies (family Tabanidae) that frequent numerous hosts over a short period of time, can transmit blood-borne and pathogens mechanically (Desquesnes *et al*., 2013).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1.1.2 Biological transmission

The most important type of transmission by arthropods is biological transmission. In this mode of transmission, pathogens must undergo some type of biological development in the body of the arthropod vector in order to complete its life cycle. The four types of biological transmission include propagative transmission which occurs when the pathogen is ingested with the bloodmeal undergoes simple multiplication in the body of the arthropod. Arboviruses replicate extensively in various tissues of mosquitoes, and are transmitted to a new host in the salivary fluid of the arthropod when it takes a bloodmeal (Kuno and Chang, 2005). Cyclopropagative transmission is where the pathogen undergoes a developmental cycle (changes from one stage to another) and multiplication in the body of the arthropod. Malaria parasites in mosquito enters as gametocytes

and eventually give rise to >200,000 sporozoites (Gubler, 2009). Cyclodevelopmental transmission occurs when pathogen undergoes developmental changes from one stage to another, but does not multiply. Microfilaria ingested by a mosquito may result in only one third-stage infective larva. In most instances, however, the number of infective larvae is significantly lower than the number of microfilariae ingested with the bloodmeal. Some viruses and rickettsiae are transmitted from the female parent arthropod through the eggs to the offspring. If the pathogen actually infects the developing egg, this is termed 'transovarial transmission'. With some arboviruses, however, only the ovarial sheath and oviduct are infected, and the egg becomes infected as it passes down the oviduct and is inseminated. This type is distinguished from transovarial transmission and is called 'vertical transmission'. In either case, the newly hatched arthropod larval stages are infected with the pathogen, which is then transmitted to subsequent development stages of the arthropod ('trans-stadial transmission'). Venereal transmission of certain viruses like LaCrosse virus in mosquitoes has also been documented (Tesh *et al*., 1972). Thus, male mosquitoes that become infected transovarially or vertically can transfer the virus to uninfected female mosquitoes in the seminal fluid during copulation (Tesh *et al*., 1972). Finally, certain arboviruses have been shown to infect their tick or mosquito vectors when infected and uninfected arthropods co-feed in close proximity to each other on the same vertebrate host in the absence of viremia in that host. The virus is apparently attracted to the uninfected arthropod through a chemo-tactic response to the salivary fluid injected into the bite wound thus getting into the system of unfected arthropod to make it infected (Gubler, 2009).

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1.2 Vector-borne disease transmission cycles

Vector-borne disease transmission cycles typically involve a set of important pathogen(s), arthropod vector(s), vertebrate host(s), and occur within a variety of particular environments

(Figure 1). Vector-borne diseases in general are especially ecologically sensitive since environmental conditions can have dramatic effects on the vectors, pathogens, and potential hosts involved in transmission (Campbell-Lendrum et al., 2005).

Vector-borne disease transmission cycles.

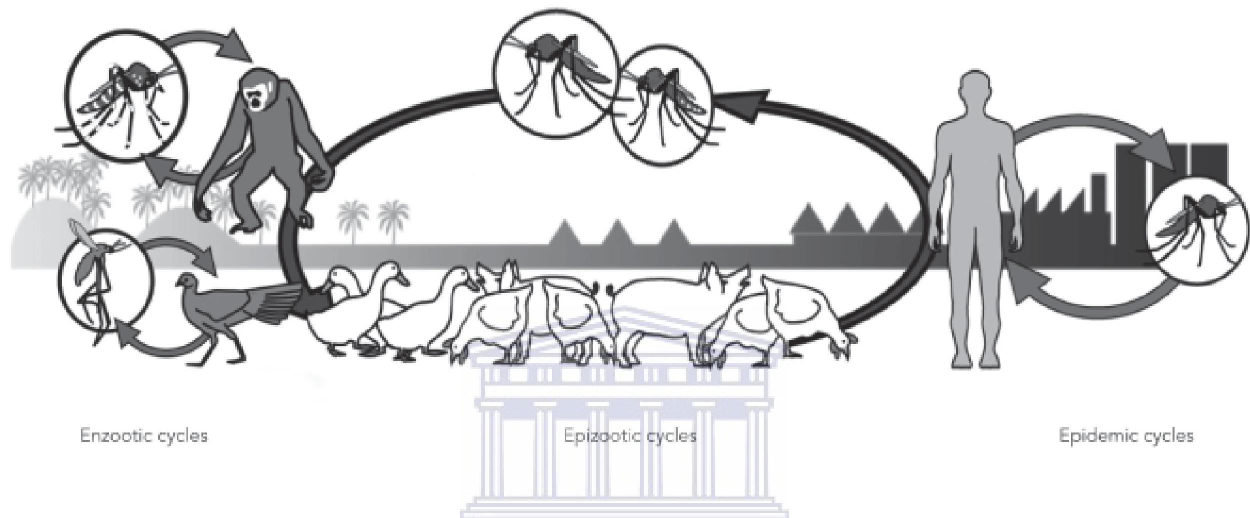

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Figure 1: General model of vector-borne disease transmission occur in three environments and involve diversity of pathogens, hosts and vectors. The three environments and transmission cycles can be classified as natural (enzootic) that occur in the forests, rural (epizootic) and urban (epidemic) (Ellis and Wilcox, 2009).

1.3 Evolution of vector-borne pathogens

Facets of globalization that include increased human populations, global change in land use and their effects on climate change exert selective pressures on pathogens, causing them to evolve to take advantage of the new environments, including hosts and vectors. Both West Nile Virus (WNV), Chikungunya virus (CHIKV), as well as other RNA viruses, evolve rapidly after being introduced to new locations and encountering new anthropophilic vectors (Holmes, 2003).

The evolution of these viruses makes them more adaptable to transmission by vectors. This has been observed in new stains of WNV, which exhibit increased transmission efficiency in *Culex pipiens and Cx. tarsalis* mosquitoes (Moudy *et al*., 2007, Kilpatrick *et al*., 2008). What has been less appreciated is the selective pressure currently imposed on zoonotic pathogens to adapt to being transmitted efficiently amongst humans by competent vectors like *Anopheles gambiae* and *Aedes aegypti*, and to a slightly lesser extent, by vectors that sometimes feed on non-human mammals and birds (Kilpatrick and Randolph, 2012).

1.4 Key mosquito vectors that spread human and animal pathogens

Kenya has abundant ecological zones that are favorable for mosquito vectors including the genus *Anopheles.* Over 140 *Anopheles* species have been recorded with at least eight considered to be effective vectors of malaria (Gillies and Coetzee 1987, Gillies and de Meillon 1968). Two of the most efficient vectors of human malaria, *Anopheles gambiae sensu stricto* and *Anopheles arabiensis* are members of the *An. gambiae* complex (White 1974). Other recognized species of the complex are *Anopheles merus*, *Anopheles melas*, *Anopheles quadriannulatus*, *Anopheles quadriannulatus* B and *Anopheles bwambae*. *Anopheles merus* and *An. melas* are associated with salt-water with a localized distribution along the eastern and western coasts of Africa, respectively, while *An. bwambae* has only been found breeding in mineral springs in the Semliki forest in Uganda (Coluzzi 1984). *Anopheles quadriannulatus*, found in south-east Africa (Coluzzi 1984) and *An. quadriannulatus* B, which has been described in Ethiopia (Hunt *et al*., 1998) are not considered vectors of human malaria as they are generally zoophilic. In addition to the *An. gambiae* complex, other species known to be important in malaria transmission in Kenya include *Anopheles nili*, *Anopheles moucheti* and *Anopheles funestus* which belongs to the Funestus group of which there are two African subgroups (Funestus subgroup includes *Anopheles aruni, Anopheles*

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confusus, Anopheles funestus, Anopheles parensis, Anopheles vaneedeni; Rivulorum subgroup includes Anopheles brucei, Anopheles fuscivenosus, Anopheles rivulorum, and *An. rivulorum*-like species) (Gillies 1987, Harbach 2004). Several of these vector species are found to occur in sympatry in much of Africa and their importance in malaria transmission varies depending on behaviour (e.g. biting activity, feeding and resting preferences), seasonal prevalence and vectorial capacity (Coluzzi 1984, Fontenille *et al*., 2004). These differences contribute to the varied malaria epidemiological patterns observed in Africa and, subsequently, different areas may require different tools and strategies for optimal vector control.

Other mosquito vectors common in Kenya are those of Rift Valley fever (RVF), which is usually transmitted to mammals by mosquitoes, and mainly depends on the availability of competent vectors, susceptible hosts, and suitable ecological and environmental conditions that favor mosquito survival and reproduction (Fontenille et a., 1998, Diallo *et al*., 2000). RVF vectors can be classified into two major groups, namely primary and secondary vectors. In Kenya, the known primary vectors, *Aedes mcintoshi* Huang and *Aedes ochraceus* Theobald, are believed to serve as reservoirs for the virus (Fontenille et a., 1998, Fontenille et a., 1994, Traore-Lamizana *et al*., 2001). Breeding of these vectors has mostly been associated with characteristic shallow depressions on land called "dambos" (Linthicum *et al*., 1985). The dambos are usually flooded after heavy rainfall, resulting in mass emergence of floodwater *Aedes* mosquitoes (Linthicum *et al*., 1985, Linthicum *et al*., 1984). The primary vectors maintain RVFv transovarially by transmitting the virus through to the eggs (Linthicum *et al*., 1985). The infected eggs can enter diapause in dry dambos for long periods and hatch into infectious mosquitoes during periods of extended rainfall. This may result in transmission of the virus to nearby animals and human beings when the vectors seek blood meals. Once primary transmission of the virus has taken place, secondary vectors belonging to the genera *Culex*, *Anopheles* and *Mansonia*, which take over

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flooded grounds for breeding, contribute to the amplification of the virus due to their ubiquitous biting patterns, consequently resulting in outbreaks (Woods *et al*., 2002, McIntosh and Jupp 1981, Linthicum *et al*., 1999, Anyangu *et al*., 2007).

Arboviral diseases such as dengue, yellow fever and chikungunya fever are transmitted from one susceptible host to another, principally by *Aedes aegypti*. These emerging and resurging diseases cause global concern (WHO 2012, Kyle 2008). The global incidence of dengue has risen rapidly in recent decades and the disease is now endemic in more than 100 countries in Asia, Africa, and the Americas. Infections from arboviral diseases have also risen and are now estimated at 50–100 million infections every year, with 21,000 fatalities (WHO 2009). This puts some 3.6 billion people, that is, half of the world's population, mainly in the urban centers of the tropics and subtropics at risk (Gubler 2002, Beatty *et al*., 2011). *Aedes aegypti* has long been assumed to have originated in Africa, where the ancestral form was likely a generalist, zoophilic treehole breeder (Mattingly 1957; Tabachnick 1991). Such zoophilic populations of *Ae. aegypti* still exist today on the African continent as the subspecies *Aedes aegypti formosus*. The better-known specialized domestic form, *Aedes aegypti aegypti*, is found in close association with human habitats throughout the tropical and subtropical world outside of Africa.

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Mosquitoes of the genus *Culex* have been reported as the most important vectors of West Nile virus (WNV), with *Culex pipiens* as the dominant vector (Rossi *et al*., 2010). Although many different species of mosquito are capable of maintaining WNV, The natural transmission cycle of the virus involves principally *Culex* species, with birds acting as reservoirs of the virus (Rossi *et al*., 2010). Not all infected mosquitoes preferentially feed upon birds, which can lead to other animals, including humans, becoming infected. Humans (and horses) are incidental or “dead-end” hosts in this cycle since the concentration of virus within the blood (viremia) is insufficient to

infect a feeding naïve mosquito (Rossi *et al*., 2010). *Culex* spp. mosquitoes have also been implicated in WNV transmission in Europe, Australia, and South Africa (Balenghien *et al*., 2008, Jupp 1974 Mackenzie *et al*., 1994 Muñoz *et al*., 2012). Mosquitoes of the genus *Aedes* which are the transmission vector for related flaviviruses, may also serve as important bridge vector for WNV (CDC 2009, Turell *et al*., 2005). While experimental transmission of WNV by ticks has been demonstrated, a role for ticks in natural transmission and maintenance of WNV has not been determined (Abbassy *et al*., 1993, Formosinho and Santos-Silva 2006, Hutcheson *at al*., 2005, Lawrie *et al*., 2004, Lwande *et al*., 2013).

Ticks transmit numerous protozoan, viral, bacterial (including rickettsial), and fungal pathogens to human and animals. They are characterized in two broad categories of hard (Ixodid) and soft (Argasid) ticks. Hard ticks are the most notorious disease vectors and incude species like *Amblyomma variegatum* that occurs in areas with a wide variety of climates, from rain forest, temperate (highland), savanna through to steppe (Walker *et al*., 2003). It is widely distributed through West, Central and East Africa and in southern Africa extends into Zambia, north eastern Botswana, the Caprivi Strip of Namibia, north western Zimbabwe and central and northern Mozambique. Its spread southwards appears to be limited by interspecific competition with *Amblyomma hebraeum* with which it shares similar habitats, hosts and sites of attachment. *Amblyomma variegatum* transmits the bacterium *Ehrlichia ruminantium* which causes heartwater in cattle, sheep and goats (Walker *et al*., 2003). It also transmits the *Ehrlichia bovis*, causing bovine ehrlichiosis, and the protozoans *Theileria mutans* and *Theileria velifera* causing benign bovine theileriosis (Walker *et al*., 2003). Heavy infestations suppress the immunity of cattle, making worse the bacterial skin disease dermatophilosis. Heavy infestations also damage teats and reduce productivity (Walker *et al*., 2003).

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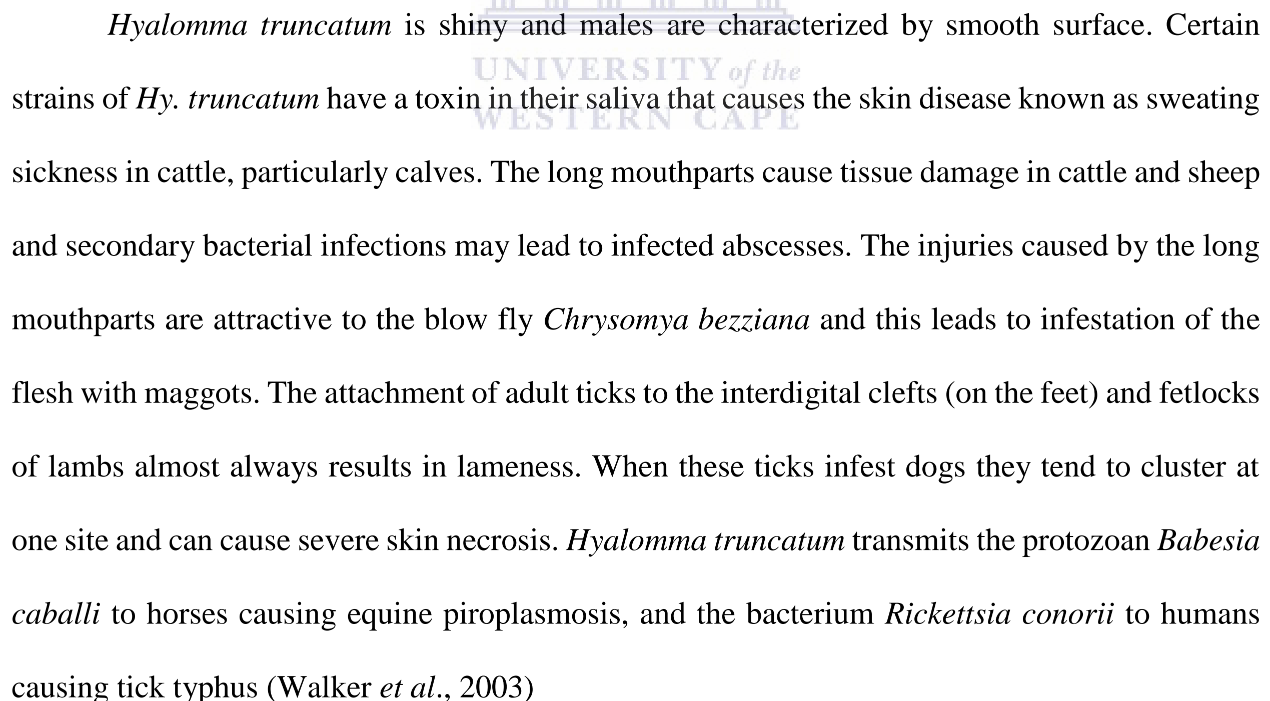
Hyalomma marginatum marginatum is also known as the Mediterranean *Hyalomma.* It is one of the important *Hyalomma* species in North Africa and is notorious as the vector of the virus causing Crimean-Congo haemorrhagic fever in humans (Walker *et al*., 2003). *Hyalomma m. marginatum* transmits the protozoan *Babesia caballi* causing babesiosis in horses and it is known to transmit *Theileria annulata* under laboratory conditions. Similar to *Hyalomma m. marginatum* is *Hyalomma marginatum rufipes* also known as the hairy *Hyalomma* or the coarse-legged *Hyalomma*. It is the most widespread *Hyalomma* in Africa and is notorious as a vector of the virus causing Crimean-Congo haemorrhagic fever in humans. It also transmits the bacterium *Anaplasma marginale* to cattle causing bovine anaplasmosis, *Rickettsia conorii* causing tick typhus in humans and the protozoan *Babesia occultans* to cattle (Walker *et al*., 2003). The feeding of adults on cattle causes large lesions at the attachment sites, leading to the formation of severe abscesses (Walker *et al*., 2003)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Hyalomma truncatum is shiny and males are characterized by smooth surface. Certain strains of *Hy. truncatum* have a toxin in their saliva that causes the skin disease known as sweating sickness in cattle, particularly calves. The long mouthparts cause tissue damage in cattle and sheep and secondary bacterial infections may lead to infected abscesses. The injuries caused by the long mouthparts are attractive to the blow fly *Chrysomya bezziana* and this leads to infestation of the flesh with maggots. The attachment of adult ticks to the interdigital clefts (on the feet) and fetlocks of lambs almost always results in lameness. When these ticks infest dogs they tend to cluster at one site and can cause severe skin necrosis. *Hyalomma truncatum* transmits the protozoan *Babesia caballi* to horses causing equine piroplasmosis, and the bacterium *Rickettsia conorii* to humans causing tick typhus (Walker *et al*., 2003)

Rhipicephalus appendiculatus is also known as the brown ear tick because of its colour and preference for feeding on the ears of cattle. It has been the subject of many studies aimed at its control because of its association with East Coast fever of cattle (Walker *et al*., 2003), *Rhipicephalus appendiculatus* transmits the protozoan *Theileria parva*, the cause of East Coast fever in cattle, and it transmits the different strains of *Th. parva* that cause Corridor or Buffalo disease and Zimbabwean theileriosis. It transmits *Theileria taurotragi* causing benign bovine theileriosis. Bacteria transmitted are *Anaplasma bovis* causing bovine ehrlichiosis, and *Rickettsia conorii* causing tick typhus in humans. It also transmit the virus causing Nairobi sheep disease. Heavy infestions cause reduction of weight gain and immune suppression. *Rhipicephalus evertsi evertsi* is also known as the red-legged tick. It is widely distributed and common on livestock throughout much of Africa. *Rhipicephalus e. evertsi* transmits the protozoans *Babesia caballi* and *Theileria equi* to horses, both causing forms of equine piroplasmosis. This tick transmits the bacterium *Anaplasma marginale* to cattle causing bovine anaplasmosis (gallsickness). The saliva of female ticks contains a toxin that causes paralysis, particularly in lambs, but it may also affect calves and adult sheep. This toxicosis is known as spring lamb paralysis because of its seasonal occurrence. *Rhipicephalus praetextatus* a characteristic *Rhipicephalus* species of cattle in East Africa and North East Africa This species of tick can transmit the virus of Nairobi sheep disease. The feeding of this tick species can cause toxicosis in cattle, resulting in paralysis. *Rhipicephalus pulchellus* is also known as the zebra tick. It is very well known where it occurs because it is so conspicuous, common and readily infests a wide range of hosts, including humans. It is called the zebra tick because of its white on black stripes and its use of zebras as a favourite host. This tick species transmits the protozoan *Theileria taurotragi* which causes benign bovine theileriosis. It also transmits Nairobi sheep disease virus causing the disease of the same name in sheep. It can be a risk to humans because of its transmission of the bacterium *Rickettsia conorii*, causing tick

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typhus, and transmission of the virus of Crimean-Congo haemorrhagic fever. It may occur on some hosts in sufficient numbers to cause direct parasitic harm (Walker *et al*., 2003).

Argas persicus is an argasid tick that has spread widely because of its close association with domestic birds. It is known as the fowl tick or the fowl tampan. Argas persicus transmits the bacterium *Borrelia anserina* causing avian spirochaetosis, and the bacterium *Aegyptianella pullorum*. Very large populations of ticks can build up rapidly in untreated poultry houses and severe anaemia can develop. Heavy infestations of poultry may also cause toxicosis. The larval ticks produce a toxin that causes paralysis in chickens and ducks similar to that seen in botulism (Walker *et al*., 2003).

1.5 Control of vector-borne pathogens

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Vector control is among the key strategies that are widely promoted by the World Health Organization (WHO) and the Roll Back Malaria Partnership (RBM) for prevention and reduction of malaria and other VBPs (WHO 2006, WHO 1993, RBM 2008). The other strategies include early diagnosis and prompt treatment of disease cases. Vector control protects people by preventing, reducing or interrupting the transmission of pathogen (WHO 2006). There are many different methods used for malaria vector control available include insecticide-treated nets (ITNs), long-lasting ITNs (LLINs), indoor residual spraying (IRS) and use of acaricides (Okumu and Moore 2011, Lengeler 2004, Pluess *et al*., 2010, WHO 2009). While ITNs, LLINs and IRS involve the use of chemical insecticides, some of the other methods of controlling larval or adult mosquitoes apply biological control techniques or environmental management (Rozendaal 1997, WHO 1995). WHO recommends the use of appropriate combinations of non-chemical and chemical methods of vector control in the context of integrated vector management (IVM) (WHO

2004). An IVM approach is pragmatic in that it offers a menu of vector control methods, which can be applied in various combinations to suit different ecological and socioeconomic settings. By using a range of different methods, it is possible to effectively target vectors at different stages in their life cycle, for instance, as larvae and pupae in mosquito breeding habitats, or at certain times during the host-seeking and resting behaviour of adult mosquitoes (Townson *et al*., 2005). On the other hand, reliance on only one vector control method is, in the long term, usually unsustainable for a variety of reasons, most notably insecticide resistance and adverse health and environmental impacts in the case of the use of chemical control (Rozendaal 1997, WHO 1995).

In 2004, WHO published the "Global Strategic Framework for Integrated Vector Management", spelling out the principles, objectives and requirements of IVM. The document underscores the purpose of IVM as improving the efficacy, cost-effectiveness, ecological soundness and sustainability of vector control (WHO 2004). IVM is defined in the document and in a subsequent 'WHO Position Statement on IVM' as "a rational decision-making process for the optimal use of resources for vector control" (WHO 2008). This current paradigm of IVM identifies several key elements for successful implementation of the approach (WHO 2004, Beier *et al*., 2008). They include: integration of non-chemical and chemical vector control methods and their integration with other disease-control measures; evidence-based decision making using methods based on sound knowledge of factors influencing local vector biology, disease transmission and morbidity; capacity building including development of adequate human resources, training and career structures at national and local level to manage IVM programmes; strengthening collaboration within the health sector and with other public and private sectors whose actions and policies might have important implications for vector control; engaging local communities and other stakeholders; and, creating a public health regulatory and legislative framework to reinforce IVM. However, very few programmes have harnessed this approach for mounting a rational,

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effective and integrated operational offensive to combat multiple vector-borne diseases. Mounting a formidable offensive against an array of chronic and debilitating vector-borne diseases is highly compromised by various reasons: environmental, socio-cultural, socio-economic, technical and programmatic; a weak health system; limited access to health services; lack of accurate entomological and epidemiological data to guide vector control planning and response; pesticide management and the threat of insecticide resistance development; weak planning and coordination amongst disease control programmes; a severely constrained skilled human resource base to drive the vector control agenda forward (Chanda *et al* 2013)

Novel introductions and increases in incidence of endemic VBPs highlight the need for effective control and treatment of individuals suffering from associated diseases. A key challenge in attempting to control many VBPs is that they are zoonotic and transmission intensity is driven primarily by possible wildlife reservoirs and climate. As a result, the dominant tools used against directly transmitted pathogens are vaccines that only protect individuals with financial and logistical access to them and have no impact on underlying transmission intensity. Managing or treating zoonoses of wildlife is a difficult task, especially in sub-Saharan Africa (SSA), and eradication is nearly impossible or prohibited (Barrett and Higgs, 2007).

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Climate variability has a large impact upon the incidence of vector-borne diseases: directly via the development rates and survival of both the pathogen and the vector, and indirectly through changes in the vegetation and the land-surface characteristics; this is particularly the case in Africa (Boko *et al*., 2007, Thomson *et al*., 2006). A significant relationship between RVF outbreaks, rainfall variability, land surface conditions and the El Niño Southern Oscillation has been highlighted over Kenya at the seasonal time scale (Linthicum *et al*., 1999). The potential climatic risk of VBPs including RVF is exacerbated by the presence of a large host reservoir (cattle, goats, sheep and to less extent camels) for these regions (Caminade *et al*., 2011).

In many cases, the most effective public health strategies for control of VBPs is to avoid infection by limiting vector feeding and disease transmission. This usually requires integration between researchers, public health agencies, the government and the public. Understanding the ecology of vectors and the pathogens that they transmit through surveillance is an important approach to creating awareness on possible transmission risks. This is evident by a recent detection in 2006 of Kupe virus in tick populations sampled in slaughter houses in Nairobi County of Kenya (Sang *et al*., 2006). It is therefore imperative to understand vector and VBP ecology and putative hosts that are important in the epidemiology of vector borne diseases. In this study, evaluation of the bionomics of VBPs was carried out in terms of i) vertebrate hosts represented in mosquito bloodmeals and presence of arboviruses among blood-fed mosquitoes sampled along the mainland shores and adjacent islands of Lakes Victoria and Baringo of Kenya, ii) tick borne pathogens in tick populations sampled from livestock, dogs, monitor lizards and free ranging tortoises in close proximity to human habitations in both study area, and iii) identification of acute arbovirus infections among patients with undiagnosed acute febrile illnesses observed at rural health clinics in Homa Bay County, western Kenya where malaria is highly endemic.

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1.6 Detection of vector-borne pathogens and their limitation

All diagnostic tests for detection of VBPs have inherent advantages and limitations. Serology relies on an immunologically appropriate and diagnostically detectable host immune response against one or more VBPs. As antibodies can persist for variable intervals after a pathogen is immunologically or therapeutically eliminated, serology does not confirm active or persistent infection in the patient, which is a diagnostic disadvantage. However, serology can be used to retrospectively confirm recent infection, by demonstrating seroconversion (i.e. a four-fold change in the patient's antibody titer between acute and convalescent serum samples) (Nilsson *et*

al., 2005). The persistence of circulating antibodies can also be an advantage of serology in that antibodies may be detectable during chronic intravascular VBP infections, when a pathogen may be circulating below the limit of PCR detection or sequestered in tissues that are not routinely submitted for PCR testing. Another potential limitation of serology includes diminished specificity, due to antibody cross-reactivity within or between VBP genera (Knobloch *et al*., 1985, Mann *et al*., 2003, Da Costa *et al* 2005, Ravnik *et al*., 2011, Rawlins *et al*., 2005, Sander *et al*., 2005, Vermeulen *et al*., 2010). Nevertheless, cross-reactivity between *Anaplasma*, *Bartonella*, *Ehrlichia*, and *Rickettsia* genera seems to be very unlikely from experimentally-infected dogs with these pathogens as they develop very specific antibodies that do not cross react among genera (Pappalardo *et al*., 1997, Solano-Gallego *et al*., 2004, Hegarty *et al*., 2014, Alleman *et al*., 2001, Beall *et al*., 2012, Beall *et al*., 2008, Chandrashekar *et al*., 2010). Conversely, serological antigens chosen or available for assays may be too specific or mismatched to the etiological pathogens resulting in false negative results. These factors can result in the inability to accurately identify the infecting species or strain, which can have therapeutic implications for the patient. A technical limitation for the development of some VBP serological assays is the inability to produce antigens in sufficient quantities to be used in indirect fluorescent antibody (IFA) or enzyme-linked immunoabsorbent assays (ELISA) (examples include *Anaplasma platys*, *Ehrlichia ewingii*, *Babesia* spp. and *Hepatozoon* spp.), although the use of synthetic peptides may help overcome this limitation. With the advent of PCR testing, it has also become obvious that some patients do not mount a detectable antibody response, despite persistent intravascular infection with VBPs (Breitschwerdt and Maggi 2009, Cherry *et al*., 2011, Diniz *et al*., 2007, Perez *et al*., 2011, Varanat *et al*., 2011). Similar to serology, PCR has advantages and disadvantages for the diagnosis of VBPs. PCR has the distinct advantage over serology of detecting “active” infection in a single sample from a single time-point. Additionally, PCR can be used to specifically target a pathogen

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at the species or strain level by using different PCR primer sets or by sequencing PCR products. While some a priori knowledge or assumptions about the DNA sequence of a pathogen are needed to design PCR-based assays, PCR does not require definitive knowledge of the pathogen DNA sequence. Additionally, PCR does not require that the pathogen(s) be isolated or their antigens produced to achieve the development and validation of an assay. Multiple pathogens or species can be detected using multiplex PCR assays (Ecker *et al*., 2009, Ecker *et al*., 2008, Sampath *et al*., 2007), but these assays can be more challenging in the context of achieving optimal sensitivity for all pathogens targeted in the panel. Co-infecting pathogens may cause competition in the PCR reaction process (Maggi and Breitschwerdt 2005, Maggi *et al*., 2005). Substantially higher concentrations of one pathogen compared to the other(s) can result in detection of only one organism despite the presence of a co-infection. The main limitation to PCR testing is the requirement for adequate template (nucleic acid of the target organism) in samples to achieve amplification of the target DNA sequence. For vector-borne pathogens such as *Anaplasma* spp., *Borrelia burgdorferi*, and *Bartonella* spp., it is well documented that the number of intravascular organisms fluctuates over time following transmission (Solano-Gallego *et al*., 2004, Hegarty *et al*., 2014, Beall *et al*., 2012, Beall *et al*., 2008). Therefore, PCR testing at a single time point may produce a false negative result for an infected patient. Some other technical disadvantages of PCR-based testing include potential false negative results due to the presence of PCR inhibitors that were not removed during the nucleic acid purification process and the potential for laboratory contamination resulting in false positive reactions in patients that are not infected. The latter disadvantages can be minimized by the use of appropriate techniques, reagents and the incorporation of appropriate controls. Unless mechanisms are developed to overcome all of the limitations of PCR-based testing, PCR is unlikely to serve as a stand-alone assay for the diagnosis of many vector-borne infections.

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1.7 Determination of mosquito bloodmeals and arboviruses in blood-fed mosquitoes

Arthropod-vectored pathogens contribute to the greatest diversity of neglected tropical diseases (NTDs) that significantly impact human health and livestock-based food security in developing countries and also threaten human and livestock health in developed countries (Gubler, 2002). Arthropod disease vectors may feed on a variety of vertebrate hosts, including wildlife that may represent unknown pathogen reservoirs, not only of known NTDs, but also of emerging infectious diseases (EIDs) (Daszak *et al*., 2001). Bloodmeal identification of field-collected vectors is pivotal to disentangling disease transmission dynamics, identifying ecological reservoirs during inter-epidemic periods, and developing appropriate disease control and response strategies (Zirkel *et al*., 2011). Molecular boodmeal identification is widely performed by using standard methods as Sanger sequencing and methods that analyze mitochondrial (mt) genes for cytochrome b (cytb), 12S or 16S ribosomal ribonucleic acid (12S rRNA, 16S rRNA) or cytochrome oxidase I (COXI) (Alacs *et al*., 2010, Alonso *et al*., 2006). These molecular markers are highly conserved between species and are robust in interspecies differentiation (Alacs *et al*., 2010). These analyses become more superior when performed as a closed-tube assays in a real-time PCR followed by a melting curve analysis (MCA) that will minimise drawbacks such as contamination and multi step processes. The melt curve analysis uses the possibility of differentiating PCR products by their characteristic melting temperatures (Tm) at which the transition of dsDNA to ssDNA occurs depending on fragment length and sequence composition (Reed *et al*., 2007, Montomery *et al*., 2007). Enhanced technologies as high-resolution melting (HRM) improve the temperature resolution down to 0.1°C/step and thereby lead to a more accurate monitoring of the melting behavior increasing the discrimination of sequence variants between PCR products. Additionally, improved analysis methods do not only consider the absolute Tm but also the shape of the melting

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curve, facilitating PCR fragment differentiation (Reed *et al*., 2007). Peña *et al*., (2012) developed an improved, cost-effective and rapid approach based on high resolution melting analysis (HRM) of cytochrome b (*cyt b*) Polymerase Chain Reaction (PCR) products to identify vertebrate bloodmeals of triatomine bugs (*Rhodnius* and *Triatoma* species) in the Caribbean region of Colombia (Pena *et al*., 2012). However, preliminary analysis found that HRM analyses of these *cyt b* amplicons alone could not reliably differentiate the diversity of potential mosquito bloodmeal host species in East Africa, where humans, livestock and wildlife are often in close proximity.

To improve the vertebrate host species resolution of PCR-HRM based mosquito bloodmeal analysis in the East African context an additional pair of vertebrate specific PCR primers flanking short polymorphic mitochondrial 16S rRNA sequences amenable to robust HRM species typing was designed. Both *cyt b* and 16S HRM analysis was used to resolve and identify mosquito blood-feeding sources found at the geographical interface of wildlife with livestock farming and of aquatic/wetland with terrestrial ecosystems along the shores and adjacent islands of Lakes Victoria and Baringo in Kenya. As both regions have documented recent arbovirus activity and support a diversity of vertebrate hosts that are critical to arthropod pathogen transmission (Okiro *et al*., 2009, Mala *et al*., 2011, Mease *et al*., 2011), the samples were also screened for arboviruses in the blood-fed mosquitoes.

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1.8 Molecular detection of tick-borne pathogens around Lakes Baringo and Victoria, Kenya

Tick borne pathogens (TBPs) are responsible for some of the most serious emerging infectious diseases facing SSA and the rest of the world today (Dunster *et al*., 2002, Parola *et al*., 2005). In Kenya, tick borne viral diseases (arboviruses) like Crimean Congo haemorrhagic fever (CCHF), Kupe and Hazara as well as protozoan and bacterial agents of babesiosis, theileriosis and

rickettsiosis are major impediments to improved livestock productivity and public health (Parola, 2006, Sang *et al*., 2011, Lwande *et al*., 2014, Kiara *et al*., 2014).

Baringo and Homa Bay Counties of Kenya are both characterized by unique land-lake biogeographies with fluctuating fresh water levels of Lakes Baringo and Victoria, respectively. These ecosystems form shallow lagoons with abundant aquatic and terrestrial vegetations that are favourable for diverse vertebrate host species and disease vectors (Minakawa *et al*., 2012). The interaction of domestic animals (mainly livestock, dogs, chickens and cats), migratory birds, monitor lizards, various rodents, humans and wildlife has the potential to facilitate the spread of zoonotic pathogens, including TBPs. In Baringo County, the abundance of ticks inflicts unacceptable health and economic burdens on the pastoralist community (Munyua *et al*., 2010, Kamani *et al*., 2015), while TBP diseases remain understudied and go unnoticed. Along the shores of Lake Victoria in the neighbouring Siaya County, a recent human exposure to *Rickettsia felis* infection was found to be exceptionally high (>57%) based on immunoglobulin G (IgG) seropositivity among febrile patients visiting a local health clinic and *Rickettsia africae* has been isolated in *Amblyomma variegatum* (Maina *et al*., 2012, Maina *et al*., 2014). In Uganda, around the Lake Victoria basin, severe anaplasmosis, East Coast Fever (ECF) and babesiosis have been documented as causes of livestock morbidity and mortality (Rubaire-Akiiki *et al*., 2004, de la Fuente *et al*., 2008). Therefore, intensification of research efforts and development of novel tools for surveillance, disease detection and control of ticks will be critical in forestalling outbreaks of TBP infections and informing public health decisions, including early warning and response strategies in both study areas (de la Fuente *et al*., 2008).

Contemporary molecular biology techniques were utilized to screen field collected tick sampled along the shores and adjacent islands of Lakes Baringo and Victoria in Baringo and Homa Bay Counties of Kenya, respectively. The presence and possible transmission of bacterial and

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protozoan disease agent that are important to public health is reported. These disease agents are responsible for ehrlichiosis, anaplasmosis, rickettsiosis, babesiosis, theileriosis and hepatozoonosis that could result in zoonotic diseases that form bulk of undiagnosed febrile illnesses observed in these sub Saharan regions (Tigoi *et al*., 2015, Crump *et al*., 2013). The findings are important to public health in mitigating ticks and TBP infection and possible disease outbreaks in these foci, as well as other areas of Kenya, with wider geographical implications.

1.9 Arboviruses occurrence among patients with acute febrile illnesses visiting rural health clinics in Homa Bay County

There has been an unprecedented worldwide increase in arbovirus activity in the last half century, with several outbreaks occurring in the eastern SSA region, among them Yellow Fever, Dengue, Rift Valley fever (RFV), O'nyong-nyong, Crimean Congo Hemorrhagic fever, West Nile fever, and Chikungunya (Devaux, 2012, Soghaier *et al*., 2013, Munyua *et al*., 2010). Although the existence of novel viruses is relatively poorly researched in East Africa, recent detection of Kupe virus not described before in a brief surveillance activity is an indicator of the likely presence and circulation of novel agents (Sang *et al*., 2006). Most arbovirus infections in humans are characterised by the onset of systemic febrile illness accompanied by headache, myalgia and malaise that can progress to severe haemorrhagic fever and meningoencephalitis (WHO, 1985). Even though arboviral infections are highly prevalent in SSA, considerable clinical cases of systemic febrile illness, including undiagnosed fevers are usually attributed to familiar diseases such as malaria and typhoid fever, and thus arbovirus infections are underappreciated (Animut *et al*., 2009, Crump *et al*., 2013, Kipanga *et al*., 2014). Detection of arboviral diseases in most health facilities in this region is hampered by non-specific clinical presentation, lack of laboratory diagnostic capacity, cross-reactions during serologic testing, and passive and inconsistent

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surveillance in most countries (Tigoi *et al*., 2015, Henderson *et al*., 1970, Reyburn *et al*., 2004, Coleman, 1998).

In the determination of occurrence of arboviruses among patients with acute febrile illness, it was hypothesized that arboviruses comprised a proportion of undiagnosed febrile illnesses observed at rural health clinics along the shores and adjacent islands of Lake Victoria in Homa Bay County of Western Kenya and sought to isolate arboviruses in patients with acute febrile fevers. The shores of Lake Victoria have previously witnessed arbovirus activities with outbreak of O'nyong nyong fever having been reported as early as 1960s (Haddow *et al*., 1960) and in a recent infection in 2013 of a tourist visiting the Lake Victoria region who suffered unknown febrile illness that was later diagnosed as O'nyong-nyong (Haddow *et al*., 1960, Tappe *et al*., 2014). Seroepidemiological and entomological data have also reported presence and possible circulation of diverse arboviral families along the shores of Lake Victoria (Mease *et al*., 2011, Ochieng *et al*., 2013). The land-lake topography with fluctuating water levels form swampy ecosystems with diverse flora and fauna that make the region favorable for disease vectors to flourish (Minakawa *et al*., 2012). Western Kenya is endemic for malaria and schistosomiasis that are characterized by febrile illnesses (Olanga *et al*., 2015, Butler *et al*., 2012, Davis *et al*., 2015). In addition, respiratory viruses like influenza and para-influenza, salmonellosis and Q fever infection have previously been reported as causes of febrile illness in this region (Knobel *et al*., 2013, O'Meara *et al*., 2015, Onchiri *et al*., 2015), while cultural beliefs of polygamy and wife inheritance perpetuates high transmission of HIV (Afrane *et al*., 2014, Musuva *et al*., 2014, Dworkin *et al*., 2013). Valuable information on the presence and possible circulation of Sindbis and Bunyamwera viruses is reported as possible etiologies of febrile illness observed at Mfangano Island of Homa Bay County. Improving surveillance and laboratory capacity for detection of arboviruses in the area will be integral to understanding the disease burden posed by these agents in the region.

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1.10 Problem statement

Lakes Victoria and Baringo in Kenya share unique land-lake biogeographies with abundant flora and fauna. These include diverse mosquitoes and tick species that parasitize human, livestock and wildlife that interface with aquatic and terrestrial habitats. The interaction of vectors, VBPs and diverse vertebrate hosts, including migratory birds, wildlife, livestock and humans, is a mix that could lead to outbreaks of zoonotic diseases.

1.11 Objectives

To understand the bionomics/ecology of vector borne diseases along the shores and adjacent islands of Lakes Victoria and Baringo in Kenya

1.11.1 Specific Objectives

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1. To determine mosquito bloodmeal hosts and the presence of arboviruses in blood-fed mosquitoes sampled along the shores and adjacent islands of Lakes Victoria and Baringo, Kenya.
2. To design and optimize PCR conditions of oligonucleotide primers for mosquito bloodmeal and VBP detection.
3. To identify tick-borne pathogens circulating along the shores and adjacent islands of Lakes Victoria and Baringo, Kenya
4. To identify arboviruses associated with acute febrile illnesses observed among patients visiting rural health clinics along the shores and island of Lake Victoria, Homa Bay County.

1.12 Justification

Understanding the bionomics of VBPs in terms of their presence and possible transmission, vectors and vertebrate hosts involved in perpetuating their transmission is a critical step towards forestalling possible disease outbreaks observed along the shores and adjacent islands of Lakes Victoria and Baringo of Kenya. Mitigation of VBP transmission to human and livestock is a priority to public health.

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CHAPTER TWO

MATERIALS AND METHODS

2.1 Determination of mosquito bloodmeals and arboviruses in blood-fed mosquitoes

2.1.1 Mosquito sampling areas and ethical statement

Determination of mosquito bloodmeals and arboviruses in blood-fed mosquitoes was carried out in Kenya, along the shores and adjacent islands of Lake Victoria in Homa Bay County and Lake Baringo in Baringo County. Before sampling, ethical clearance was obtained for the study from the Kenya Medical Research Institute (KEMRI) ethics review committee (Approval Ref: Non-SSC Protocol #310). Sampling was conducted twice over a year, during the wet seasons (March-May) and (October-December) of 2012-2013, on unprotected public land.

The region is characterized by an equatorial tropical climate with an average minimum temperature of 16°C and an average maximum temperature of 28°C. The area experiences two rainy seasons: the long rainy season between March and June and the short rainy season between October and December. The average annual rainfall during the sampling period 2012-2013 was 1,536 mm (*icipe*-TOC meteorological station).

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Baringo County is located in the Rift Valley Province of Kenya, 250 km northwest of Nairobi. The area is semi-arid and consists of Acacia-Commiphora bushlands and impenetrable thickets of *Prosopies juliflora* that are displacing native trees and reducing grazing areas. Harsh physical and climatic conditions have led to a sparsely populated district where the local communities rely on pastoralism and on limited crop production. Lake Baringo (0°36'56.4"N 36°03'36.8"E, elevation ~980 m) has an annual rainfall range of 300 to 700 mm, and the daily temperature varies between 16°C and 42°C.

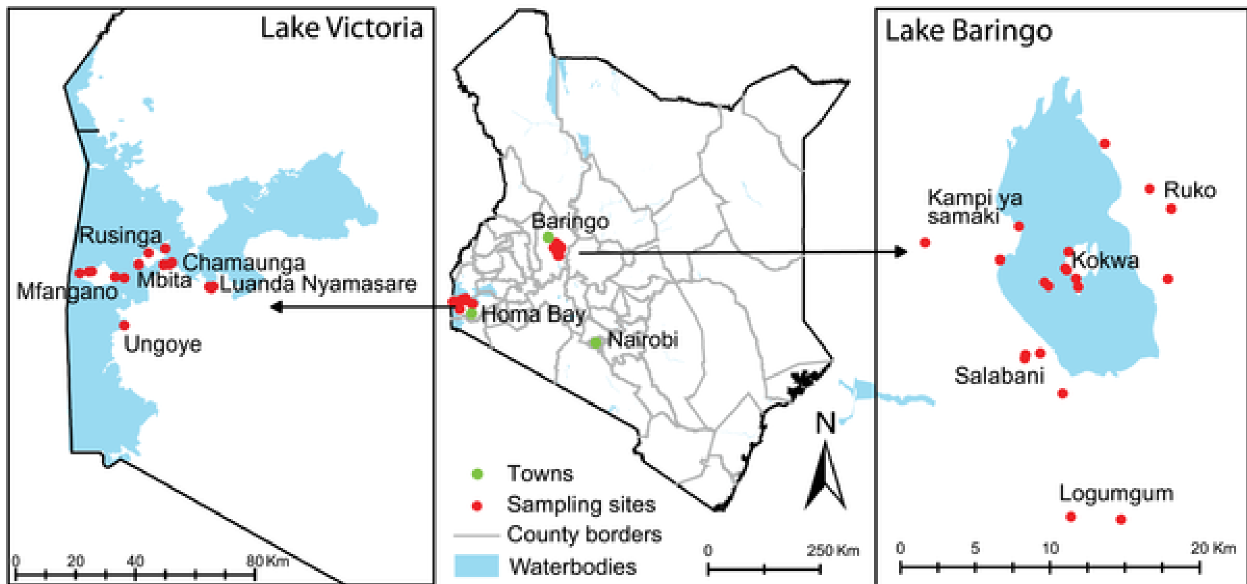

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Figure 2: Map of mosquito sampling areas near Lakes Victoria and Baringo in Kenya (Omondi *et al*., 2015).

2.1.2 Mosquito trapping

Mosquitoes were trapped outdoors, proximal to human habitation, in villages with CO_2-baited CDC light traps placed between 5:30-6:30pm and collected between 6:00am and 9:00am the following morning. This was effective for collection of evening and night biting mosquitoes. BG sentinel traps with BG-Lure (Human skin scented) were set at 8:00am and collected in the evenings between 5:00pm and 6:00pm were appropriate for trapping day time biting mosquitoes.

In Homa Bay County, a total of five carbon dioxide baited CDC light traps (John W. Hock Company, Florida. U.S.A.) and two BG sentinel traps (BioQuip Products, CA. USA) were set over two trap nights per season at each of six sampling areas on the mainland shores of Lake Victoria

in Ungoye, Luanda Nyamasare and Mbita (0°26’06.19” S, 34°12’53.13”E; elevation ~1,137 m) and the adjacent islands of Mfangano, Rusinga, and Chamaunga (Figure 2).

In Baringo County, a total of 21 CDC light traps and two BG sentinel traps were deployed over two trap nights per season in the five sampling areas along the shores of Lake Baringo in Kampi ya Samaki, Salabani, and Ruko Conservancy and Logumgum (a remote area with an oxbow lake filled with thick swampy marsh that is slightly distant from Lake Baringo, but was a hotspot for 2006-2007 RVF outbreak), as well as the Island of Kokwa (Figure 2).

After collection, mosquitoes were anesthetized using triethylamine (O'Guinn and Turell, 2002) and identified on a chilled surface (paper towels over -80°C icepacks) to species level based on their morphological features (Edwards, 1941). Blood-fed mosquitoes were individually separated in vials and cryopreserved in liquid nitrogen for transportation to the laboratory, where they were stored for bloodmeal analysis in -80° freezers.

2.1.3 Nucleic acid extraction

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DNA was extracted from frozen intact blood-fed mosquitoes using two extraction protocols, DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) extraction protocol was used initially, and the MagNA 96 Pure DNA and Viral NA Small Volume Kit (Roche Applied Science, Penzberg, Germany) was subsequently used in an automated MagNA Pure 96 extraction system (Roche Applied Science) to increase throughput. The first batch of mosquitoes (from Homa Bay County) was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) extraction protocol. The 258 engorged abdomens were separated from the rest of the body using sterile dissection pins and homogenized in phosphate buffered saline (PBS). Blood was extracted following the manufacturer’s instructions. In the second batch of 214 samples from Baringo County, the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Applied Science, Penzberg, Germany) was used. Individual blood-fed mosquitoes were homogenized whole for 5 sec in 0.5 ml screw-cap

tubes (Sarstedt, Newton, NC) filled with 750 mg of 2.0 mm, 150 mg of 0.1 mm zirconia/yttria stabilized zirconium oxide beads (Glen Mills, Clifton, NJ), and 350 µl of PBS before DNA extraction in an automated MagNA Pure 96 extraction system (Roche Molecular Systems, Pleasanton, CA).

2.1.4 Primer design

Although *cyt b* primers have already been used successfully for HRM-based identification of Chaga's disease vectors (Triatominae bugs; *Triatoma sp.* and *Rhodnius sp.*) (Pena *et al*., 2012), preliminary investigations found that these amplicons could not reliably differentiate some of the diversity of potential mosquito bloodmeal host species in East Africa. To improve the resolution of specific bloodmeal host identifications, primers were designed for PCR-HRM analysis to compliment HRM analyses based on *cyt b* primers (Boakye *et al*., 1999). Multiple alignment was generated of complete vertebrate mitochondrial genomes (Table 1), identified regions of high and low conservation across broad taxa and manually designed primers in conserved regions that flank highly polymorphic sequences of no more than 300 base pairs (bp). Multiple alignment of obtained sequences were then performed alongside invertebrate mitochondrial genomes (GenBank Accessions: AY140887, HM132112, EU352212, NC_006817, NC_014574, NC_015079, NC_014275, HQ724614, DQ146364, MSQMTCG) and identified primer pairs that could discriminately amplify vertebrate sequences in an arthropod vector background. Empirical identification of a set of primers that amplify a 190-250 bp fragment was done, depending on the vertebrate species, of the 16S ribosomal (r) RNA gene that generates distinct HRM profiles for a broad range of bloodmeal hosts.

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Table 1: Vertebrate mitochondrial genomes aligned for primer design for the detection of mosquito bloodmeals.

Vertebrate Class	Common name	Species	GenBank Accession
Mammalia	Human	*Homo sapiens*	KC569547
	Cow	*Bos taurus*	KC153975
	Goat	*Capra hircus*	NC_005044
	Dog	*Canis lupus familiaris*	NC_002008
	Horse	*Equus caballus*	NC_001640
	Zebra	*Equus zebra hartmannae*	JX312719
	Greyvi zebra	*Equus grevyi*	JX312725
	Baboon	*Papio cynocephalus*	JX946199
	Mouse	*Mus musculus*	JX945979
	Cane rat	*Thryonomys swinderanius*	NC_002658
	Hyrax	*Dendrohyrax dorsalis*	NC_010301
	Elephant	*Loxodonta africana*	NC_000934
	Hippopotamus	*Hippopotamus amphibius*	NC000889
Aves	Chicken	*Gallus gallus*	AY235570
Amphibia	Asian common toad	*Bufo melanostictus*	NC_005794
	African common toad	*Amietophrynus regularis*	DQ158485
	Seoul frog	*Rana chosenica*	NC_016059
Reptilia	Galapagos tortoise	*Geochelone nigra*	JN999704
	Nile monitor	*Varanus niloticus*	NC_008778

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2.1.5 Molecular bloodmeal identification

For identification of vertebrate hosts in mosquito bloodmeals, complimented HRM profile differences observed using the established 383 bp *cyt b* gene amplicon primers with the 200 bp amplicons obtained using newly designed 16S rRNA gene primers (Table 2). Using mosquito DNA extracts as negative controls, PCRs in final volumes of 10 µl with 0.5 µM concentrations of each primer, using 5X Hot Firepol Evagreen HRM Mix (Solis BioDyne, Tartu, Estonia) and 1 µl of DNA template was carried out. The thermal cycling conditions used for *cyt b* primers were as follows: Initial denaturation was done for 1 min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 20 sec, and extension at 72°C for 30 sec followed by a final extension at 72°C

for 7 min. Cycling conditions for 16S ribosomal DNA fragment were similar to those of *cyt b* except for an annealing temperature of 56°C. All PCR reactions were conducted on an HRM capable Rotor-Gene Q real time PCR thermocycler (QIAGEN, Hannover, Germany).

Table 2 Oligonucleotide primers used for the detection of mosquito bloodmeals and arboviruses in blood fed mosquitoes

Target	Primer name	Primer sequence	Citation
Vertebrate *cyt b*	Cytb For	5'-CCC CTC AGA ATG ATA TTT GTC CTC A-3'	(Boakye *et al*., 1999)
	Cytb Rev	5'-CAT CCA ACA TCT CAG CAT GAT GAA A-3'	
Vertebrate 16S	Vert 16S For	5'-GAG AAG ACC CTR TGG ARC TT-3'	Omondi *et al*., 2015
	Vert 16S Rev	5'-CGC TGT TAT CCC TAG GGT A-3'	
Phlebovirus	Phebo F1	5'-AGT TTG CTT ATC AAG GGT TTG ATG C-3'	(Lambert and Lanciotti, 2009)
	Phlebo F2	5'-GAG TTT GCT TAT CAA GGG TTT GAC C-3'	
	Phlebo R	5'-CCG GCA AAG CTG GGG TGC AT-3'	
Orthobunyavirus	BUN F	5'-CTG CTA ACA CCA GCA GTA CTT TTG AC-3'	(Lambert and Lanciotti, 2009)
	BUN R	5'-TGGAGGGTAAGACCATCGTCAGGAACTG-3'	
Alphavirus	Vir 2052 F	5'-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3'	(Eshoo *et al*., 2007)
	Vir 2052 R	5'-TAC GAT GTT GTC GTC GCC GAT GAA-3'	
Flavivirus	1NS5F	5'-GCA TCT AYA WCA YNA TGG G-3'	(Vazquez *et al.,* 2012)
	1NS5R	5'-CCA NAC NYN RTT CCA NAC-3'	
	2NS5F	5'-GCN ATN TGG TWY ATG TGG-3'	
	2NS5R	5'-TRT CTT CNG TNG TCA TCC-3'	

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Following PCR, HRM analysis of amplicons was conducted by gradually increasing the temperature in 0.1°C, 2 second increments from 65°C to 90°C and recording and plotting changes in fluorescence with changes in temperature (dF/dT). DNA extracted from known vertebrate blood samples were used as standard reference controls and included cow, goat, sheep and human clinical samples from Suba district (within afore-mentioned ethics approval), as well as Swiss mouse, rabbit, and chicken blood samples sourced from *icipe*'s animal rearing unit. DNA extracted from *Culex pipiens* and *Anopheles* sp. legs were used as negative controls. PCR-HRM protocols were validated for accuracy and sensitivity using reference controls. Ten microliters of human, mouse, rabbit and chicken reference control blood were serially diluted ten-fold in PBS to 10^{-7}. DNA from

three 1 µl replicates of each diluent was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) extraction protocol. HRM analysis was carried out using the Rotor-Gene Q software v2.1 with normalization regions between 76.0–78.0°C and 89.50–90.0°C. Bloodmeal sources were identified by comparison of melting profiles with those of the reference control species. Representative samples that generated unknown HRM curves either from the *cyt b* or 16S PCR amplicons, that did not match standard reference controls were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) to remove unincorporated dNTPs and PCR primers before submission for amplicon sequencing using the same primers for amplification at Macrogen (Seoul, South Korea). The returned sequences were edited and aligned using MAFFT (Katoh *et al*., 2002) plugin in Geneious software (version 8.1.4) (Kearse *et al*., 2012). Sequence identity was revealed by querying in the GenBank nr database using the Basic Local Alignment Search Tool BLAST (Altschul *et al*., 1990). For identification of specific vertebrate host species, homology cut-off of 97%-100% identity with a GenBank *e*-value threshold of 1.0*e*-130 for the *cyt b* sequences and 1*e*-75 for the 16S sequences were used. Based on multiple alignments, particularly among the bird samples, some identification were limited to higher taxonomic classifications.

2.1.6 Identification of arbovirus infections

Identification of arbovirus infections was performed on 214 mosquito samples comprising the second batch from Baringo. Fifty microlitre aliquots of homogenates were inoculated on 24-well culture plates (Nunc Culture Treated Multidishes, Thermo Scientific, USA) with confluent monolayers of Vero cell line grown in growth media (minimum essential medium with 10% fetal bovine serum (FBS), 2% glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1µl/ml amphotericin B) and observed for virus induced cytopathology for 14 days after incubation at 37°C in a humidified incubator with 5% CO_2. Culture wells that showed cytopathic effects were harvested and RNA extracted using the MagNA 96 Pure DNA and Viral NA Small Volume Kit

(Roche Applied Science) in a MagNA Pure 96 (Roche Applied Science) automated extractor, followed by reverse transcription using the High Capacity cDNA Reverse Transcription Kit (Life technologies, Carlsbad, CA). Using published primers (Table 2), phleboviruses, orthobunyaviruses (Lambert and Lanciotti, 2009) and alphaviruses (Eshoo *et al*., 2007) were screened by PCR, as well as flaviviruses by nested PCR (Vazquez *et al*., 2012) in 10 µl reactions using 1µl cDNA template, 5 µl MyTaq HS master mix (Bioline Reagents Limited, London, UK) and 1 µl of 50 µM SYTO-9 saturating intercalating dye (Life technologies, Carlsbad, California). Laboratory arboviral stocks of diverse Kenyan isolates (Ochieng *et al*., 2013) were used as standard reference controls. Viral amplicons were initially identified by HRM analysis and then confirmed by sequencing at Macrogen (Seoul, South Korea) after amplicon purification with ExoSAP-IT (USB Corporation, Cleveland, OH).

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2.2 Molecular detection of tick borne pathogens around Lakes Victoria and Baringo, Kenya

2.2.1 Tick borne pathogen study locality

Tick-borne pathogen survey was conducted in 2012-2013 along the shores and adjacent islands of Lakes Baringo and Victoria in Baringo and Homa Bay Counties of Kenya, respectively. Three indigenous agro-pastoralists communities that live in Baringo County rely mainly on livestock, with some irrigated crop production along the Perkerra, Molo and Kerio Rivers. In Homa Bay County, most inhabitants belong to the Luo and Suba ethnic groups whose main socio-economic activities are fishing and small scale mixed farming, which includes keeping of livestock (mainly cattle, sheep and goats).

2.2.2 Tick Sampling

Live ticks were collected twice in 2012 and 2013 in the months of April-May and October-December from domesticated animals, cattle (76), goats (117), sheep (54), poultry houses (17) dogs (15), tortoises (12) and monitor lizards (4) found around human habitats. Tortoises and Monitor lizard were chosen as they are laden with ticks and are common around human habitats and therefore present a risk of unknown diseases. Tick sampling was conducted in the same areas that have previously been described for mosquito sampling (Omondi *et al*., 2015) (Figure 3). Before sampling, ethical clearance for the study was obtained from the Kenya Medical Research Institute (KEMRI) ethics review committee (Approval Ref: Non-SSC Protocol #310). Sampling was conducted on unprotected public land and no protected species were sampled.

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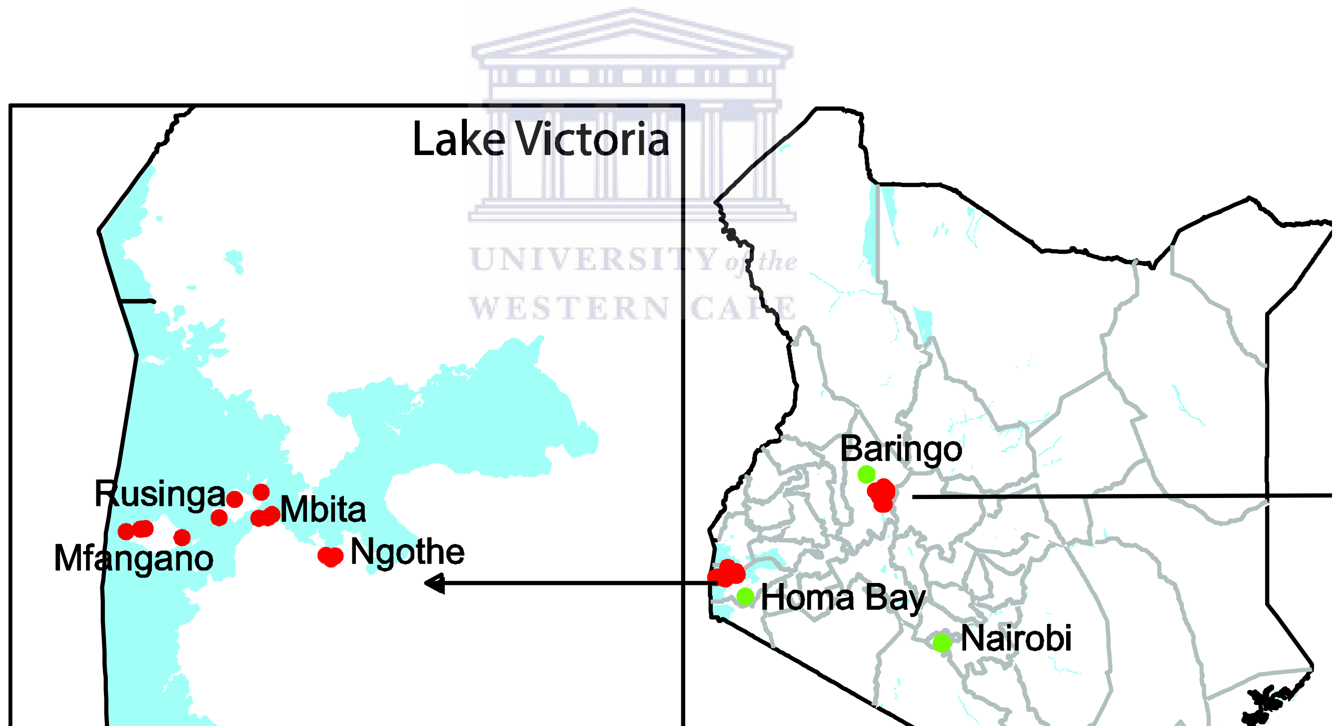

Figure 3: Map of Kenya showing tick sampling areas

After collection, ticks were frozen in liquid nitrogen and transported to the Martin Lüscher Emerging Infectious Disease (ML-EID) Laboratory in Nairobi where they were analyzed. Ticks were identified morphologically to species level based on taxonomic keys developed by Walker *et al*. (2003) (Walker and Olwage, 1987) and pooled (≤8 individuals) by species, collection date and site.

2.2.3 Molecular tick borne pathogen identification

Tick pools were homogenized for 25 sec in 0.5 ml screw-cap tubes (Sarstedt, Newton, NC) filled with 750 mg of 2.0 mm, 150 mg of 0.1 mm zirconia/yttria stabilized zirconium oxide beads, (Glen Mills, Clifton, NJ) (Crowder *et al*., 2010), 650 µL of phosphate buffered saline (PBS) before total nucleic acid extraction in an automated MagNa Pure 96 extraction system (Roche Molecular Systems, Pleasanton, CA) using the small volume DNA/Viral RNA kits (Roche). Tick borne pathogens were detected and characterized by PCR and high resolution melting (HRM) analyses. All gene fragments were amplified in an HRM capable RotorGene Q thermo cycler (QIAGEN, Hannover, Germany) to a final volume of 10 µl using primers listed in Table 1. For identification of *Ehrlichia*, *Anaplasma* and *Rickettsia* we utilized the shorter (<200 bp) 16S rRNA and outer membrane protein (ompB) precursor, all previously described by Tokarz (Tokarz *et al*., 2009) and unresolved samples were subsequently analyzed by amplification and sequencing of a longer (>300bp) 16S rRNA fragment using primer set *Ehrlichia* 16S rRNA JV (Table 3). For *Rickettsia*, the *rpmE*/$tRNA^{fMet}$ intergenic spacer typing (Zhu *et al*., 2005) was added. *Theileria*, *Babesia* and *Hepatozoon* were amplified and resolved using previously described primers (Gubbels *et al*., 1999) (Table 1). The thermal cycling conditions used for amplification were as follows: Initial denaturation at 95°C for 5 min, followed by 10 cycles of 94°C for 20s, step-down annealing from 63.5°C decreasing by 1°C per cycle for 25 sec, and primer extension at 72°C for 30 sec; 25 cycles of denaturation at 94°C for 25 sec, annealing at 50.5 for 20 sec and extension at 72°C for 30 sec,

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followed by a final extension at 72°C for 7 min. Following PCR, high resolution melting analyses of amplicons through gradual increase in temperature from 75°C to 90°C at 0.1°C/s increments was done. Changes in fluorescence with time (dF/dT) were plotted against changes in temperature (°C). Positive samples/amplicons were detected by observation of melting curves and peaks, and then purified with ExoSAP-IT (USB Corporation, Cleveland, OH) to remove unincorporated dNTPs and PCR primers before sequencing, which was outsourced from Macrogen (South Korea). The returned sequences were edited and aligned using MAFFT (Katoh *et al*., 2002) plugin in Geneious software (version 8.1.4) (Kearse *et al*., 2012) sequence identity was revealed by querying in the GenBank nr database using the Basic Local Alignment Search Tool BLAST (Altschul *et al*., 1990). For identification of specific pathogens, homology cut-off of 94%-100% identity with a GenBank *e*-value threshold of 1.0*e*-50 was used. Study sequences > 200bp were submitted to GenBank. Based on multiple alignments, some pathogens were not fully characterized to species.

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Table 3: Primers used for TBPs detection on pools of field collected ticks of Baringo and Homa Bay counties of Kenya

Pathogen /gene target	Primer Pair	Amplicon size	References
Anaplasma/16S rRNA	Fwd: GGGCATGTAGGCGGTTCGGT Rev: TCAGCGTCAGTACCGGACCA	195bp	Tokarz *et al*., 2009
Ehrlichia/ 16S rRNA	Fwd: CGTAAAGGGCACGTAGGTGGACTA Rev: CACCTCAGTGTCAGTATCGAACCA	195bp	Tokarz *et al*., 2009
Rickettsia/*ompB*	Fwd: ATACAAAGTGCTAATGCAACTGGG Rev: GTAAAATTACCGGTAAGGGTTATAGC	856bp	Tokarz *et al*., 2009
Rickettsia rpmE/tRNA^{fMet}	Fwd: TTCCGGAAATGTAGTAAATCAATC Rev: TCAGGTTATGAGCCTGACGA	175-402bp	Zhu *et al*., 2005
Theileria & Babesia/16S rRNA	Fwd: GAGGTAGTGACAAGAAATAACAATA Rev: TCTTCGATCCCCTAACTTTC	450bp	Gubbels *et al*., 1999
Anaplasma 16S rRNAS	Fwd: GGGCATGTAGGCGGTTCGGT Rev: TCAGCGTCAGTACCGGACCA	300bp	Tokarz *et al*., 2009
Ehrlichia 16S rRNAS	Fwd: GCAACCCTCATCCTTAGTTACCA Rev: TGTTACGACTTCACCCTAGTCAC	300bp	Tokarz *et al*., 2009

2.3 Arbovirus occurrence among patients with acute febrile illnesses visiting rural health clinics in Homa Bay County

2.3.1 Study design

This was a longitudinal qualitative study involving patients with acute febrile illnesses that presented at four rural health clinics at Homa Bay County. The study was carried out between January, 2012 and June, 2014 after ethical clearance from the Kenya Medical Research Institute (KEMRI) ethics review committee (Approval Ref: Non-SSC Protocol #310).

2.3.2 Human febrile illness sampling area

This study was conducted in Homa Bay County, Kenya along the shores and two adjacent islands of Lake Victoria. Four rural health clinics were chosen at random around *icipe*-Thomas Odhiambo campus (0° 25’49.57"S, 34°12' 24.20"E; e1200m above sea level). These health clinics included Sena clinic located on Mfangano Island, Tom Mboya clinic on Rusinga Island, Kitare and Kisegi health clinics both located on the mainland shore of Lake Victoria. Most inhabitants belong to the Luo and Suba ethnic groups whose main socio-economic activities are fishing and small scale mixed farming, which includes keeping of livestock (mainly cattle, sheep, and goats).

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2.3.3 Human Subject Selection

Using a brief close-ended questionnaire, project staff explained the study to the participants in Swahili or the local language and provided participants with study information. Written informed consent was obtained from all sampled febrile illness patients with fever >38° C. Adults accompanying patients between 12 and 17 years old consented on behalf of the patients. Patients presented with case definition for acute febrile illness with other symptoms (Table 4) were selected and screened for the presence of *Plasmodium* parasites by microscopy (thick blood smear), and rapid diagnostic tests (RDTs) (CareStart™ Malaria HRP2(Pf)). RDTs (supplied by Ministry of

Health) are occasionally used if available to screen for the presence of *Plasmodium*. Patients who provided samples with detectable *Plasmodium* parasites using these approaches were treated for malaria and excluded from the study. Serum and blood (3 mL each) was drawn from consenting patients (12 years and above). All patients included in the study were further screened and selected for those without history of antimalarial treatment two weeks prior to seeking medical attention. Vacutainer collection tubes (BD Vacutainer, New Jersey) were used to collect patients' fresh blood and serum samples. Blood and Serum collected from each patient was separated into three aliquots of 1mL each and stored labelled cryovials before immediately storing in liquid nitrogen shippers. To protect patient anonymity, all samples and questionnaires were labelled with barcode identifiers. Filled shippers and questionnaires were transported to Martin Lüscher Emerging Infectious Disease Laboratory in Nairobi for analysis. The samples were collected between May 2012 and June 2014, facilitated by staff in the Division of Disease Surveillance and Response (DDSR) of the Kenyan Ministry of Public Health and Sanitation.

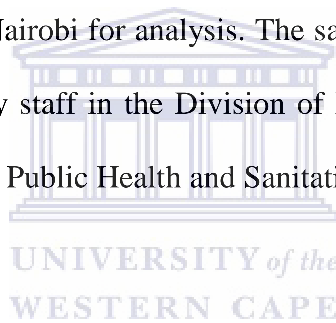
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Table 4: Case definition used to detect patients with acute febrile illness visiting rural health clinics of Homa Bay County of Kenya (Jentes *et al*., 2010)

Major Signs	Minor signs
Bleeding of nose, mouth and vagina	Headache
Conjunctival haemorrhage	Sore throat
Jaundice	General Malaise
Buzzing in the ears	Muscle Pain
	Joint pain
	Vomiting
	Cough
	Abdominal pain
	Diarrhoea

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3.3.4 Testing Blood samples for presence of *Plasmodium* using PCR-HRM

DNA was extracted from blood aliquots and amplified using nested PCR, targeting 18S rRNA genes in *Plasmodium* DNA. For the primary amplification step, forward (PL-1459-F: CTG GTT AAT TCC GAT AAC), and reverse (PL-1706-R: TAA ACT TCC TTG TGT TAG AC) primers were designed using Primer3 software (Untergasser *et al*., 2012). The second pair of primers used for the nested amplification reaction were PL-1473-Forward (3'-TAA CGA ACG AGA TCT TAA-5') and PL-1679-Reverse (3'-GTT CCT CTA AGA AGC TTT-5'), which targeted a 204–270 base pair fragment (depending on the *Plasmodium* species) of a polymorphic region in the 18S rRNA gene (Mangold *et al*., 2005). Ten-fold dilutions of the primary PCR products were used as templates for the nested reactions. Each of the two amplification reactions were carried out in 10 µL final reaction volumes consisting of 1 µL DNA template, 2 µL Hot Firepol® HRM mix kit (Solis BioDyne, Estonia), 0.5 µL of 0.5 µM of both primers and 6 µL nuclease free PCR water. The touchdown PCR thermal conditions consisted of an initial

denaturation at 95°C for 5 minutes, 45 cycles of denaturation at 94°C for 20 seconds, decreasing annealing temperatures from 65°C to 50°C for 25 seconds (cycles 1–5), 50°C for 40 seconds (cycles 6–10), 50°C for 50 seconds (cycles 11–45), and extension at 72°C for 30 seconds. A final extension of 72°C for 3 minutes was included before HRM analysis. Upon completion, the nested PCR process was transitioned into the melting phase (HRM) in the same closed tube system yielding distinct melting profiles in a Rotor-Gene Q® machine (QIAGEN, Germany). The set of conditions for HRM included 0.2°C incremental temperature increases from 75°C to 90°C, with fluorescence acquisition at the end of each 2 second temperature increment. DNA extracts from *P. falciparum* infected blood and deionized water were used as positive and negative controls, respectively. Products that produced curves distinct from the P. falciparum positive control were sequenced by Macrogen (Seoul, Korea) and aligned with reference sequences for *Plasmodium malariae* [GenBank:AB489193] and *Plasmodium ovale* [GenBank:AJ001527] using Geneious 6.1.6 software (Kearse *et al*., 2012). Protocols for nPCR (Snounou *et al*., 1993) and dPCR-HRM (Mangold 2005) were also separately used on all samples for comparison purposes.

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2.3.5 Inoculation in Vero cell lines and virus propagation

Fifty microlitre of serum was diluted in 50µL Minimum Essential Medium (MEM) and inoculated in a confluent monolayer of Vero cell line in order to facilitate viral expansion through replication. Prior to the inoculation, Vero cells were grown in Minimum Essential Eagle's Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 2% L-glutamine and antibiotic mixture (fungizone, 100 U/mL penicillin, and 100 U/mL streptomycin). Cells were grown in 25 and 75 cm^2 cell culture flasks (Corning Incorporated, Corning, NY 14831) and incubated at 37°C in a humidified incubator with 5% CO_2. Upon attaining a confluent monolayer, the cells were trypnised and 1 mL used to seed every well in a 24 well cell culture plates. This was incubated over night for attachment of the cells to take place. To each well, 50 µL of the serum was seeded including

positive of Ndumu virus (Lutomiah *et al*., 2014b) and negative controls of neat MEM and incubated at 37°C for attachment to occur then 1 mL Minimum Essential Eagle's Medium (MEM) supplemented with 2% fetal bovine serum (FBS), 2% L-glutamine and antibiotic mixture (fungizone, 100 U/mL penicillin, and 100 U/mL streptomycin) was added to each well. The inoculated cell culture plates were incubated at 37°C in a humidified incubator with 5% CO_2 in enhanced bio-safety level 2 (BSL-2) facility. They were observed daily through 14 days for cytopathic effects (CPE), which signifies viral growth. Wells showing cytopathic effects or suspected cytological changes were noted and harvested by centrifugation at 8000 rpm for 15 minutes. All negative cultures were blind passaged once and observed daily for 4 additional days for any CPE before discarded. Two hundred microlitre aliquots of harvested positive culture were used for specific RT-PCR and the remainders were stored in liquid nitrogen for future use.

2.3.6 Passage of confirmed virus isolates

Two hundred micro-litres of the infected cells that had been harvested from the original inoculation were re-inoculated into confluent monolayer in 25 cm^2 tissue culture flask (T-25 flasks) and monitored until the CPE was reproduced. The flasks were then frozen at -80°C for a day then thawed and the contents transferred into 15ml centrifuge tubes. The tubes were centrifuged at 2500-3000rpm for 10min, the supernatant collected and aliquoted into cryovials of 1 ml each then stored at -80°C.

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2.3.7 Total RNA extraction, Reverse Transcription and Polymerase chain reaction

Total RNA was extracted from positive cultures as well as all blood aliquots of 339 patients in fully automated MagNA Pure LC extraction system robot (MagNA 96 Pure RNA and Viral NA Small Volume Kit (Roche Applied Science, Penzberg, Germany). Following extraction, reverse transcription was performed using High Capacity cDNA Reverse Transcription Kit (Applied

Biosystems) and multiplex real-time PCRs for flavivirus, alphavirus, phleboviruses, and othobunyaviruses were carried out in 5x *HOT FIREPol® EvaGreen®* HRM Mix *(no ROX)* (Solis BioDyne, Estonia). Using published primers (Table 5), phleboviruses (Lambert and Lanciotti, 2009), orthobunyaviruses (Lambert and Lanciotti, 2009) and alphaviruses (Eshoo *et al*., 2007) were screened by PCR, as well as flaviviruses were amplified based on nested PCR as previously described (Vazquez *et al*., 2012) in 10 µl reactions using 1µl cDNA template, 5 µl MyTaq™ HS master mix (Bioline Reagents Limited, London, UK) and 1 µl of 50 µM SYTO-9 saturating intercalating dye (Life technologies, Carlsbad, California). Laboratory arboviral stocks of diverse Kenyan isolates (Ochieng *et al*., 2013) were used as standard reference controls. Viral amplicons were initially identified by HRM analysis and then confirmed by sequencing at Macrogen (Seoul, South Korea) after amplicon purification with ExoSAP-IT (USB Corporation, Cleveland, OH). The thermal cycling conditions used for amplification were as follows: Initial denaturation at 95°C for 5 min, followed by 10 cycles of 94°C for 20s, step-down annealing from 63.5°C decreasing by 1°C per cycle for 25 sec, and primer extension at 72°C for 30 sec; 25 cycles of denaturation at 94°C for 25 sec, annealing at 50.5 for 20 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Following PCR, high resolution melting analyses of amplicons through gradual increase in temperature from 75°C to 90°C at 0.1°C/s increments was done. Changes in fluorescence with time (dF/dT) were plotted against changes in temperature (°C). Positive samples/amplicons were detected by observation of melting curves and peaks, and then purified with ExoSAP-IT (USB Corporation, Cleveland, OH) to remove unincorporated dNTPs and PCR primers before sequencing, which was outsourced from Macrogen (South Korea). The returned sequences were edited and aligned using MAFFT (Katoh *et al*., 2002) plugin in Geneious software (version 8.1.4) (Kearse *et al*., 2012) sequence identity was revealed by querying in the GenBank nr database using the Basic Local Alignment Search Tool BLAST (Altschul *et al*., 1990).

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For identification of specific pathogens, homology cut off of 94%-100% identity with a GenBank *e*-value threshold of 1.0*e*-50 was used.

Table 5: Oligonucleotide primers used for the detection of arboviruses in patients with acute febrile illnesses visiting rural health clinics in Homa Bay County.

Target	Primer name	Primer sequence	References
Phlebovirus	Phebo F1	5'-AGT TTG CTT ATC AAG GGT TTG ATG C-3'	(Lambert and Lanciotti, 2009)
	Phlebo F2	5'-GAG TTT GCT TAT CAA GGG TTT GAC C-3'	
	Phlebo R	5'-CCG GCA AAG CTG GGG TGC AT-3'	
Orthobunyavirus	BUN F	5'-CTG CTA ACA CCA GCA GTA CTT TTG AC-3'	(Lambert and Lanciotti, 2009)
	BUN R	5'-TGGAGGGTAAGACCATCGTCAGGAACTG-3'	
Alphavirus	Vir 2052 F	5'-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3'	(Eshoo *et al.*, 2007)
	Vir 2052 R	5'-TAC GAT GTT GTC GTC GCC GAT GAA-3'	
Flavivirus	1NS5F	5'-GCA TCT AYA WCA YNA TGG G-3'	(Vazquez *et al.*, 2012)
	1NS5R	5'-CCA NAC NYN RTT CCA NAC-3'	
	2NS5F	5'-GCN ATN TGG TWY ATG TGG-3'	
	2NS5R	5'-TRT CTT CNG TNG TCA TCC-3'	

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2.3.8 Determination of viral titer (Plaque assay)

Plaque assay was used to determine known amounts of virus (viral titer) that produced recommended plaque forming units per well. Due to limited resources, this was only possible for limited samples from Mfangano and Rusinga islands. To achieve about 800pfu/ml (80plaques in a well when 100µl of virus is added per well), tenfold dilutions of each virus were prepared as described at (www.bdbiosciences.com › Resources › Baculovirus Protein Expression). Briefly, fifty microlitres of a viral stock (e.g. Sindbis) was added onto 450 µl 2% MEM (Sigma Aldrich, St. Louis, USA) over a tenfold dilution series upto 10^{-10}. A hundred microlitre of these dilutions was then plated onto their respective confluent Vero cells in 6 well plates. After one hour incubation at 37°C, 3 ml of methyl cellulose overlay medium was added to each well and incubated at 37°C, 5% CO_2 for three days for Sindbis virus and four days for Bunyamwera virus. After the overlay was removed, 10 % formaldehyde was added to the wells and placed under UV for 30 min

for fixing and to inactivate the virus. The plates were placed under slow running tap water to remove the formaldehyde and stained immediately with 0.5 % methyl violet dye then washed off and left overnight to dry.

The plaques were then counted and plaque forming units per ml (pfu/ml) calculated using the formula:

$$\text{Plaque forming units per ml} = \text{number of plaques/volume of diluted virus per well}$$

2.3.9 Determination of Neutralization Activity (Plaque Reduction Neutralization Test)

All the sera samples were heat inactivated at 56 °C for 30 min. Twelve microlitres of a serum sample was diluted into 108 µl of 2 % MEM (1:10 dilution) virus. A previously unthawed vial of virus was thawed on ice. The virus stock was then diluted as previously determined from the plaque assay. Sixty microlitres of the diluted virus was then mixed with an equal volume of the serum in a 24 well plate and incubated for one hour. Other controls incubated included a negative control (MEM alone), backtitrated controls (10^{-9} and 10^{-8} for Bunyamwera and Sindbis viruses respectively) and a working dilution of the virus. A 100 µl of the incubated mixture and the controls were inoculated onto confluent cells in 6 well plates and incubated for one hour again. Three millilitres of methyl cellulose overlay medium was finally added to the wells and the plates incubated for three days for Sindbis and four days for Bunyamwera virus and later stained as described above (3.2.6).

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CHAPTER THREE

RESULTS

3.1 Results on determination of mosquito bloodmeals and arboviruses in blood-fed mosquitoes

Greater numbers of mosquitoes were sampled in Baringo County (68.3%) than in Homa Bay County (31.7%). Out of the total collection, 472 (0.8%) mosquitoes were blood-fed (258 from Homa Bay County, shores of Lake Victoria; 214 from Baringo County) and analysed. In Homa Bay, blood-fed mosquito samples were composed of 15 species in all the six sampling sites with the highest proportion from the Mfangano Island (27.1%), while Rusinga Island had the least (5.8%). *Culex naivei* (9.3%), and *Culex pipiens* (5.4%) were the predominant blood-fed species in Mfangano, while *Aedes mettalicus* (0.4%) was the least abundant (Table 6). In Baringo County, the samples constituted 11 mosquito species in all five sampling areas with the highest frequency of blood-fed mosquitoes in Logumgum (37.9%). On average, blood-fed *Mansonia africana* was abundant in all sampling areas, while *Ae. aegypti* (0.9%) sampled at Salabani and *Ae. vittatus* (0.9%) sampled from Ruko wildlife conservancy were the least abundant blood-fed species from Baringo. The percentage abundance of blood-fed mosquitoes sampled in both study areas are represented in Table 6.

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Table 6: Numbers of blood-fed mosquito species captured at different sampling sites in Homa Bay and Baringo Counties of Kenya**.**

	Homa Bay County							Baringo County					
	Mainland sites			Island sites				Mainland sites					
Species	Mbita	Ungoye	Luanda Nyamasare	Mfangano	Chamaunga	Rusinga	Homa Bay Total	Kampi ya samaki	Ruko	Salabani	Logumgum	Kokwa Island	Baringo Total
Ad. africana											17(7.94%)		17 (7.94%)
Ae. aegypti				4 (1.55%)		1 (0.38%)	5 (1.94%)			2(0.93%)			2 (0.93%)
Ae. hirsutus				3 (1.16%)			3 (1.16%)						
Ae. mettalicus				1 (0.38%)	6 (2.32%)		7 (2.71%)						
Ae. vittatus								1 (0.46%)	2 (0.93%)				3 (1.40%)
An. coustani			4 (1.55%)	10 (3.87%)	8 (3.1%)	4 (1.55%)	26 (10.08%)	3 (1.4%)	11 (5.14%)		15 (7.01%)	3 (1.4%)	32 (14.95%)
An. funestus					2 (0.77%)		2 (0.78%)	1 (0.46%)					1 (0.47%)
An. gambiae	5 (2.33%)		7 (2.71%)	7 (2.71%)		1 (0.38%)	20 (7.75%)	2 (0.93%)	2 (0.93%)		4 (1.87%)		8 (3.74%)
An. pharoensis	2 (0.77%)						2 (0.78%)						
Cx. naivei				24 (9.3%)			24 (9.30%)						
Cx. pipiens	14 (5.42%)	6 (2.32%)	14 (5.42%)	14 (5.42%)		3 (1.16%)	51 (19.77%)	3 (1.4%)		4 (1.87%)		7 (3.27%)	14 (6.54%)
Cx. poicilipes					14 (5.42%)		14 (5.43%)		4 (1.87%)				4 (1.87%)
Cx. univitatus	9 (3.48%)	1 (0.38%)	7 (2.71%)		6 (2.32%)		23 (8.91%)	1 (0.46%)		9 (4.2%)	5 (2.33%)		15 (7.01%)
Cx. vansomerini	5 (2.33%)						5 (1.94%)						
Ma. africana	14 (5.42%)	3 (1.16%)	11 (4.26%)	7 (2.71%)		1 (0.38%)	36 (13.95%)	15 (7.01%)	15 (7.01%)	13 (6.07%)	32 (14.95%)	5 (2.33%)	80 (37.38%)
Ma. uniformis	9 (3.48%)		13 (5.03%)		2 (0.77%)	5 (2.33%)	29 (11.24%)	9 (4.2%)	3 (1.4%)	6 (2.8%)	8 (3.74%)	12 (5.6%)	38 (17.76%)
Mm. splendens	11 (4.26%)						11 (4.26%)						
Totals	**69 (26.74%)**	**10 (3.87%)**	**56 (21.7%)**	**70 (27.13%)**	**38 (14.72%)**	**15 (5.81%)**	**258**	**35 (16.35%)**	**37 (17.29%)**	**34 (15.88%)**	**81 (37.85%)**	**27 (12.61%)**	**214**

Denominator totals represent total blood fed mosquito per site.

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Bloodmeal sources were identified from 445 mosquitoes, representing 94.3% of captured blood-fed mosquitoes with the remaining 5.7% not conclusively determined due to either inability to be amplified by both primers or sequenced poorly, making them difficult to analyse. In Homa Bay, bloodmeal sources of mosquitoes (Table 7) ranged from humans (15.9%) to domestic (reared by humans) (53.5%) and peridomestic (common within homesteads) (11.2%) animals, as well as diverse species of wild birds (13.6%). Bloodmeal sources from domestic animals included goats (15.9%; *Capra hircus*), cows (15.5%; *Bos taurus*), sheep (6.9%; *Ovis aries*), dogs (5.4%; *Canis lupus*), chicken (5.4%; *Gallus gallus*) and donkeys (4.3%; *Equus asinus*), while peridomestic bloodmeal sources included rodents (2.3%; *Arvicanthis niloticus*, *Rattus norvegicus* and *Mus musculus*), frogs (6.2%; *Ptychadena nilotica* and *Ptychadena anchietae*), toads (1.9% *Bufo regularis*) and two bat species (*Rhinolophus ferrumequinum* and *Micropteropus pusillus*). Wildlife included cane rats (2.3%; *Thryonomys swinderianus*) and wild birds (11.2%) comprising Muscovy ducks (*Cairina moschata*), crows (Family: Corvidae), grey heron (*Ardea sp.*), doves (Family: Columbidae), two weaver bird species (Family: Ploceidae), blue-naped mousebirds (*Urocolius macrourus*), *cattle egrets* (*Bubulcus ibis*) and two passerine bird species (Order: Passeriformes) (Table 8). Similarly, in Baringo, bloodmeal sources of mosquitoes (Table 9) ranged from human (11.21%), to domestic (63.1%) and peridomestic animals (6.1%), but included pigs (*Sus scrofa*) and more wildlife species (12.2%) like baboons (*Papio sp.*), hippopotamus (*Hippopotamus amphibius*) and crested porcupine (*Hystrix cristata),* black bird (Family: Icteridae), Pheasant (Subfamily: Phasianinae) and rabbit (*Orycytolagus sp.*). Sequence identity e-values with specific Blast matches to GenBank sequences, as well as GenBank accessions of bloodmeal *cyt b* sequences are indicated in Table 10. The alignments of the shorter 16S sequences with their closest Blast hits are shown in appendix 1

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Table 7: Number of bloodmeal sources of mosquito species sampled in Homa Bay County.

Sampling area	Species	N	Human	Chicken	Cow	Dog	Donkey	Goat	Sheep	Cane rat	Toad	Rodent	Birds	Wild Duck	Frog
Mbita	*Cx. pipiens*#	14	5	-	1	2	-	5	-	-	-	-	2	-	-
	Ma. africana	14	2	-	6	1	1	3	1	0	-	-	-	-	-
	Ma. uniformis	9	-	1	2	-	1	3	1	-	-	-	-	1	-
	Cx. univittatus	9	2	-	-	1	2	-	1	-	1	-	2	-	-
	An. gambiae	5	3	-	-	1	-	-	-	-	-	1	-	-	-
	Mm. splendens	11	-	-	-	-	-	-	-	-	-	-	2	-	9
	Cx. vansomerini	5	1	1	1	-	-	1	-	-	-	-	1	-	-
Luanda Nyamasare	*An. coustani*	4	-	-	2	-	-	-	1	-	-	-	1	-	-
	An. gambiae	7	5	-	1	-	-	1	-	-	-	-	-	-	-
	Cx. pipiens	14	4	2	3	-	-	3	1	-	-	-	1	-	-
	Cx. univittatus	7	2	-	1	-	1	-	-	-	-	-	3	-	-
	Ma. africana	11	-	-	6	-	2	3	-	-	-	-	-	-	-
	Ma. uniformis	13	1	-	4	-	-	4	4	-	-	-	-	-	-
Ugoye	*Cx. pipiens*	6	2	-	1	-	-	2	-	-	-	-	-	-	-
	Cx. univittatus	1	-	-	-	-	-	1	-	-	-	-	-	-	-
	Ma. africana	3	-	-	1	-	-	2	-	-	-	-	-	-	-
	An. pharoensis	2	1	-	-	-	-	-	1	-	-	-	-	-	-
Mfangano	*Cx. naivei*	24	6	2	2	3	-	2	1	1	-	1	4	-	-
	Cx. pipiens	14	4	2	-	-	1	3	1	-	-	-	-	-	1
	An. coustani	1	-	1	3	1	1	2	2	-	-	-	-	-	-
	An. gambiae	7	1	-	-	1	-	1	1	2	1	-	-	-	-
	Ma. africana	7	2	1	1	1	-	-	1	-	1	-	-	-	-
	Ae. aegypti	4	-	-	1	1	-	-	1	-	-	1	-	-	-
	Ae. hirsutus	3	-	2	-	-	-	-	-	-	-	-	-	-	1
	Ae. mettalicus	1	-	1	-	-	-	-	-	-	-	-	-	-	-
Chamaunga	*Cx. poicilipes*	14	-	-	-	-	1	1	-	3	1	2	5	-	-
	An. coustani	8	-	-	-	-	1	1	-	-	-	1	1	-	1
	Cx. univittatus	6	-	-	1	-	-	1	-	-	-	-	2	1	-
	Ae. metalicus	6	-	-	1	1	-	-	-	-	1	-	1	1	1
	An. funestus	2	-	-	-	-	-	-	-	-	-	-	1	-	1
	Ma. uniformis	2	-	-	-	-	-	-	-	-	-	-	1	1	-
Rusinga	*An. coustani*	4	-	-	-	-	-	-	-	-	-	-	-	2	1
	Ma. uniformis	5	-	1	1	-	-	1	-	-	-	-	1	-	1
	Ma. africana	1	-	-	1	-	-	-	-	-	-	-	-	-	-
	Cx. pipiens	3	-	-	-	-	-	1	1	-	-	-	1	-	-
	An. gambiae	1	-	-	-	-	-	1	-	-	-	-	-	-	-
	Ae. aegypti	1	-	-	-	1	-	-	-	-	-	-	-	-	-
Total		**249**	**41 (15.89%)**	**14 (5.42%)**	**4- (15.5-%)**	**14 (5.43%)**	**11 (4.26%)**	**42 (16.28%)**	**18 (6.98%)**	**6 (2.33%)**	**5 (1.94%)**	**6 (2.33%)**	**29 (11.24%)**	**6 (2.33%)**	**16 (6.2-%)**

N = Number of mosquitoes analyzed, ND = Not determined. #mixed blood meal cases.

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Table 8: Bird species represented in mosquito bloodmeals in both study areas

Birds represented in mosquito bloodmeal	Mosquito species	Study site	GenBank Accession (% identity; *e*-value)
Weaver bird (*Ploceus baglaflecht reichnowi*)	*Cx. naivei* (2)	Mfangano	AY283898 (97% 16S; 9*e*-76)
	An. gambiae (1)	Logumgum	
	Ae. metalicus (1)	Chamaunga	
	An. funestus (1)	Chamaunga	
Weaver bird (Family: Ploceidae)	*Cx. pipiens* (1)	Luanda Nyamasare	AY283898 (96% 16S; 9*e*-76)
	Cx. pipiens (1)	Kokwa	
	An. coustani (3)	Kokwa	
	Ma. uniformis (1)	Logumgum	
Blackbird (Family: Icteridae)	*Ma. uniformis* (1)	Logumgum	JX516070 (94% 16S; 4*e*-54)
	Ma. africana (1)	Logumgum	
Swallow (*Hirundo rustica*)	*Mm.* splendens (2)	Mbita	AB042382 (99% 16S; 7*e*-82)
Blue-naped mousebird (*Urocolius macrourus*)	*Cx. vansomereni* (1)	Mbita	AF173589 (99% 16S; 3*e*-90)
	Cx. pipiens (1)	Mbita	
	Cx. univittatus (3)	Luanda Nyamasare, Chamaunga	
	Ma. uniformis (1)	Rusinga	
Cattle egret (*Bubulcus ibis*)	*Cx. poicilipes* (2)	Chamaunga	KJ190945 (99% 16S; 1*e*-890)
	Ad. africana (1)	Logumgum	
Chicken (*Gallus gallus*)	*Ma. uniformis* (1)	Mbita	AY236430 (100% 16S; 2*e*-82)
	Cx. vansomereni (1)	Mbita	
	Cx. pipiens (8)	Luanda Nyamasare, Mfangano, Kampi ya Samaki, Kokwa	
	Cx. naivei (2)	Mfangano	
	An. coustani (6)	Mfangano, Kokwa, Logumgum	
	Ma. africana (8)	Mfangano, Kampi ya Samaki, Salabani, Logumgum	
	Ae. hirsutus (2)	Mfangano	
	Ae. metalicus (1)	Mfangano	
	Ma. uniformis (6)	Rusinga, Kampi ya Samaki, Salabani, Mbita	
	An. gambiae (1)	Kampi ya Samaki	
	Ae. aegypti (1)	Salabani	
	Cx. univittatus (1)	Logumgum	
	Cx. poicilipes(1)	Ruko	
Pheasant (Subfamily: Phasianinae)	*Ma. uniformis* (3)	Kokwa	EU165707 (92% 16S; 2*e*-51)
Grey heron (*Ardea cinerea*)	*Ma. uniformis*	Salabani	KJ190947 (100% 16S; 2*e*-77)
	Cx. poicilipes (3)	Salabani	
Muscovy duck (*Cairina moschata*)	*Cx. univittatus* (1)	Kampi ya Samaki	EU755254 (100% 16S; 2*e*-81)
	Ma. uniformis (2)	Mbita, Chamaunga	
	An. coustani (2)	Rusinga	
Passerine bird (Order: Passeriformes)	*Cx. naivei* (1)	Mfangano	KM078809 (96%; 2*e*-71)
	Cx. coustani (1)	Chamaunga	
	Cx. univittatus(1)	Logumgum	
	Cx. univittatus (1)	Logumgum	
Passerine bird (Order: Passeriformes)	*Cx. naivei* (1)	Mfangano	FJ465224 (94%; 6*e*-68)
	Cx. pipiens (1)	Mbita	
	Ma. uniformis (1)	Chamaunga	
Dove (Family: Columbidae)	*Cx. univittatus* (3)	Ruko, Mbita	KC984248 (96% 16S; 5*e*-86)
	Ma. africana (1)	Ruko	
	An. coustani (2)	Ruko	
Crow (Family: Corvidae)	*Cx. univittatus* (1)	Salabani	AF171067 (91% *cyt b*, 1*e*-112)

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Table 9: Number of bloodmeal sources of mosquito species sampled in Baringo County

Sampling area	Species	N	Human	Chicken	Cow	Dog	Donkey	Goat	Sheep	Pig	Frog	Hippo	Baboon	Bird	Rabbit	Porcupine
Kampi ya samaki	*Ma. africana*	15	2	1	6	-	2	2	2	-	-	-	-	-	-	-
	Ma. uniformis	9	-	1	2	-	-	3	2	-	-	-	-	-	-	-
	Cx. pipiens	3	1	1	-	-	-	-	-	-	-	-	-	-	-	1
	An. coustani	3	1	1	-	-	-	1	-	-	-	-	-	-	-	-
	An. gambiae	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-
	Cx. univittatus	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-
	Ae. vittatus	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-
	An. funestus	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Ruko	*Ma. africana*	15	1	1	6	1	1	2	-	-	1	-	1	1	-	-
	An. coustani	11	1	1	2	-	1	1	-	1	2	-	-	2	-	-
	Cx. poicilipes	4	-	1	-	-	-	1	-	-	1	-	-	-	-	-
	Ma. uniformis	3	1	1	-	-	-	-	-	-	-	-	-	-	-	-
	An. gambiae	2	1	-	-	-	-	1	-	-	-	-	-	-	-	-
	Ae. vittatus	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Salabani	*Ma. africana*	13	1	3	2	-	2	3	1	-	1	-	-	-	-	-
	Cx. univittatus	9	1	-	1	-	2	1	-	-	-	-	1	1	-	-
	Cx. pipiens	4	3	-	-	-	-	-	1	-	-	-	-	-	-	-
	Ma. uniformis	6	-	2	-	1	-	-	1	-	-	-	-	1	-	-
	Ae. aegypti	2	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Kokwa	*Ma. uniformis*	12	1	-	2	-	-	1	2	-	-	1	-	3	-	-
	*Cx. pipiens**	7	1	1	-	-	-	-	-	-	2	-	-	2	-	-
	Ma. africana	5	-	-	1	-	1	1	1	-	1	-	-	-	-	-
	An coustani	3	-	1	-	-	-	-	1	-	-	-	-	1	-	-
Logumgum	*Ma. africana*#*	32	3	2	1	1	3	9	4	1	1	1	1	1	-	1
	*Ad. africana**	17	2	-	3	1	-	1	4	1	1	-	-	1	1	-
	*An. coustani**	15	1	1	1	1	2	-	3	2	-	1	-	1	-	-
	Ma. uniformis	8	-	1	1	-	-	1	2	-	-	-	-	1	-	-
	Cx. univittatus	5	1	1	-	1	-	-	-	-	-	-	-	1	-	-
	An. gambiae	4	-	-	1	1	-	1	-	-	-	-	-	1	-	-
Total		**214**	**24 (11.21%)**	**25 (11.21%)**	**30 (14.02%)**	**8 (3.74%)**	**14 (6.54%)**	**29 (13.55%)**	**25 (11.68%)**	**5 (2.34%)**	**10 (4.67%)**	**3 (1.40%)**	**3 (1.40%)**	**17 (7.94%)**	**1 (0.47%)**	**2 (0.93%)**

N = Number of mosquitoes analysed, ND = Not determined. #mixed blood meal cases. *virus positive cases

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Table 10: Sequence identity and e-values of all mosquito bloodmeal sources with specific BLAST matches to GenBank sequences (**Accessed May 25, 2015).**

Blood meal sources	Study *cyt b* sequence	*cyt b* (% identity; *e*-value)	16S (% identity; *e*-value)
Human (*Homo sapiens*)	KP858485	KJ801974 (100%; 1*e*-150)	KP218948 (99%; 4*e*-79)
Goat (*Capra hircus*)	KP858486	FM205715 (100%; 2*e*-153)	KP195268 (100%; 2*e*-72)
Cattle (*Bos taurus*)	KP858487	EU365345 (100%; 1*e*-151)	KJ789953 (99%; 3*e*-70)
Sheep (*Ovis aries*)			KP998473 (98%; 4*e*-79)
Dog (*Canis familiaris)*	KP858493*	DQ309764* (100%; 5*e*-150)	KF661075 (99%; 3*e*-91)
Donkey (*Equus asinus*)			KM881681 (99%; 3*e*-85)
Pig (*Sus scrofa*)	KP858488	KJ652503 (100%; 9*e*-148)	
Rat (Subfamily: Murinae)			AF141228 (92%; 4*e*-59)
Baboon (*Papio sp.*)	KP858489	JX946199 (100%; 1*e*-146)	
House mouse (*Mus musculus*)	KP858490	KP260517 (100%; 7*e*-144)	
Brown rat (*Rattus norvegicus*)	KP858491	KP241960 (100%; 1*e*-146)	
Cane rat (*Thryonomys sp.*)	KP844654	AJ301644 (97%; 5*e*-140)	
Rabbit (*Oryctolagus sp.*)	KP858492	HG810791 (100%; 4*e*-146)	
Crested porcupine (*Hystrix cristata*)	KP858484	FJ472572 (100%; 7*e*-144)	
Fruit bat (*Micropteropus pusillus*)			JN398183 (100%; 6*e*-72)
Hippopotamus (*Hippopotamus amphibius*)	KP844655	AP003425 (99%; 3*e*-132)	
Weaver bird (*Ploceus baglaflecht reichnowi*)			AY283898 (97%; 9*e*-76)
Weaver bird (Family: Ploceidae)			AY283898 (96%; 9*e*-76)
Blackbird (Family: Icteridae)			JX516070 (94%; 4*e*-54)
Swallow (*Hirundo rustica*)			AB042382 (99%; 7*e*-82)
Blue-naped mousebird (*Urocolius macrourus*)			AF173589 (99%; 3*e*-90)
Cattle egret (*Bubulcus ibis*)			KJ190945 (99%; 1*e*-89)
Chicken (*Gallus gallus*)			AY236430 (100%; 2*e*-82)
Pheasant (Subfamily: Phasianinae)			EU165707 (92%; 2*e*-51)
Grey heron (*Ardea cinerea*)			KJ190947 (100%; 2*e*-77)
Muscovy duck (*Cairina moschata*)			EU755254 (100%; 2*e*-81)
Passerine bird (Order: Passeriformes)			FJ465224 (94%; 6*e*-68)
Passerine bird (Order: Passeriformes)			KM078809 (96%; 2*e*-71)
Dove (Family: Columbidae)			KC984248 (96%; 5*e*-86)
Crow (Family: Corvidae)	KP844653	AF171067 (91%, 1*e*-112)	
African toad (*Bufo regularis*)			AF220889 (100%; 7*e*-92)
Grass frog (*Ptychadena nilotica*)			DQ525928 (100%; 8*e*-86)
Anchieta's ridged frog (*Ptychadena anchietae*)			GQ183598 (100%; 1*e*-84)

**cyt b* like pseudogene

Distinct HRM profiles for the two markers of diverse bloodmeal species are shown in Figure 4. Detection of mixed blood meals was evaluated for accuracy and sensitivity from 1 µl triplicate aliquots of ten-fold serial dilutions (up to 10^{-7}) of pure and mixed blood. The sensitivity of the two markers in identifying the standard controls is tabulated in Table 11. Detection of mixed and pure blood showed slight variations in melting profiles (Figure 5). Mixed bloodmeals in the field-collected samples were detected in *Cx. pipiens* gut from Mbita that had fed on goat and human blood, and two *Ma. africana* sampled in Baringo that both had bloodmeals of rat (*Arvicanthis niloticus*) in addition to human or baboon (*Papio sp*.) (Figure 6). Although the *cyt b* marker could more efficiently identify mixed bloodmeals in field collection than the 16S marker (Figure 6), there were instances where sequencing of *cyt b* amplicons resulted in mosquito-specific sequences (<90% sequence homology) with lower melting temperature ranges than vertebrates (Figure 7). Such was observed in *Ae. hirsutus*, *Ae. metallicus*, *Ae. aegypti*, *Ae. vittatus* and *An. coustani* that were among the 27 samples in which mosquito DNA amplified instead of their host's DNA. These were clearly resolved based on 16S HRM analysis, resulting in correct identification of vertebrate host species. Melting profiles of some pairs of vertebrate host species was found to appear somewhat similar when using products of either *cyt b* or 16S alone, but not across both markers (Figure 4). For instance, pairs of host species such as pig and wild rabbit (*Oryctolagus sp.*) or human and baboon (*Papio sp.*) could be differentiated based on their 16S HRM profiles, but not based on their *cyt b* HRM profiles. Likewise, similar 16S HRM profiles were observed between crested porcupine (*Hystrix cristata*) and goat (*Capra hircus*) and between passerine bird and mousebird bloodmeals that could be differentiated based on their *cyt*

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b HRM profiles. Therefore, the comparisons of HRM profiles from both loci allowed for more robust species differentiation and identification among many species (Figure 4).

Table 11: Dilution factor detection limits of cyt b and 16S rRNA markers of pure and mixed serially diluted blood.

Host	**Cytochrome b**	**16S rRNA**
Chicken	10^{-4} (0.1 nl)	10^{-4} (0.1 nl)
Human	10^{-6} (1 pl)	10^{-6} (1 pl)
Rabbit	10^{-4} (0.1 nl)	10^{-4} (0.1 nl)
Swiss mouse	10^{-6} (1 pl)	10^{-5} (10 pl)
Human-Rabbit Mix	10^{-4} (0.1 nl)	10^{-5} (10 pl)
Chicken-Swiss mouse mix	10^{-3} (1 nl)	10^{-5} (10 pl)

Volumes of blood extracted at different dilutions are indicated in brackets.

nl=nanoliters, pl=picoliters

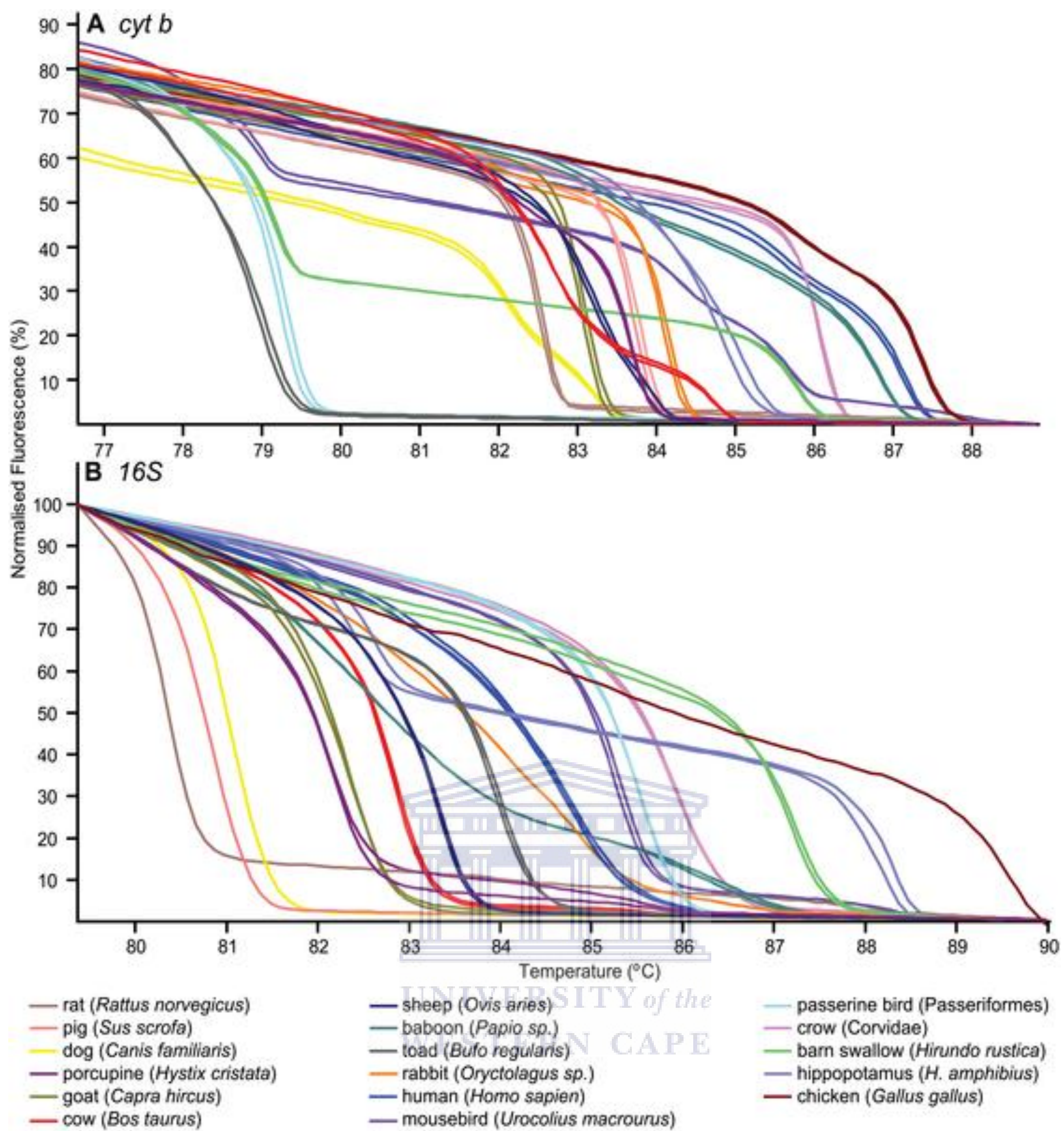

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Figure 4: HRM profiles of selected mosquito bloodmeal sources using *cyt b* (A) and 16S rRNA (B). Vertebrate species in the legend are ordered from their lowest to highest 16S rRNA melting temperatures.

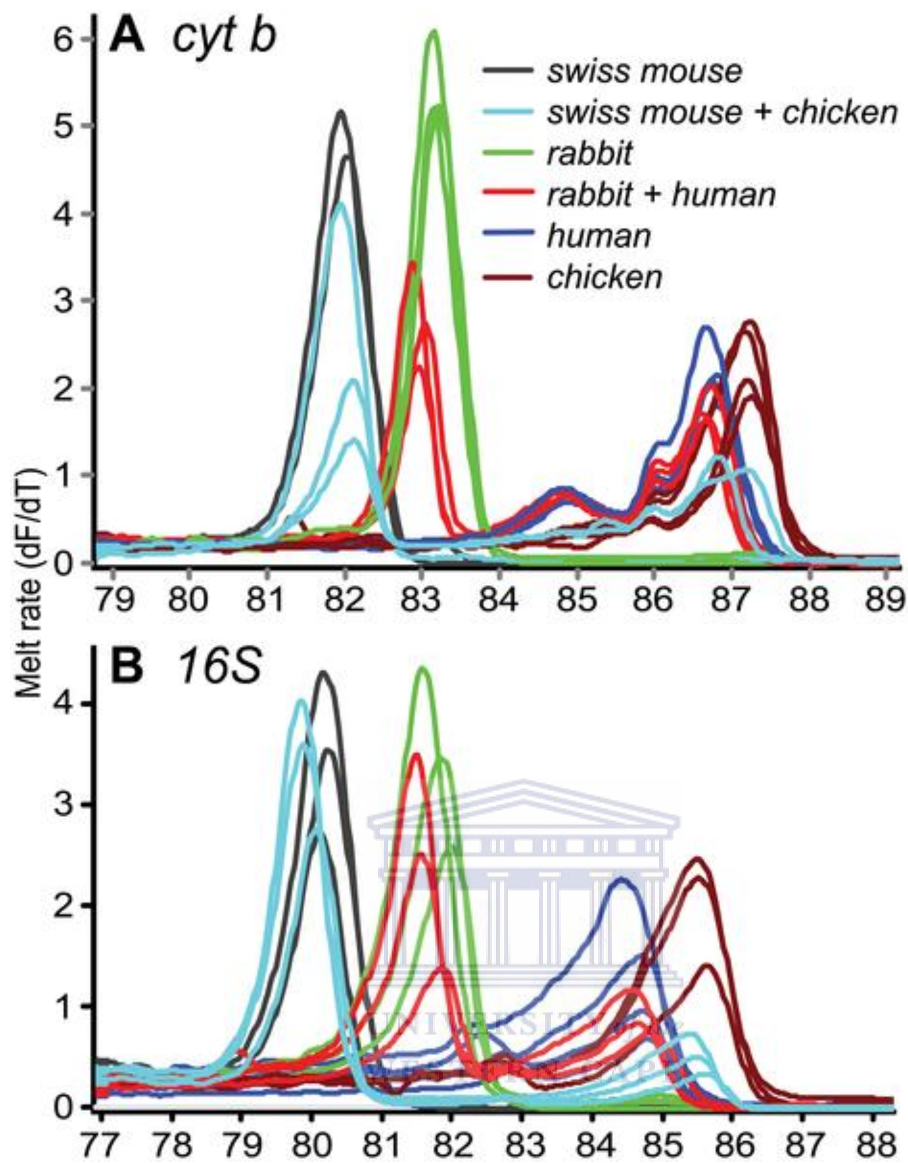

Figure 5: Melt rates of serially diluted pure and mixed blood for calibration of identifications and sensitivity validations using *cyt b* (A) and 16S rRNA (B). Vertebrate species in the legend are ordered from their lowest to highest melting temperatures.

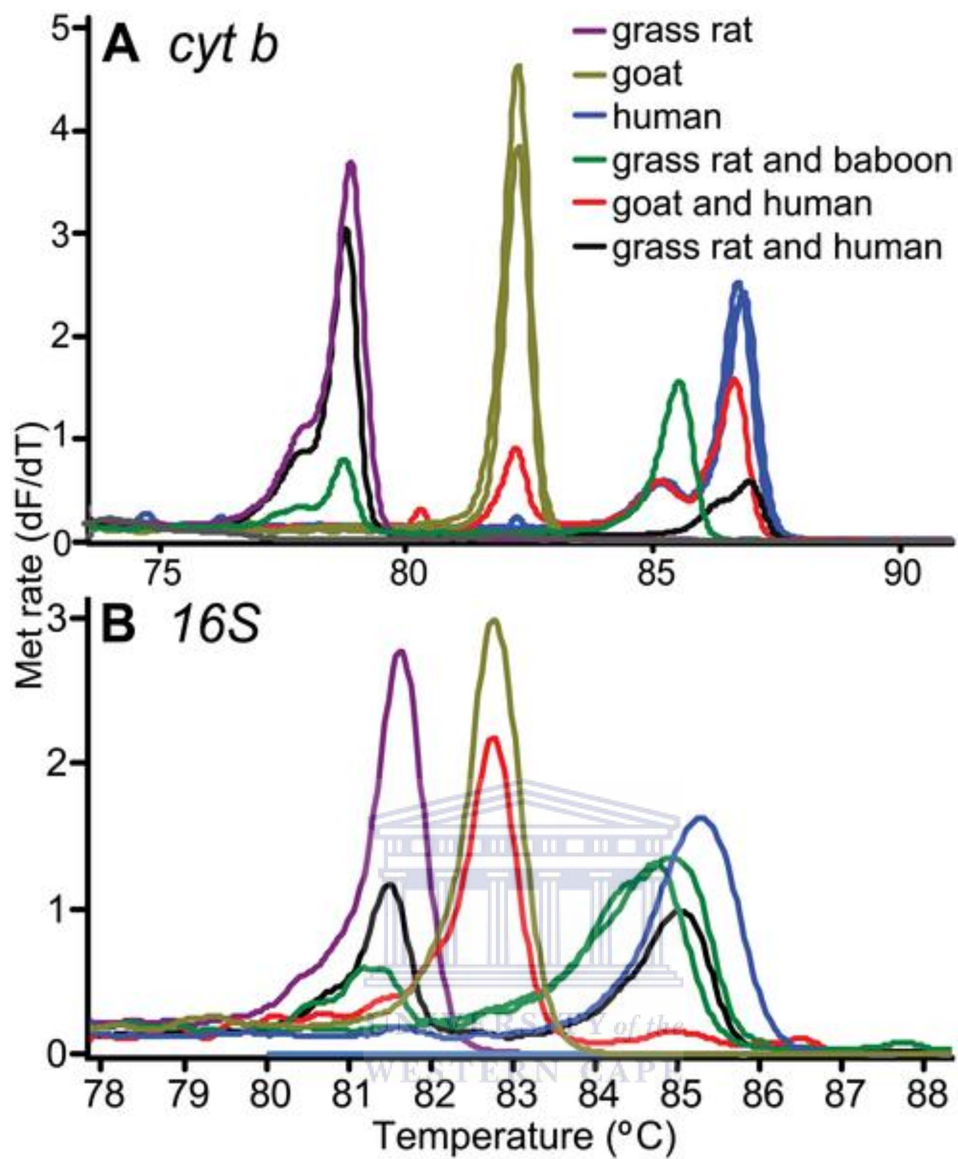

Figure 6: Melt rates of field collected mixed mosquito blood meals using *cyt b* (A) and 16S rRNA (B). Vertebrate species in the legend are ordered from their lowest to highest melting temperatures.

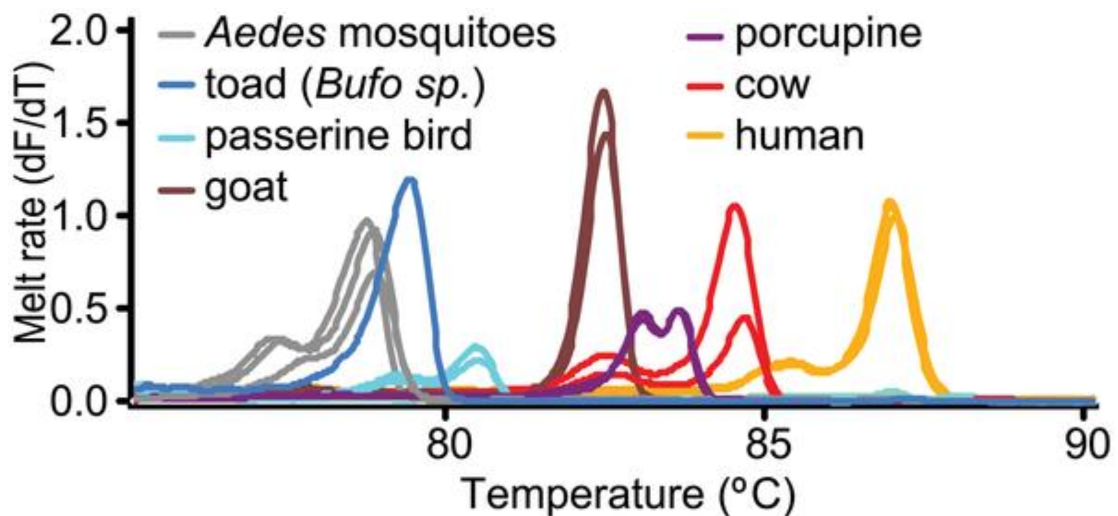

Figure 7: Melt rates of mosquito *cyt b* amplicons alongside selected vertebrate bloodmeal amplicons. Species in the legend are ordered from their lowest to highest melting temperatures.

Seven out of 214 (3.3 %) cell culture wells inoculated with blood-fed mosquito homogenates from Baringo that were suspected to be positive for virus induced cytopathology. Three isolates were successfully amplified and resolved as Bunyamwera Virus by HRM (Figure 8) and confirmed by amplicon sequencing of 199bp non-structural gene fragment (98% identity to GenBank accession KM507344; *e*-value 8*e*-92). These Bunyamwera Virus positives included *Aedeomyia africana* that had fed on cattle, *An. coustani* that had fed on sheep and *Ma. africana* that had fed on man all from Logumgum. From a *Cx. pipiens* from Kokwa Island that had fed on human, a 91bp fragment of Sindbis virus was also sequenced (95% identity to GenBank accession KF737350; *e*-value 1e-30). The rest were not fully characterized, as the samples did not amplify. PCR screening of *phleboviruses* and *flaviviruses* were all negative.

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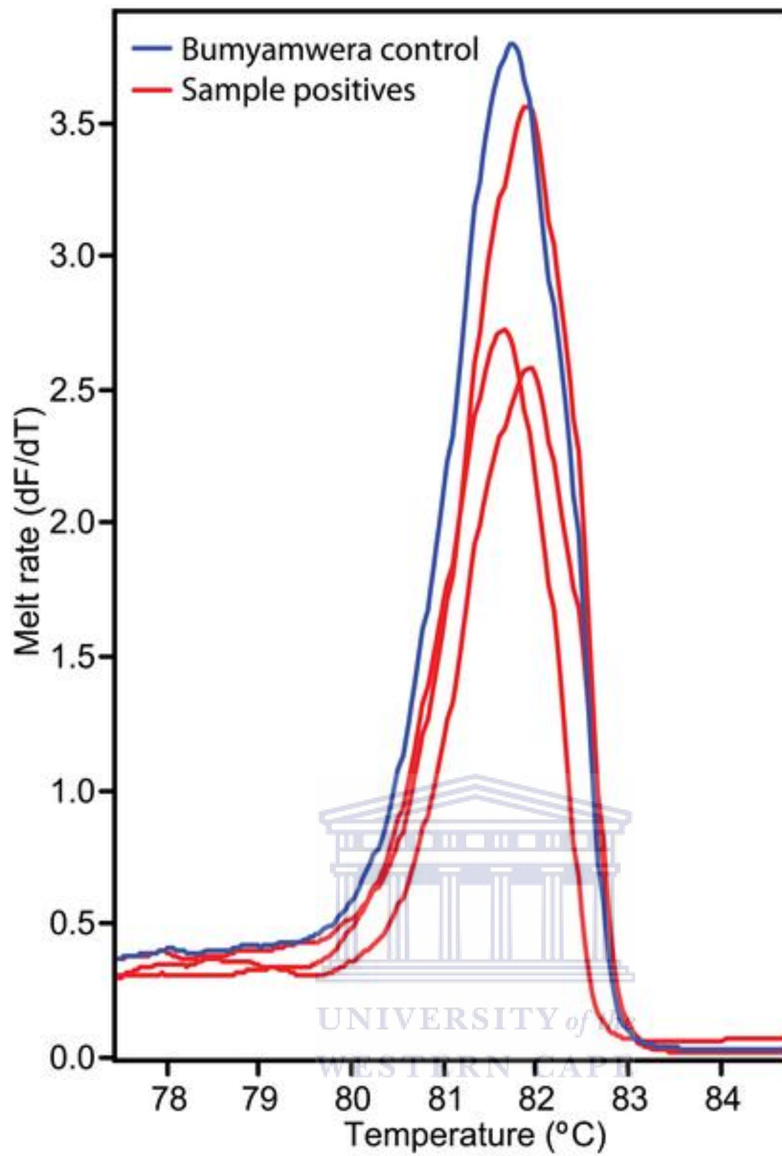

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Figure 8: Melt rate profiles of Bunyamwera S segment amplicons detected in blood-fed mosquitoes.

3.2 Results on molecular detection of tick borne pathogens around Lakes Victoria and Baringo, Kenya

A total of 585 tick pools representing 4,126 ticks of 14 species were collected and analysed from both study areas. There were more ticks sampled in Baringo County (80.5%) as a result of higher sampling effort compared to Homa Bay County (19.5%). In Baringo County, 12 tick species sampled from cattle, goats, sheep, poultry houses and free ranging tortoises were analysed (Figure 9). *Rhipicephalus pravus* (22.0%) sampled from domestic ruminants and dogs from Logumgum were the most frequent species sampled, while *Am. nuttalli* (0.2%) sampled from free ranging tortoises at Kampi ya Samaki was the least frequent species sampled. Among the 12 tick species sampled from Baringo County, 11 were hard ticks (Family; *Ixodidae*) and one was a soft tick (*Argas persicus*, 7.0%) that was sampled from a poultry house in Kampi ya Samaki. The proportion of all tick species sampled from different hosts in the four study areas of Baringo County is represented in Figure 10 and Table 12. In Baringo County, 1,084 ticks representing 32.6% of the total collection were sampled from goats, indicating that goats were more heavily parasitized than any other animal though high number of goats (117) was in relation to their high frequency as natives of Baringo prefer domesticating goats over any other animal (Table 12).

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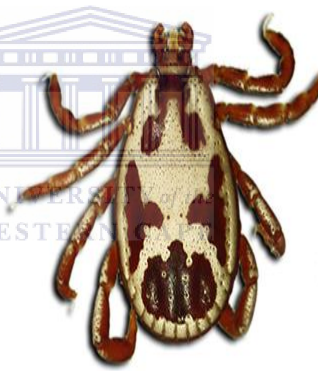
Am. gemma *Am. variegatum* *Am. sparsum* *Am.falsomarmoreum* *Am.nuttalli*



Rh. pravus *Rh. evertsi evertsi* *Rh. pulchellus* *Hy. truncatum* *Hy. rufipes*

Argas persicus

Figure 9: *Amblyomma*, *Rhipicephalus*, *Hyalomma* and *Argars* tick genera sampled from Baringo County (Amblyomma species were taken by camera enabled Leica microscope. Leica Microsystems Buffalo Groove, Illinois). The other species were obtained at http://www.barcodeoflife.org/.

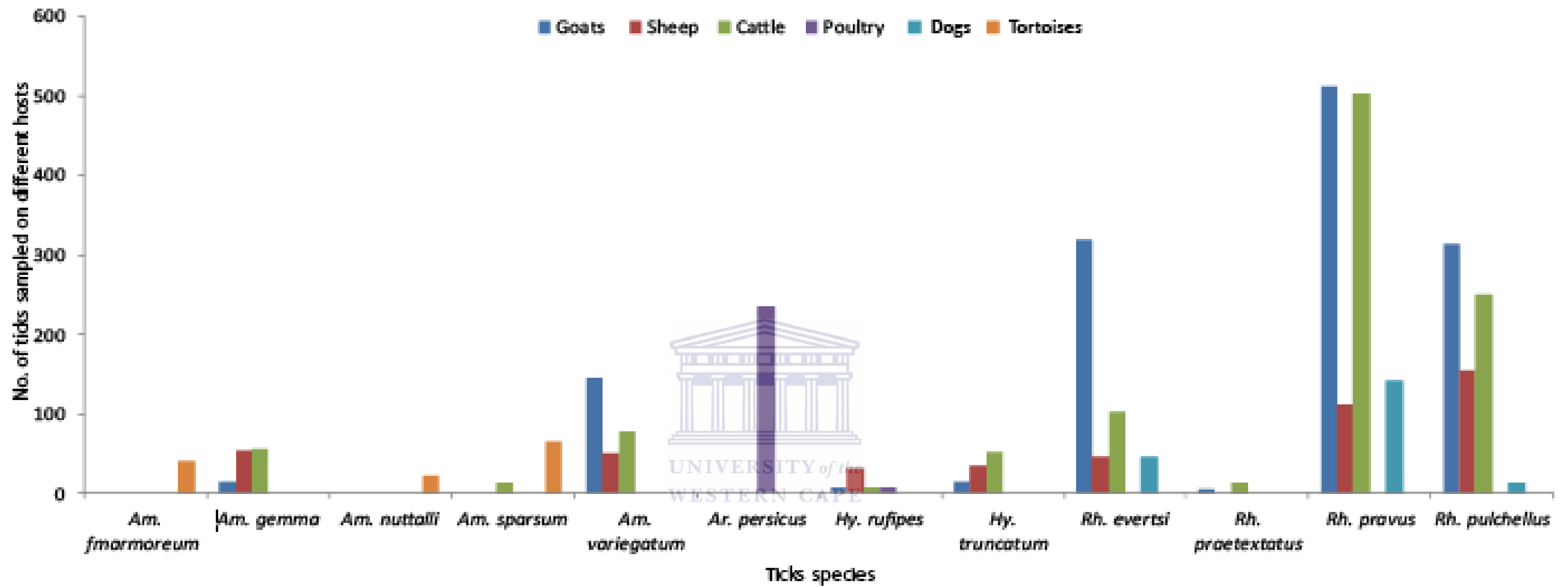


Figure 10: Distribution of ticks sampled from different hosts in Baringo County.

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Am. aponoma latum

Rh. appendiculatus

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Figure 11: Two tick species that were sampled in Homa Bay County, but not Baringo County (*Am. aponoma latum* was taken by camera enabled Leica microscope. Leica Microsystems Buffalo Groove, Illinois) *Rh. appendiculatus* picture was obtained at http://www.barcodeoflife.org/.

Table 12: Tick sampling from different hosts in four study areas of Baringo County

Study Area	Tick species	Pools	n	Goats	Sheep	Cattle	Poultry	Dogs	Tortoises	Totals
Kampi Ya Samaki	*Ar. persicus*	34	234				234 (7.04%)			234 (7.04%)
	Am. gemma	4	17	6 (0.18%)	3 (0.09%)	8 (0.24%)				17 (0.51%)
	Am. nuttalli	1	6						6 (0.18%)	6 (0.18%)
	Am. sparsum	2	13			13 (0.39%)				13 (0.39%)
	Am. variegatum	5	33	5 (0.15%)	9 (0.27%)	19 (0.57%)				33 (0.99%)
	Rh. evertsi	17	133	66 (1.98%)	38 (1.14%)	24 (0.72%)		5 (0.15%)		133 (4%)
	Rh. pravus	51	401	186 (5.6%)	95 (2.86%)	82 (2.47%)		38 (1.14%)		401 (12.07%)
	Rh. pulchellus	31	204	98 (2.95%)	58 (1.74%)	46 (1.38%)		2 (0.06%)		204 (6.14%)
Ruko Conservancy	*Am. gemma*	5	36	11 (0.33%)	7 (0.21%)	18 (0.54%)				36 (1.08%)
	Am. nuttalli	2	16						16 (0.48%)	16 (0.48%)
	Am. sparsum	4	32						32 (0.96%)	32 (0.96%)
	Am. variegatum	8	52	6 (0.18%)	5 (0.15%)	41 (1.23%)				52 (1.56%)
	Rh. evertsi	28	219	46 (1.38%)	68 (2.04%)	59 (1.77%)		46 (1.38%)		219 (6.59%)
	Rh. praetextatus	8	15	5 (0.15%)		10 (0.3%)				15 (0.45%)
	Rh. pravus	31	235	128 (2.85%)	55 (1.65%)	52 (1.56%)				235 (7.07%)
	Rh. pulchellus	27	209	46 (1.38%)	30 (0.9%)	114 (3.43%)		19 (0.57%)		209 (6.29%)
	Hy. rufipes	7	47	7 (0.21%)	6 (0.18%)	28 (0.84%)	6 (0.18%)			47 (1.41%)
	Hy. truncatum	13	101	14 (0.42%)	35 (1.05%)	52 (1.56%)				101 (3.04%)
Logumgum	*Am. falsomarmoreum*	6	41						41 (1.23%)	41 (1.23%)
	Am. gemma	6	47	5 (0.15%)	23 (0.69%)	19 (0.57%)				47 (1.41%)
	Am. sparsum	5	30						30 (0.9%)	30 (0.9%)
	Am. variegatum	26	187	23 (0.69%)	43 (1.29%)	121 (3.64%)				187 (5.83%)
	Rh. evertsi	14	110	24 (0.72%)	15 (0.45%)	25 (0.75%)		46 (1.38%)		110 (3.31%)
	Rh. pravus	97	731	304 (9.15%)	202 (6.08%)	161 (4.85%)		64 (1.92%)		731 (22.01%)
Kokwa	*Am. gemma*	4	22	12 (0.36%)	8 (0.24%)	2 (0.06%)				22 (0.66%)
	Rh. evertsi	4	26	8 (0.24%)	9 (0.27%)	4 (0.12%)		5 (0.15%)		26 (0.78%)
	Rh. pravus	16	123	84 (2.53%)	38 (1.14%)	1 (0.03%)				123 (3.7%)
	Totals	**456**	**3320**	**1084 (32.56%)**	**747 (22.5%)**	**899 (27.07%)**	**240 (7.22%)**	**225 (6.77%)**	**125 (3.76%)**	

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In Homa Bay County, there were eight tick species with *Rh. pravus* (29.9%) being the most frequent species, other species from Homa Bay included *Rh. pulchellus* (19.0%), *Am. variegatum* (16.6%), *Rh. evertsi evertsi* (15.5%), *Am. gemma* (9.4%), *Rh. appendiculatus* (4.1%) and *Hy. truncatum* (1.7%). *Amblyomma (Aponomma) latum* (3.8%) was also sampled from parasitized monitor lizards. (Figure 12). *Amblyomma latum* and *Rh appendiculatus* (Figure 11) represents two species that were only sampled from Homa Bay County and not Baringo. Distribution of different tick species among hosts is represented in Table 13

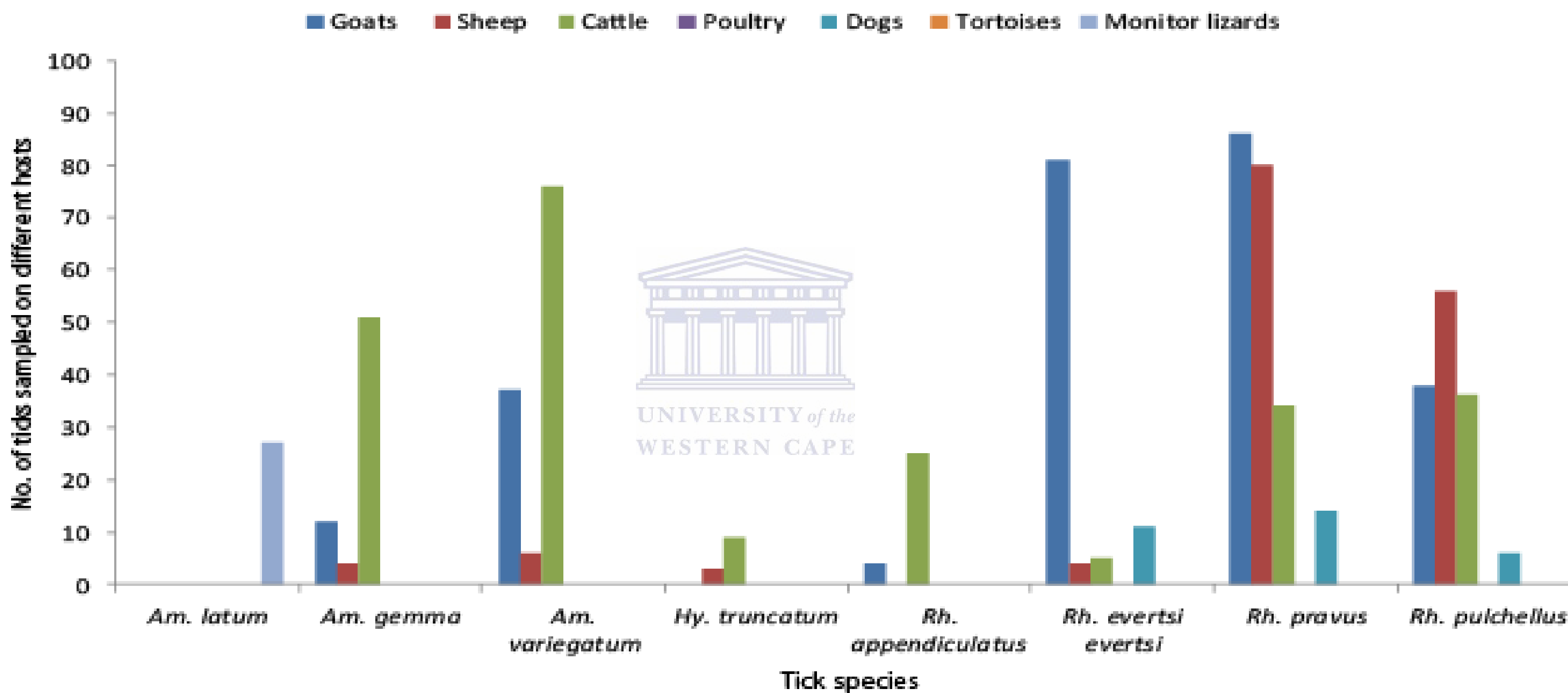

Figure 12: Distribution of ticks sampled from different hosts in Homa Bay County.

Table 13: Tick sampling from different hosts in four study areas of Homa Bay County

Study Area	Tick species	Pools	n	Goats	Sheep	Cattle	Dogs	Monitor lizards	Totals
Ngothe	*Am. variegatum*	4	18	4 (0.49%)	5 (0.62%)	9 (1.11%)			18 (2.23%)
	Rh. evertsi evertsi	8	45	30 (2.72%)	4 (0.49%)	3 (0.37%)	8 (2.23%)		45 (5.58%)
	Rh. pulchellus	9	61	23 (2.85%)	14 (1.73%)	24 (2.97)			61 (7.56%)
	Hy. truncatum	3	12		3 (0.37%)	9 (1.11%)			12 (1.48%)
Mbita	*Am. gemma*	9	67	12 (1.48%)	4 (0.49%)	51 (6.32%)			67 (8.31%)
	Am. variegatum	5	32	6 (0.74%)	10 (1.24%)	16 (1.98%)			32 (3.97%)
	Rh. evertsi evertsi	7	40	18 (2.23%)	6 (0.74%)	11 (1.36%)	5 (0.62%)		40 (4.96%)
	Rh. pravus	13	83	38 (4.71%)	28 (2.47%)	13 (1.61%)	4 (0.49%)		83 (10.29)
	Rh. pulchellus	1	6			6 (0.74%)			6 (0.74%)
Mfangano	*Am. variegatum*	6	45	14 (1.73%)	6 (0.74%)	25 (3.1%)			45 (5.58%)
	Rh. evertsi evertsi	5	26	14 (1.73%)	4 (0.49%)	5 (0.62%)	3 (0.37%)		26 (3.22%)
	Rh. pravus	14	94	37 (4.59%)	27 (2.35%)	18 (2.23%)	12 (1.48%)		94 (11.66%)
	Rh. pulchellus	8	41	19 (2.35%)	13 (1.61%)	9 (1.11%)			41 (5.08%)
Rusinga	*Am. latum*	4	27					27 (3.34%)	27 (3.34%)
	Hy. truncatum	1	6			6 (0.74%)			6 (0.74%)
	Rh. Appendiculatus	5	29	4 (0.49%)		25 (3.1%)			29 (3.59%)
	Rh. evertsi evertsi	6	23	11 (1.36%)	2 (0.24%)	4 (0.49%)	6 (0.74%)		23 (2.85%)
	Rh. pravus	14	101	57 (7.07%)	15 (1.86%)	15 (1.86%)	14 (1.73%)		101 (12.53%)
	Rh. pulchellus	7	50	26 (3.22%)	11 (1.36%)	12 (1.48%)	1 (0.12%)		50 (6.2%)
	Totals	**129**	**806**	**307 (38.08%)**	**152 (18.85%)**	**261 (32.38%)**	**53 (6.57%)**	**27 (3.34%)**	

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Tick borne pathogen gene fragments with distinct HRM profiles and representative sequence data that were at least 94% identical to a recognized TBP on GenBank were detected from Baringo (Table 14) and Homa Bay Counties (Table 15). In Baringo County, *Ehrlichia ruminantium* (12.3%) [GenBank accession NR_074155 99%; *e*-value 1e-94, 196 bp 16S rRNA] which is the causative agent of heartwater in ruminants, was isolated in 56 tick pools mainly from *Amblyomma* species and *Rh. evertsi evertsi* (Table 14). Other agents of ehrlichiosis detected from ticks sampled from Baringo included *E. canis* (9.6%). The longer fragment of *Ehrlichia canis* (555bp) was subsequently amplified and analysed using *Ehrlichia* 16S rRNA JV primers in *Rh. pravus*, *Rh. evertsi evertsi* and *Rh. pulchellus* pools sampled from livestock and dogs (Table 16). *Ehrlichia* sp. (9.4%) and subsequent analysis of longer fragment 384bp, specifically Tibet/ Xinjiang isolated from pools of *Am. gemma* (7), *Am. variegatum* (3), *Rh. evertsi evertsi* (13), *Rh. pulchellus* (9) and *Rh. praextatus* (1). *Paracoccus* sp. (8.4%) was amplified using *Ehrlichia* primers in tick pools of *Am. variegatum* (1), *Am. sparsum* (9), *Am. falsomarmoreum* (2) and *Am. gemma* (8) (Table 16). There were instances where detected a mix of *Paracoccus* sp. and *E. ruminantium* from a single tick pool was observed. Six pools of *Am. sparsum* and four of *Am. variegatum* had both *E. ruminantium* and *Paracoccus* sp., while three pools of *Am. variegatum* had a mixed infection of *Ehrlichia* sp. and *Paracoccus* sp. Distinct HRM profiles of different *Ehrlichia* and *Paracoccus* sp. are shown in Figure 13.

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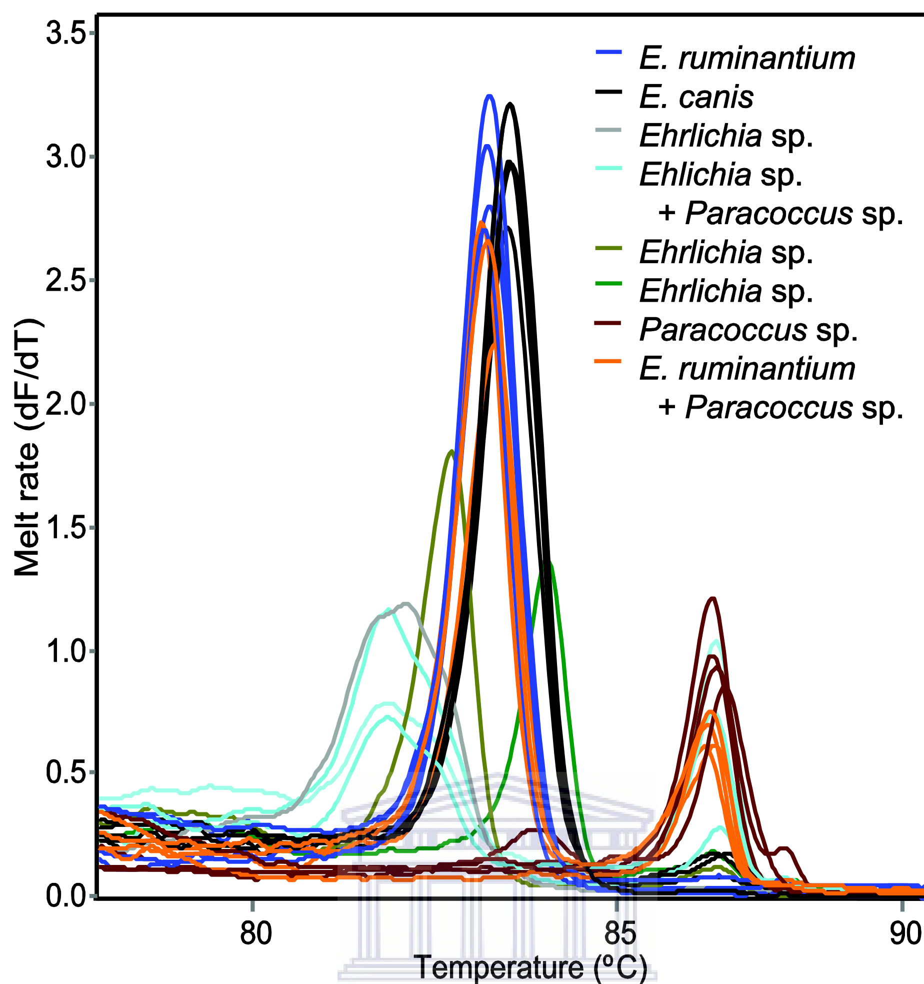

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Figure 13: Melt rate profiles of different *Ehrlichia* and *Paracoccus* sp. 16S rRNA segment amplicons detected in field collected ticks.

Agents of livestock and canine anaplasmosis were detected in Baringo County. These included *Anaplasma ovis* (7.5%) [GenBank accession KJ410246 99%; *e*-value 1e-135, 185bp] and a subsequent analysis of a longer 328 bp fragment using *Anaplasma* 16S rRNA JV primers [GenBank accession KJ410246 100%] [submitted GenBank accessions KT266581] isolated in tick pools of *Am. variegatum* (22), *Am. gemma* (7) and *Rh. pulchellus* (5) parasitizing livestock in Logumgum and Kampi ya Samaki. *A. platys* (14.0%) [GenBank accession LC018183 100%; *e*-value 5e-62, 185 bp 16S rRNA] was

detected in *Rhipicephalus* tick pools including *Rh. evertsi evertsi* (15), *Rh. pravus* (45) and *Rh. pulchellus* (4). *Anaplasma bovis* (3.9%) [GenBank: accession U03775 99%; *e*-value 1e-135, 320 bp] using *Anaplasma* 16S rRNA JV primers [submitted GenBank accession KT266580] was detected in 18 tick pools of both *Amblyomma* and *Rhipicephalus* ticks (Table 14). HRM profiles of the three different anaplasmosis agents detected in Baringo County are represented in Figure 14.

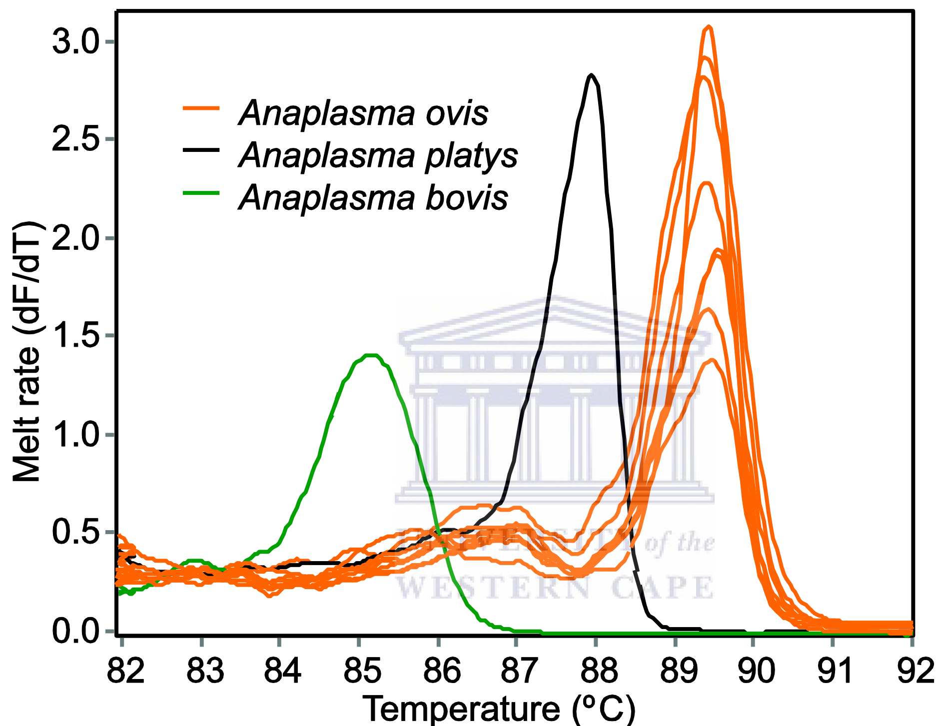

Figure 14: Melt rate profiles of three *Anaplasma* 16S rRNA segment amplicons detected in field collected ticks.

Bacterial agents of rickettsiosis were also detected from tick species of Baringo County. *Rickettsia aeschlimannii* (2.9%) [GenBank accession HQ335165 100%; *e*-value 8e-179, 344 bp *rpmE*/tRNA^{fMet}] [Submitted GenBank accessions KT266585] detected from tick pools of *Hy. truncatum* (6), *Hy. rufipes* (5) and *Rh. pulchellus* (2). *Rickettsia rhipicephali* (5.5%) [GenBank accession CP003342 99%; *e*-value 1e-171, 434bp *rpmE*/tRNA^{fMet}] [submitted GenBank accessions KT266586] was detected in tick pools

of *Rh. evertsi evertsi* (4) and *Rh. pulchellus* (21). *Rickettsia montanensis* (2.6%) [GenBank accession CP003340 99%; *e*-value 6e-170, 343 bp *rpmE*/$tRNA^{fMet}$] [submitted GenBank accessions KT266588] was detected in tick pools of *Am. gemma* (6) and *Rh. evertsi evertsi* (3). *Rickettsia japonica*-like sequences were also detected [GenBank accession AP011533 94%; *e*-value 5e-115, 338 bp *rpmE*/$tRNA^{fMet}$] [submitted GenBank accession KT266589] was isolated from two pools of *Rh. pulchellus* sampled from dogs near Ruko Conservancy. *Rickettsia* sp. (4.4%) [GenBank accession AB763430 97%; *e*-value 9e-53, 254 bp *rpmE*/$tRNA^{fMet}$] [submitted GenBank accession KT266587] were also detected in *Rh. pulchellus* pools sampled from cattle of Baringo County. *Rickettsia africae* sequences (7.9%) [GenBank accession CP001612 100%; *e*-value 2e-143, 280 bp *rpmE*/$tRNA^{fMet}$] [submitted GenBank accessions KT266590] were isolated from tick pools of *Am. gemma* (14), *Am. variegatum* (16), *Am. sparsum* (3) and *Rh. evertsi evertsi* (3). High resolution melt curves of different *Rickettsia* detected are illustrated in Figure 15.

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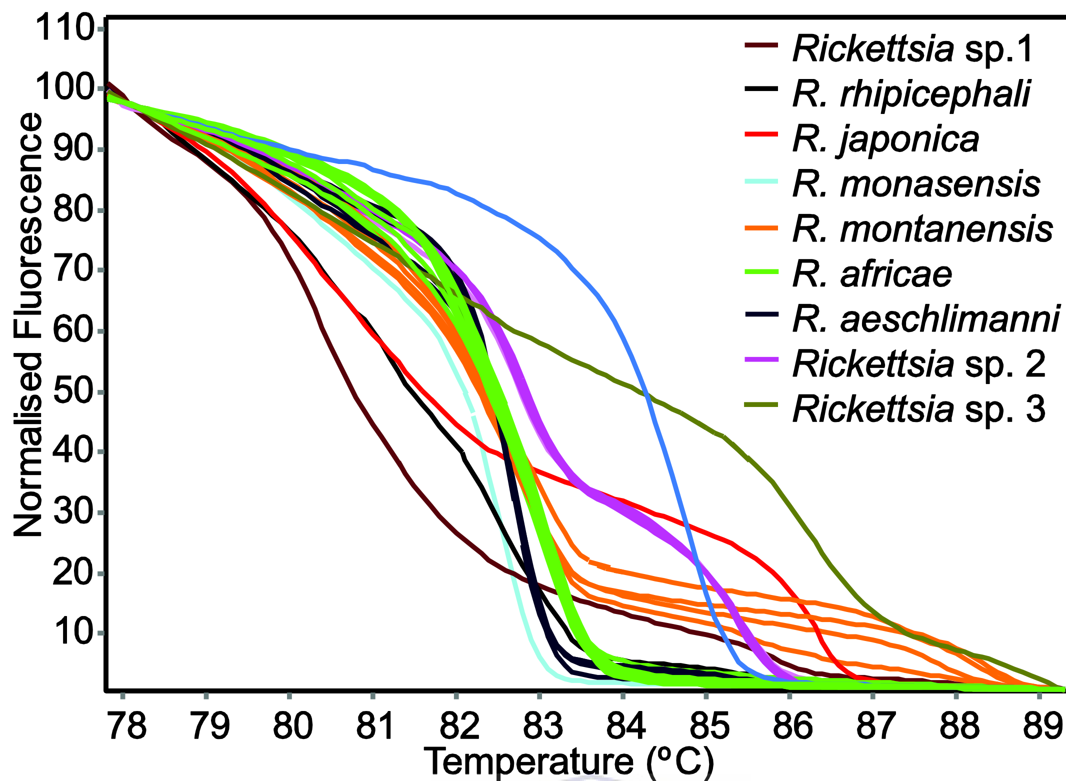
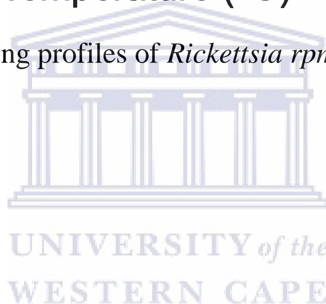


Figure 15: High resolution melting profiles of *Rickettsia rpmE*/tRNA^{fMet} gene fragments detected in field collected ticks.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Babesia caballi [GenBank accession EU642514 98%; *e*-value 8e-123, 255 bp 18S rRNA] [submitted GenBank accession KT266583] was isolated from eight pools of *Rh. pulchellus* parasitizing cattle in Kampi ya Samaki. *Hepatozoon fitzsimonsi* [GenBank accession KR069084 100%; *e*-value 0.0, 438 bp 18S rRNA] [submitted GenBank accession KT266582] was isolated in nine tick pools of *Am. sparsum* and five pools *Am. falsomarmoreum* all having been sampled from free ranging tortoises in Baringo County. *Theileria* sp. [GenBank accession KP864648; 90%; *e*-value 2e-140, 402 bp 18S rRNA] [submitted GenBank accessions KT266584] was isolated from three pools of *Rh. evertsi evertsi* parasitizing livestock of Logumgum study area of Baringo County. Distinct HRM

profiles of *Babesia caballi*, *Hepatozoon fitzsimonsi* and *Theileria* sp. is illustrated in Figure 16

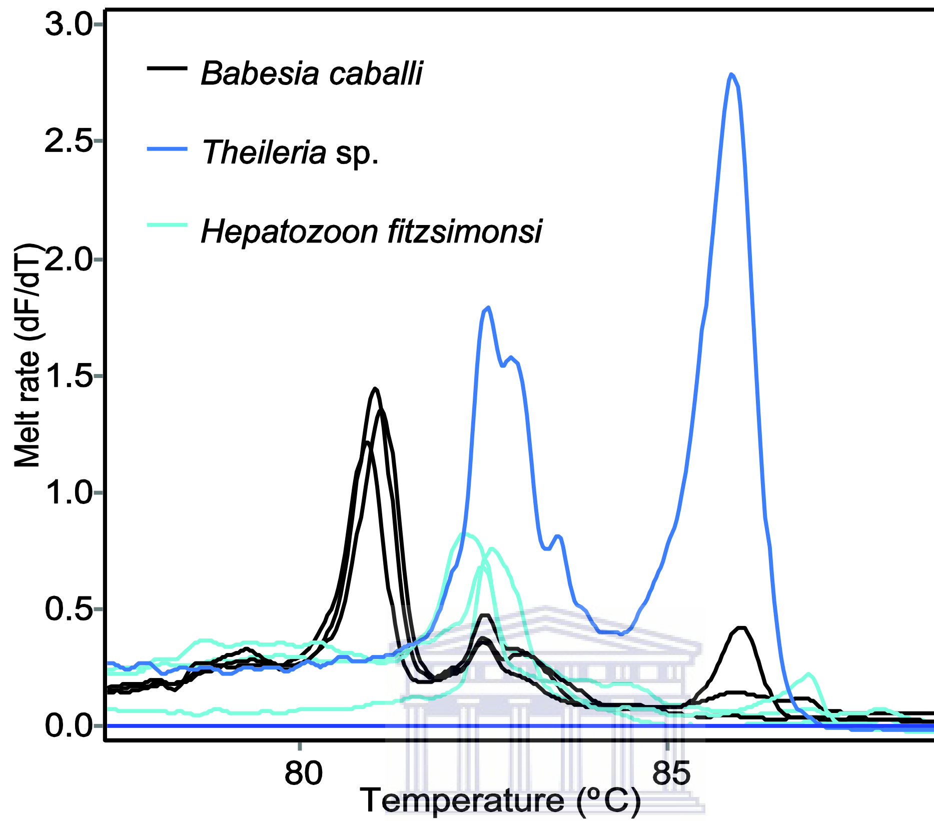

Figure 16: Melt rate profiles of three protozoa. 16S rRNA segment amplicons detected in field collected ticks.

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In Homa Bay County, a total of 129 tick pools were analysed representing 806 ticks. *Ehrlichia ruminantium* (13.2%) was detected [GenBank accession U03776 99%; *e*-value 6e-93, 196 bp 16S rRNA] from tick pools of *Am. gemma* (6), *Am. variegatum* (6) and *Rh. evertsi evertsi* (5) sampled from Ngothe, Mbita and Mfangano Island. *Anaplasma ovis* (2.3%) was isolated from three tick pools of *Rh. appendiculatus* sampled from Rusinga Island and *A*. *platys* (15.5%) was isolated from pools of *Rhipicephalus* species sampled in Homa Bay County (Table 15). *Ehrlichia canis* (6.9%) was also isolated in tick pools of *Rh. pulchellus* and *Am.* (*aponomma*) *latum* (Table 15). Summary TBPs detected in Baringo and Homa Bay Counties study areas is illustrated in Table 16.

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Table 14: Tick borne pathogens isolated from tick species collected in Baringo County

Tick species	N	*A. ovis*	*A. platys*	*A. bovis*	*E. ruminantium*	*E. canis*	*Ehrlichia sp.*	*R. aeschlimannii*	*R. africae*	*R. rhipicephalii*	*R. montanensis*	*Rickettsia sp.*	*H. fitzsimonsi*	*Paracoccus sp.*
Rh. pravus	195		23.07% (45)			6.67% (13)								
Rh. evertsi evertsi	63		23.81% (15)	9.52% (6)	9.52% (6)	31.74% (20)	20.63% (13)		4.76% (3)	6.34%(4)	4.76% (3)	12.69% (8)		
Rh. pulchellus	58	8.62% (5)	6.89% (4)	5.17% (3)		18.96% (11)	15.51% (9)	3.44% (2)	5.17% (3)	36.2% (21)		20.68% (12)		
Am. variegatum	39	56.51% (22)		10.25% (4)	58.97% (23)		7.69% (3)		41.02% (16)					2.56% (1)
Ar. persicus	34													
Am. gemma	19	36.84% (7)		5.26% (1)	68.42% (13)		36.84% (7)		73.68%(14)		31.57% (6)			42.1% (8)
Hy. truncatum	13			7.69 % (1)				46.15% (6)						
Am. sparsum	11				63.6% (7)								81.81% (9)	81.81% (9)
Rh. praetextatus	8			25% (2)			12.5% (1)							
Hy. rufipes	7			14.28% (1)				71.41% (5)						
Am. falsomarmoreum	6				66.7% (4)								83.3% (5)	33.3% (2)
Am. nuttalli	3				100% (3)									
Total	**456**	**7.45% (34)**	**14.03% (64)**	**3.95% (18)**	**12.28% (56)**	**9.64% (44)**	**7.23% (33)**	**2.85% (13)**	**7.89% (36)**	**5.48% (25)**	**1.97% (9)**	**4.38% (20)**	**3.28% (15)**	**4.38% (20)**

Table 15: Tick borne pathogens isolated from tick species collected in Homa Bay County

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Tick species	N	*A. ovis*	*A. platys*	*E. canis*	*E. ruminantium*
Rh. pravus	41		41.17% (7)		
Rh. evertsi evertsi	26		26.92% (7)		19.23% (5)
Rh. pulchellus	25		24% (6)	24% (6)	
Am. variegatum	15				35.29% (6)
Am. gemma	9				66.67% (6)
Rh. appendiculatus	5	60% (3)			
Am. latum	4			75% (3)	
Hy. truncatum	4				
Total	**129**	**2.32% (3)**	**15.5% (20)**	**6.97% (9)**	**17.52% (17)**

Table 16: Summary of tick borne pathogens detection in Baringo and Homa Bay Counties, Kenya

Pathogen detected	Tick species	Study areas	Reference GenBank Accessions	Percent identity (e-value)	locus	Sequence length (GenBank Accession
Anaplasma bovis	*Am. gemma, Am. variegatum*	Mbila	U03775	99% (1e-135)	short 16S	185 bp
				(99% (9e-164)	long 16S	320 bp (KT266580)
A. ovis	*Am. variegatum, Rh. pulchellus, Am. gemma*	Logumgum, Kampi Ya Samaki	KJ410245	99% (1e-135p)	short 16S	185 bp
				100% (7e-170)	long 16S	328 bp (KT266581)
A. platys	*Rh. evertsi, Rh. pulchellus, Rh. pravus*	Ngolthe, Mlangano, Kampi ya Samaki, Kokwa	LC018183	100% (5e-162)	short 16S	185 bp
Cowdria ruminantium	*Am. gemma, Am. variegatum, Rh. evertsi evertsi*	Ngolthe, Mlangano, Mbila	U03776	99% (6e-93)	short 16S	196 bp
Ehrlichia ruminantium	*Am. gemma, Am. variegatum, Am. sparsum, Am. evertsi evertsi*	Baringo, Logumgum	NR_074155	99% (1e-94)	short 16S	196 bp
E. canis	*Rh. pravus, Rh. evertsi evertsi, Rh. pulchellus*	Logumgum, Rusinga, Mbila	LC018188	100% (3e-96)	short 16S	196 bp
				100% (0.0)	long 16S	555 bp (KT266591)
Ehrlichia sp. (Tibet/Xinjiang)	*Rh. pulchellus*	Ruko	AF414399, JX402605	98% (3e-98)	short 16S	196 bp
				100% (0.0)	long 16S	384 bp (KT266592)
Rickettsia africae	*Am. gemma, Am. variegatum, Am. sparsum, Rh. evertsi evertsi*	Kampi Ya Samaki, Ruko, Logumgum	CP001612	100% (2e-143)	IRNA	280 bp (KT266590)
R. montanensis	*Am. gemma, Rh. evertsi evertsi*	Logumgum, Kokwa	CP003340	99% (6e-170)	IRNA	343 bp (KT266588)
R. rhipicephali	*Rh. evertsi evertsi, Rh. pulchellus*	Kampi ya Samaki, Ruko	CP003342	99% (1e-171)	IRNA	434 bp (KT266586)
R. aeschlimannii	*Hy. truncatum, Hy. rufipes, Rh. pulchellus*	Kokwa, Logumgum, Kampi ya Samaki	HQ335165	100% (8e-179)	IRNA	344 bp (KT266585)
Rickettsia sp.	*Rh. pulchellus*	Ruko	AB763430	97% (8e-53)	OmpB	198 bp
			AP011533	94% (5e-115)	IRNA	338 bp (KT266589)
Hepatozoon fitzsimonsi	*Am. falsomarmoreum, Am. sparsum*	Logumgum, Ruko	KR069084	100% (0.0)	18S	438 bp (KT266582)
Paracoccus sp.	*Am. variegatum, Am. gemma, Am. sparsum, Am. falsomarmoreum*	Mbila, Logumgum	KP003988	97% (6e-88)	short16S	195 bp
Babesia caballi	*Rh. pulchellus*	Kampi ya Samaki	EU642514	98% (8e-123)	18S	255 bp (KT266583)
Theileria sp.	*Rh. evertsi evertsi*	Logumgum	KP864848	90% (2e-140)	18S	402 bp (KT266584)

* GenBank accession numbers of submitted pathogen sequences

3.3 Results on arbovirus occurrence among patients with acute febrile illnesses visiting rural health clinics in Homa Bay County

A total of 339 patients with undiagnosed acute febrile illnesses were recruited as study participants in the four clinics of Homa Bay County. There was no significant variation in the number of selected participants (p=0.001) with Sena Clinic in Mfangano having the most participants (n=105, 31%) and Kitare health clinic at the mainland shore (n=61, 18%) the fewest. Tom Mboya health clinic in Rusinga Island (n=92, 27.1%) and Kisegi health clinic at the mainland shore (n=61, 23.9%), had more proportional study sample sizes. In general, there was a preponderance of female participants (57.2%) over their male counterparts (42.2%), with 0.6% having listed as missing gender. There was variation in the age groups of study participants with persons whose ages ranged from 21-40 being more predominant (46.9%) compared to other age groups. In terms of occupation of participants, there were more teachers and students selected as participants (31.3%) compared to fishermen (19.5%), housewives (17.1%), farmers (15%) and those doing business (10.9%).

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Five Vero cell cultures with disrupted monolayer a possible virus induced cytopathology were detected. Figure 17. There was a transformation of the confluent Vero cell line from the characteristic spindle shaped morphology (Figure 17, Panel A) to fibrillar rods with disruption of the monolayer (Figure 17, Panel B), characteristic virus induced cytopathology in the cell line. The cells rounded up and detached from the surface of culture vessel. Additionally, there was a rapid color change of the phenol red indicator from red to orange/yellow, indicative of active metabolism that are characterized by viral infection. All the five positives samples were from Sena clinic of Mfangano Island. Inoculated serum samples from the other three clinics did not differ from the negative controls suggesting absence of active virus. Second passages of all other samples also turned out negative.

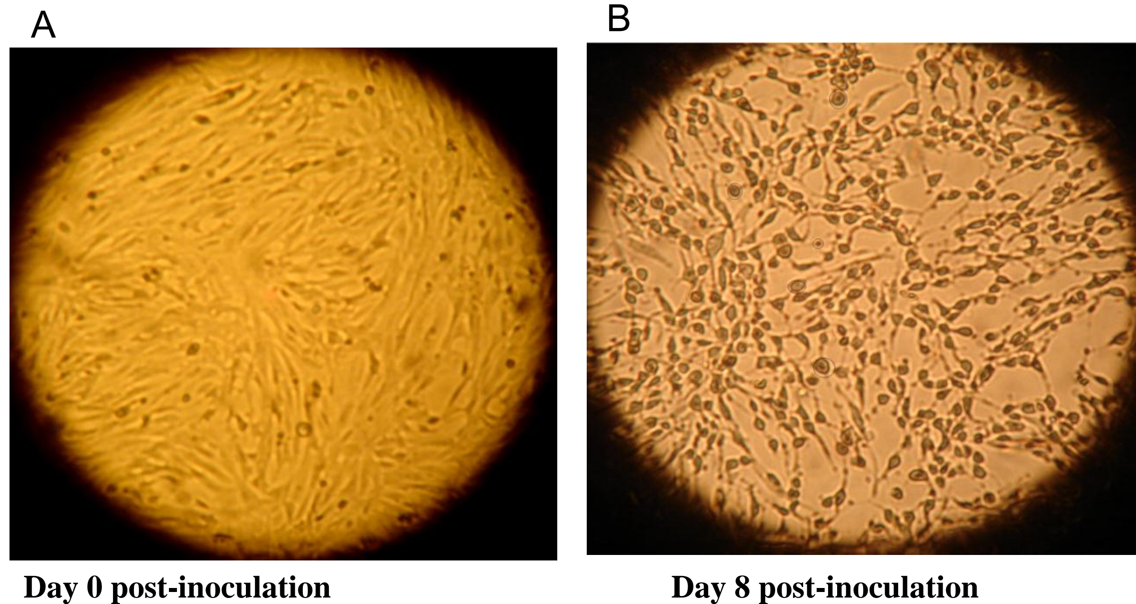

Figure 17: Bunyamwera positive sample inoculated in Vero cell lines and monitored for cytopathology. Panel A); Morphology of the monolayer of the cell line at the onset of inoculation. Panel B); virus induced cytopathology**.**

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Detection of HRM curves with profiles similar to Sindbis and Bunyamwera that have previously been described by Villinger *et al.*, 2016 was observed. (Figure 18). Two positive cultures confirmed to be Sindbis virus (Figure 19 Panel A) and three cultures were confirmed as Bunyamwera virus (Figure 19 Panel B). The summary of acute Bunyamwera and Sindbis infections and there relation with demographic data of study participants is illustrated in Table 17.

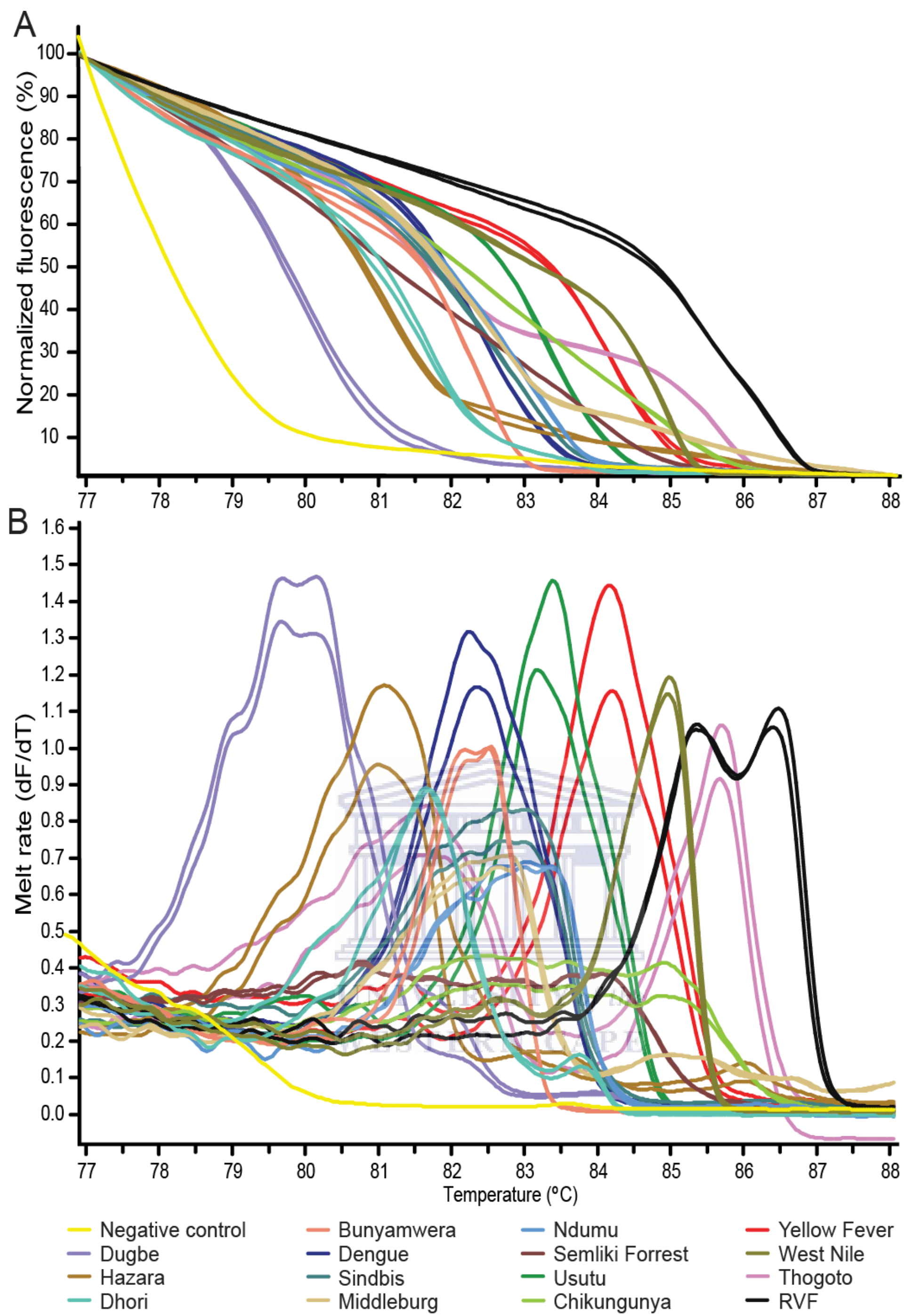
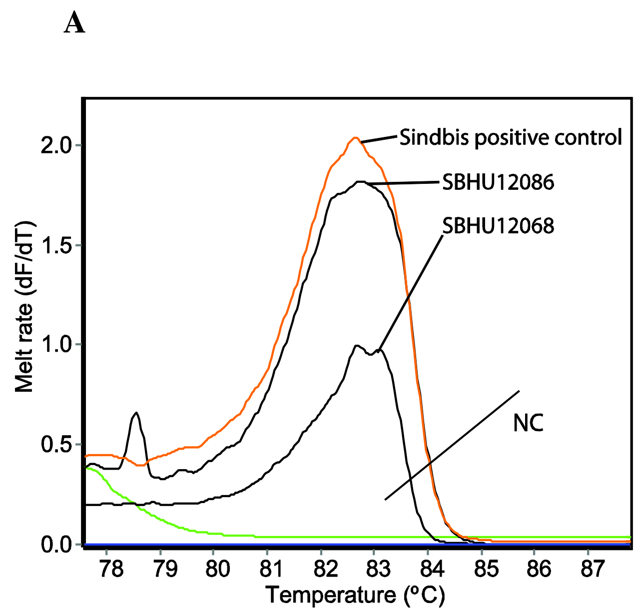


Figure 18: Optimization of multiplex PCR-HRM using different arbovirus positive controls (Villinger *et al*., 2016).



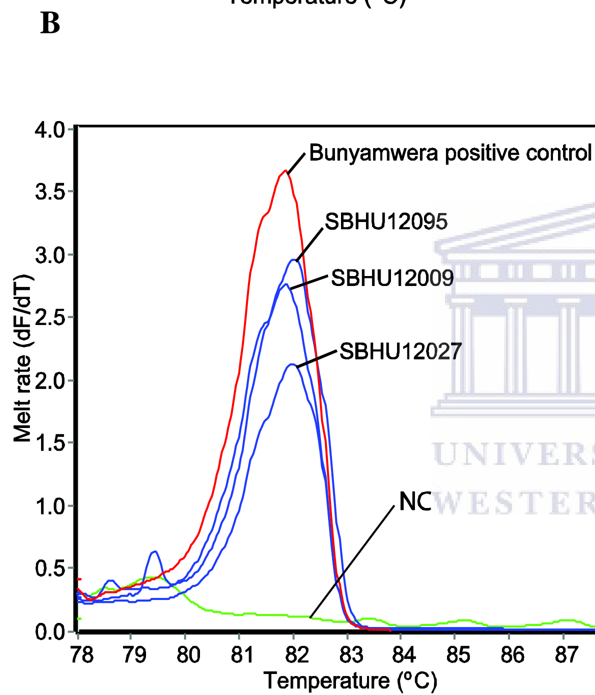


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Figure 19: Melt rates of Sindbis virus and Bunyamwera virus in blood samples collected in Sena clinic along with respective positive controls. NC is no template control.

Table 17: Demographic data and arbovirus infections among patients presenting with acute febrile illness in four rural health clinics in Homa Bay County

Characteristics	# patients (% patients)	Chi sq. value	df	P value
All patients	339			
Clinic				
Mfangano*****	105 (31.0)			
Tom Mboya	92 (27.1)			
Kisegi	81 (23.9)			
Kitare	61 (18.0)	16.4	3	0.0010
Sex				
Female***	194 (57.2)			
Male**	143 (42.2)			
Missing	2 (0.6)	14.8	1	0.0001
Age group				
≤20	117 (34.5)			
21–40 ***	159 (46.9)			
41–60	50 (14.7)			
≥ 61**	10 (2.9)			
Missing	3 (0.9)	211.8	3	<0.0001
Weight	Mean=60.59 (range: 38-94)			
Occupation				
Teacher/student	106 (31.3)			
Fishing**	66 (19.5)			
Housewife**	58 (17.1)			
Farming*	51 (15.0)			
Business	37 (10.9)			
Other	21 (6.2)	89.5	5	<0.0001

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* Sindbis virus infection * Bunyamwera infection; asterisk mean single infection in the group

None of the five participants that were positive for either Sindbis or Bunyamwera infection were positive for *Plasmodium.* This indicates that their febrile illnesses were not a result of malaria infection. Analysis of the arbovirus negative samples however resulted in 62 *Plasmodium* positives that were never detected by microscopy or RDT kits (Kipanga *et al*, 2014).

Incidence rates of malaria were generated by PCR-HRM technique. *Plasmodium* positive samples collected at Tom Mboya Health Centre also had the highest *Plasmodium* species diversity consisting of 72.2% (n=26) *P. falciparum*, 13·9% (n=5) *P. malariae*, 5·6% (n= 2) *P.*

ovale, 5·6% (n=2) double infection of *P. falciparum* and *P. ovale* and 2·8% (n=1) triple infection with *P. falciparum*, *P. malariae*, and *P. ovale*. Samples collected at Sena clinic in Mfangano had only two cases of *P. ovale* with the rest being *P. falciparum*. The unique HRM profiles of the three *Plasmodium* parasites are represented in Figure 20.

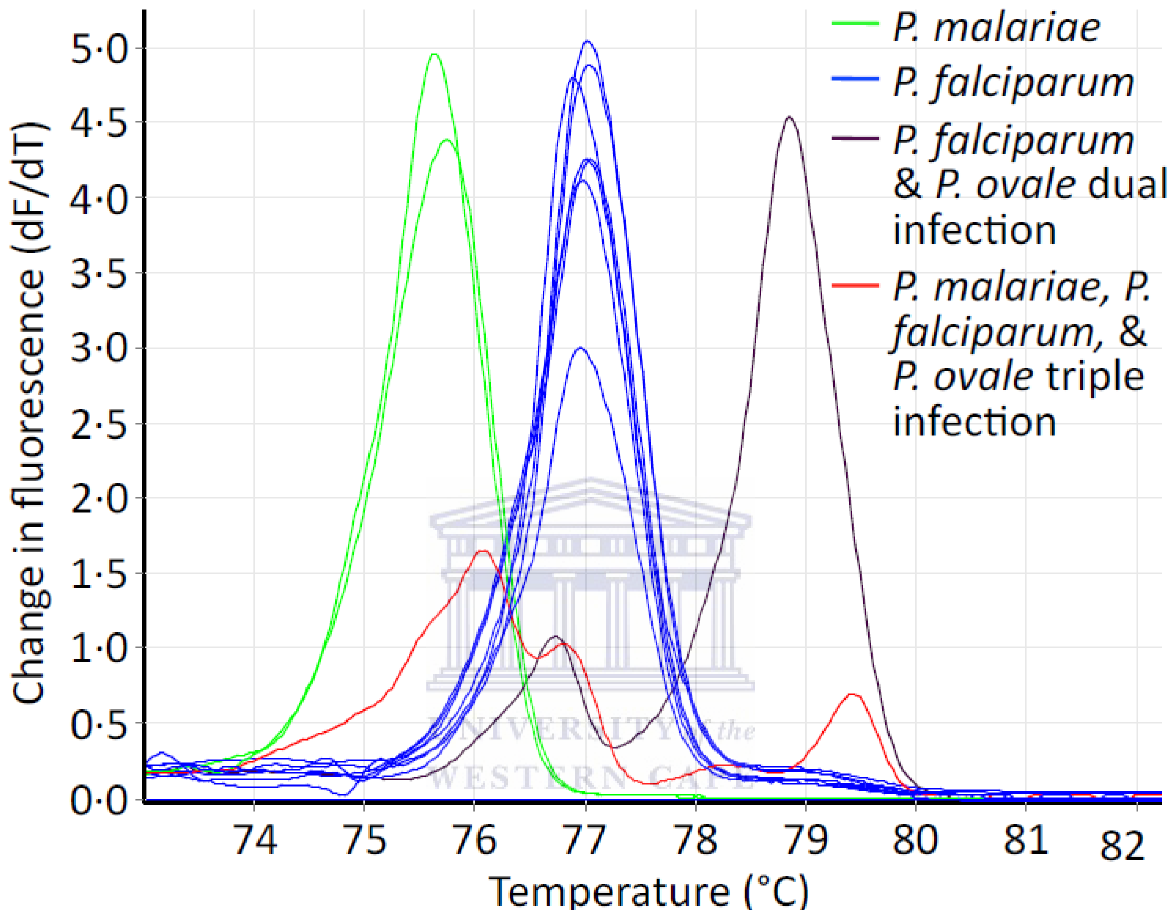

Figure 20: Three *Plasmodium* species detected in a subset of samples from Sena and Tom Mboya clinics (Kipanga *et al*., 2014).

Out of the 197 patients presenting with febrile illness enrolled in Tom Mboya and Sena clinic in Mfangano island, 152 (77.2%) were treated with antimalarial drugs. However, of the 62 patients with detectable *Plasmodium* parasites, only 42 (67.7%) patients were correctly treated with antimalarial drugs. Conversely, of the 135 patients without detectable *Plasmodium* parasites, 110 (81.5%) were incorrectly treated with antimalarial drugs. However, these patterns differed between the two Health Centres, as represented in figure 21. While in Tom Mboya Health Centre,

only 44.4% of the 36 patients with detectable *Plasmodium* parasites were correctly treated, all 26 malaria patients with detectable parasites at Sena were treated with antimalarial drugs. Yet, among the patients that did not have *Plasmodium* infection, more than half were treated with antimalarial drugs at both Tom Mboya (32 out of 56, 57.1%) and Sena clinic (78 out of 79, 98.7%).

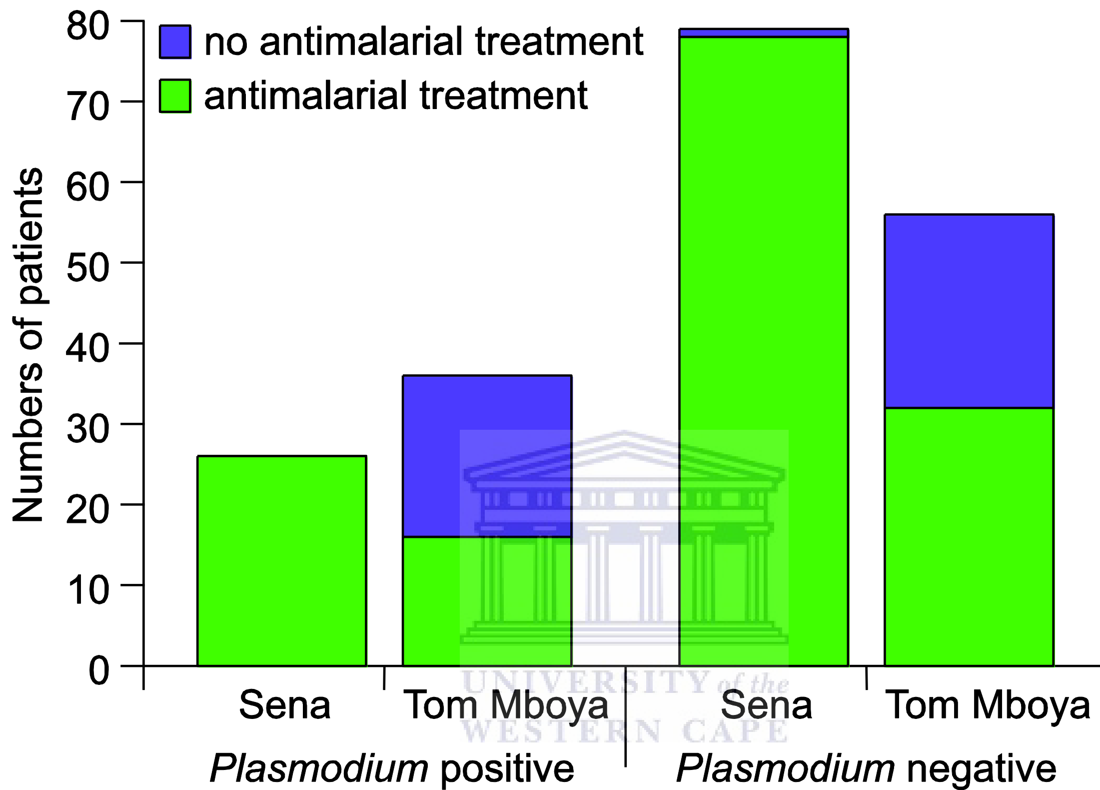

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Figure 21: Antimalarial treatment of febrile patients with and without low *Plasmodium* parasitaemia (Kipanga *et al.*, 2014).

3.4 Seroprevalence of arboviral infections in Rusinga Island

Out of the 92 patients enrolled in the study, Sindbis virus neutralizing antibodies were detected in five (5.4%; 95% CI 1.1%-9.5%) patients (Figures 22 and 23). No Bunyamwera virus neutralizing antibodies were detected. Relatively similar number of females, n=2 (2.2%) and males, n=3 (3.3%) had Sindbis virus neutralizing antibodies as shown in Table 18. Among the individuals with Sindbis virus neutralizing antibodies, three out of the five had co-infection with *Plasmodium* parasites as illustrated in Table 18 and Figure 24.

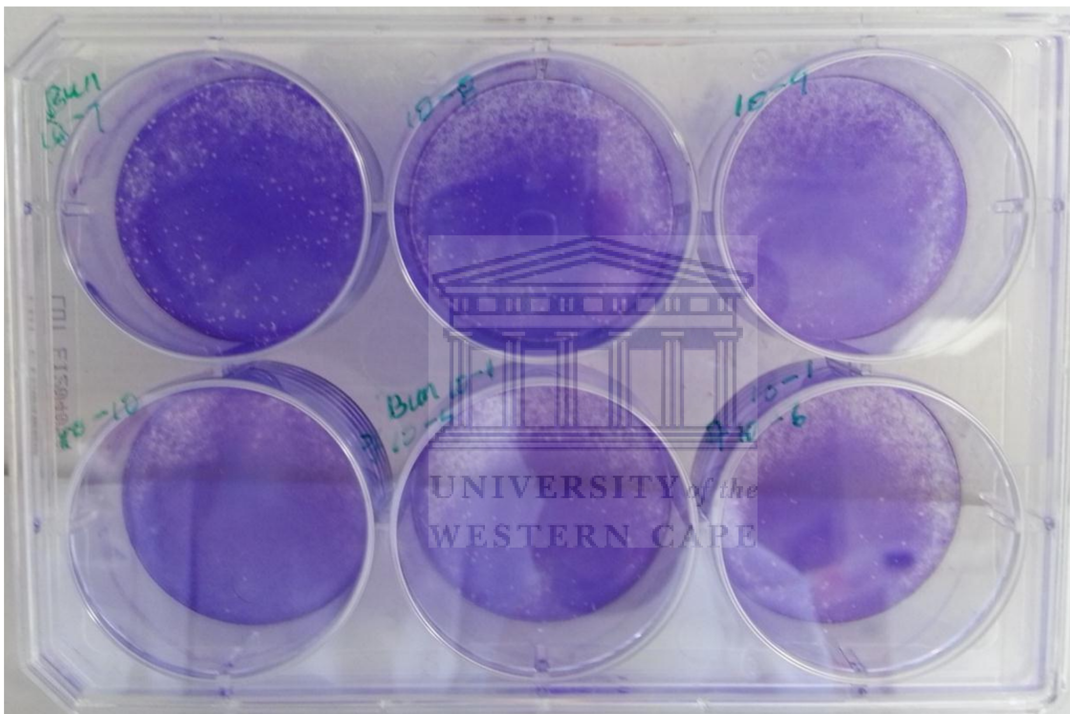
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Figure 22: Plaque assay for Sindbis virus infection in patient sample dilution from 10^{-1} to 10^{-6}.

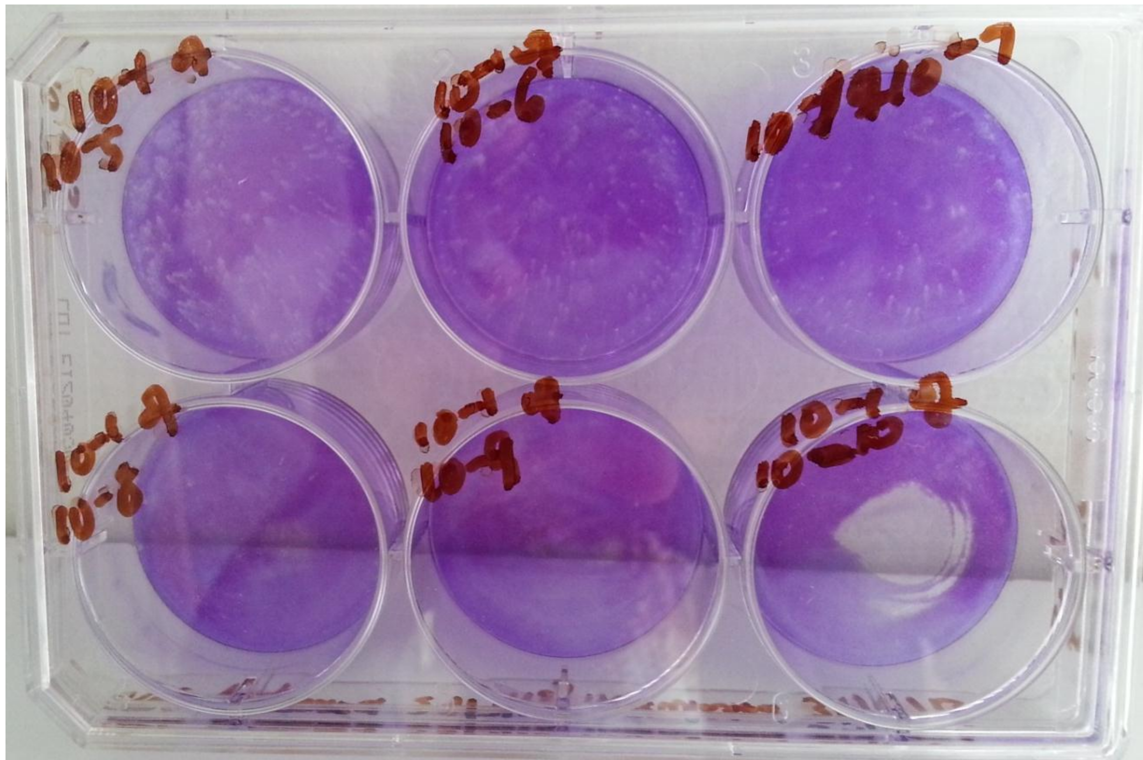

Figure 23: Continuation of Bunyamwera Plaque assay from 10^{-7}-10^{-9} dilutions.

Table 18: Data on individuals with Sindbis virus neutralizing antibodies and co-infection with malaria in Rusinga Island

Serial Number	Gender	Occupation	Treatment	Malaria status
SCHU12028	F	Housewife	antimalarial	*P. falciparum*
SCHU12029	F	Housewife	antimalarial	None
SCHU12030	M	Farming	antibiotic, amoebicide	*P. falciparum*
SCHU12083	M	Fishing	antibiotic, amoebicide	*P. falciparum, P. malariae, P. ovale*
SCHU12092	M	Businessman	antimalarial, antibiotic	None

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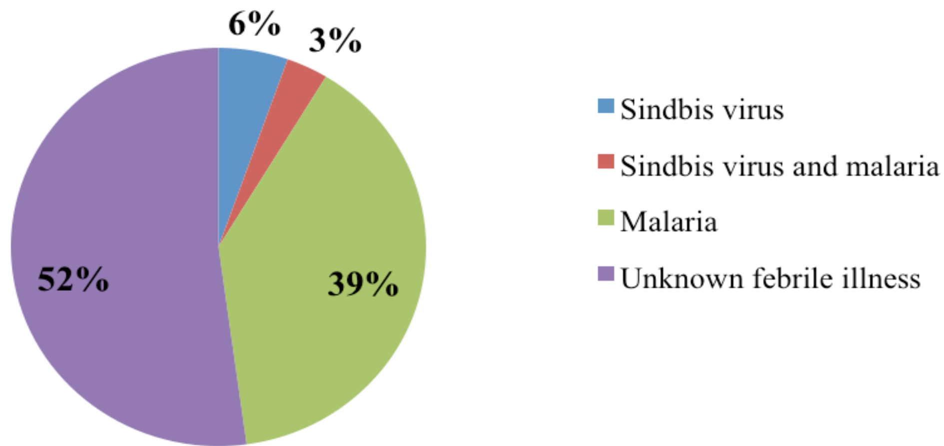

Figure 24: Pie-chart representation of the estimated infections of patients visiting Tom Mboya clinic of Rusinga Island.

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CHAPTER FOUR

DISCUSSION

4.1 Mosquito bloodmeal analysis and arbovirus detection in blood-fed mosquitoes

Blood-fed mosquitoes representing diverse vectors of malaria, arboviruses and filarial worms were sampled in both Baringo and Homa Bay counties. Thirty three (33) distinct vertebrate hosts of mosquito bloodmeals were identified based on their HRM profiles (Figure 4) across both *cyt b* and 16S gene PCR products. Bloodmeal profiles of vertebrates were specifically matched to sequenced positive controls and novel profiles were identified by sequence analysis. Previously, the *cyt b* marker had been used to resolve 14 vertebrate species as sources of triatomine bug bloodmeals in South America. However, this approach alone could not reliably differentiate the diversity of potential mosquito bloodmeal host species in East Africa. Compared to less mobile triatomine bugs, mosquitoes can fly long distances (Greenberg *et al*., 2012) seeking bloodmeals and some species feed on a range of hosts. East Africa (afrotropical) also has abundant fauna (wild animals and birds) compared to South America that has a neotropical habitat.

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Melting profiles of some pairs of vertebrate host species could only be differentiated clearly based on HRM profiles generated by either *cyt b* or 16S amplicons, but not by both markers (Figure 4). Therefore, comparisons of HRM profiles from both loci allow for more robust species differentiation and identification. Additionally, identification of hosts with mixed bloodmeal sources from individual mosquitoes based on melting profiles with double peaks was possible (Figure 6). While sequencing of DNA fragments may also resolve mixed feedings, sequence analysis of double base calls is costly, difficult and time-consuming (Metzker, 2005). These HRM validation results (Figure 5) show high sensitivity in identifying both pure and mixed vertebrate samples from below 1 nl blood volumes (Table 11). As mosquito bloodmeals range from 1nl to 6

µl (Konishi, 1989), HRM analysis can effectively identify multiple blood feeding in mosquitoes and probably other vectors.

Most bloodmeals identified were from humans and domestic animals, but also identified mosquitoes that had fed on wildlife hosts in both study areas. More wildlife bloodmeal sources were identified in Baringo County, which has a higher density of wildlife that interfaces with livestock compared to Homa Bay County. As traps were positioned proximal to human habitation and farms, bloodmeal host proportions are probably biased by increased host diversity at peridomestic and domestic sampling sites.

In Homa Bay County, blood-fed malarial vectors *An. gambiae* (7.8%) and *An. funestus* (0.8%) were less commonly collected in comparison to other blood-fed species such as *Cx. pipiens* (19.8%), *Ma. africana* (13.9%), *Ma. uniformis* (11.2%) and *An. coustani* (10.1%) (Table 7). This could be attributed in part to massive malaria eradication programs, as many studies are being conducted in the area that are focused on development of efficient tools for eliminating malaria and malaria vectors (Mukabana *et al*., 2006, Opiyo *et al*., 2007, Kawada *et al*., 2011, Worrall and Fillinger, 2011, Kipanga *et al*., 2014). Because traps were set outdoors, *An. arabiensis* (78%) were mainly among the small number of *An. gambiae s.l.* mosquitoes. This was probably due to their high abundance and exophilic tendencies compared to *An. gambiae "s.s"*, which are documented to be more endophilic in their feeding behavior (Fornadel *et al*., 2010, Mwangangi *et al*., 2013). This finding concurred with previous entomological survey that showed abundance of *An. arabiensis* over *An. gambiae "s.s"* and *An funestus s.l* around Lake Victoria and adjacent habitats (Minakawa *et al*., 2012).

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Bloodmeals of *An. gambiae "s.l",* and *Cx. pipiens* were identified from the same range of host species, including birds, with the highest proportions being from humans (Tables 7 and 8). This contrasted with previous observations that *Cx. pipiens* preferentially feed on birds than on humans (Simpson *et al*., 2009, Garcia-Rejon *et al*., 2010, Greenberg *et al*., 2013). Even during the onset of West Nile cases in humans in suburban Chicago, Illinois, the predominant bloodmeals of

its principal vector, *Cx. pipiens*, were birds (Hamer *et al*., 2009). Considering the prominent abundance human bloodmeal sources of *Cx. pipiens* (19.8%) in Homa Bay County (Table 7), it is surprising that the diverse bulk of wild birds that thrive from fish and aquatic organisms along the shores of Lakes Victoria and Baringo (Lewis A, 1989.) did not form a higher proportion of *Cx. pipiens* bloodmeals. Host feeding of *Cx. pipiens* in study areas are dynamic and dependent on the composition and proportion of available host species present. Alternatively, given that sampling was conducted in close proximity to human habitation, it could be interesting to find out if avian hosts form the bulk of *Cx. pipiens* diets in wild caught species that are not close to human habitation.

Blood-fed *An. coustani* were well represented in the sampling localities of Homa Bay and Baringo Counties (Table 7 and 9). Although this species has been implicated in malaria transmission in some countries (Fornadel *et al*., 2011, Tabue *et al*., 2014), three (Table 6) out of 32 (9.4%) (Table 7) were the only blood-fed *An. coustani* mosquitoes found with human bloodmeals in Baringo, while none (Table 9) of the 26 (Table 6) blood-fed *An. coustani* sampled from Homa Bay County had human bloodmeals. This finding was surprising considering that in Baringo, cattle herders sleep outdoors to protect their livestock from cattle rustlers and night fishing is popular in Homa Bay County and therefore inhabitants of both areas should have been prone to bites of outdoor blood-questing mosquitoes.

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Culex univittatus, which is considered a competent vector of West Nile virus in Africa was collected within the mainland sampling sites and Chamaunga Island in Homa Bay County and from Kampi ya Samaki, Salabani and Logumgum sites of Baringo County. This species contained bloodmeal sources from humans and domestic animals and interestingly, also from birds such as Muscovy duck (*Cairina moschata*) and *Corvus sp.* that have the potential of long distance migration, which could increase the risk of West Nile virus transmission (Rappole *et al*., 2000, Rappole and Hubalek, 2003). It is worthwhile noting that West Nile virus was recently isolated in North Eastern Kenya from *Culex sp.* and that in the 2006/2007 Rift Valley fever (RVF) outbreak,

RVF virus was isolated from a single pool of *Cx. univittatus* collected from Baringo (LaBeaud *et al*., 2011, Crabtree *et al*., 2009a). This is a possible indication of *Cx. univittatus* being a competent vector of WNV and RVF virus.

Blood-fed *Ma. africana* and *Ma. uniformis* were widely encountered in samples analyzed from Mbita and Luanda Nyamasare and were the predominant species in Baringo (Table 6). The abundance of *Ma. africana* (37.4%) and *Ma. uniformis* (17.8%) in Baringo observed in this study concurred with an entomological survey that was conducted in 2013 (Lutomiah *et al*., 2013). This abundance is noteworthy considering that both vectors have been implicated in RVF virus transmission in Baringo in 2007 and also detected Bunyamwera virus in *Ma. africana* that had fed on human. The two *Mansonia* species exhibited diverse feeding choices with bloodmeal sources including humans and domesticated animals like goats, sheep, donkeys and dogs. Other sources of *Mansonia* bloodmeals included hosts that could be classified as disease reservoirs such as the baboons (*Papio sp.*), rodents (*Ra. norvegicus*), amphibians (*Pt. nilotica*), crested porcupines (*Hy. cristata*) and wild migratory birds (*Ca. moschata* and *Ar. cinerea*). The diverse choices of blood hosts by these two species could increase the cross-species transmission and potential for Bunyamwera, RVF and possibly other arboviruses and filarial worms in Baringo and Homa Bay. Although findings indicate that the two species of *Mansonia* prefer to feed on domesticated animals over humans, this may be due to indiscriminate feeding patterns, as domestic animals are more common in areas where mosquitoes were sampled and are located outdoors; always susceptible to nocturnal feeding mosquitoes (Beier *et al*., 1990).

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Sindbis virus was detected in *Cx. pipiens* sampled in Kokwa Island that had fed on human and Bunyamwera virus in *Ad. africana* that had fed on cattle, *An. coustani* that had fed on sheep and *Ma. africana* that had fed on man. Although Bunyamwera virus has low pathogenicity in vertebrate hosts (Nichol, 2001), it has the propensity to reassort with other closely related orthobunyavirus leading to increased virulence in man (Eshoo *et al*., 2007, Odhiambo *et al*., 2014). As Bunyamwera virus has been isolated from diverse mosquito vectors, ticks and vertebrate hosts

in East Africa (Gerrard *et al*., 2004, Lwande *et al*., 2013), it appears to have complex transmission cycles that may facilitate the emergence of reassortant viruses (Yanase *et al*., 2006) of public health and veterinary concern.

Blood-fed mosquito species composition differed between sampling areas, probably due to adaptation to specific environmental niches or oviposition sites (Table 6). This was observed in *Mimomyia splendens* that were all sampled in close proximity to a wastewater treatment pond in Mbita and *Ad. africana* that were sampled in Logumgum, close to an oxbow lake in areas with swampy lagoons. *Mansonia sp.* were also found abundantly in these ecological sites and could migrate to homesteads nearby. Occasionally, bloodmeal sources of these groups of mosquitoes were from amphibians (e.g. frogs and toads) with 9/11 (82%) blood-fed *Mm. splendens* mosquitoes having fed on grass frog (*Pt. nilotica*) (Table 7). *Ma. africana, Cx. univittatus, Ae. metalicus* and *An. gambiae* and *Cx. poicilipes* bloodmeals also included African toad (*Bu. regularis*). This kind of feeding could be opportunistic when both amphibians and mosquitoes localize in swampy habitats. Alternatively, such feeding behaviour may be restricted to specific mosquito species as some mosquito repellents have been isolated from frog skin (Williams *et al*., 2006). Mosquitoes may possibly benefit from antimicrobial peptides found in amphibian skin to get rid of micro-organisms in their system (Conlon and Mechkarska, 2014).

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4.2 Molecular detection of tick borne pathogens around Lakes Victoria and Baringo, Kenya

We identified 14 tick species that are potential vectors of ehrlichiosis, anaplasmosis, rickettsiosis, theileriosis and babesiosis in domestic surroundings of Baringo and Homa Bay Counties, which were sampled from livestock or wild hosts near human habitation. Most tick species were taken from multiple host taxa, except for *Hy. truncatum* that was restricted to cattle, *Ar. persicus* that was restricted to poultry, *Am. latum* that was restricted to monitor lizards, and

Am. nuttalli and *Am. falsomarmoreum* that were restricted to tortoises. Overall, we identified 13 TBPs, each from multiple tick species. These TBPs include novel *Ehrlichia* sp., *Rickettsia* sp., *Paracoccus* sp., and *Theileria* sp. sequences that warrant further investigations into their potential pathogenicity. The complex pathogen-tick-host relationships presented here are important to public health in mitigating ticks, TBP transmission and possible disease outbreaks in these foci, as well as other areas of Kenya, with wider geographical implications.

While only one to three TBPs were identified in most tick species, none were identified in *Ar. persicus* sampled from poultry houses, and nine, nine and seven TBPs were identified in *Rh. evertsi evertsi*, *Rh. pulchellus*, and *Am. gemma*, respectively (Tables 4 and 5). Although all 28 pools of *Ar. persicus* were negative for TBP, its large population in poultry houses and frequent blood feeding behavior has been linked with severe anemia, paralysis and toxicosis (Rosenstein 1976). While *Rh. evertsi evertsi* and *Rh. pulchellus* have been widely implicated in TBP transmission (Mediannikov *et al*., 2010, Kumsa *et al*., 2015), *Am. gemma* (Dönitz, 1909) sampled from livestock in both study areas that were once thought to be less important as a disease vector, have been recently linked with the transmission of a number of pathogens (Ngumi *et al*., 1997, Wesonga *et al*., 1993, Burgdorfer *et al*., 1973). Laboratory studies have shown that *Am. gemma* can transmit *E. ruminantium*, the etiological agent of heartwater ehrlichiosis in livestock, from infected African buffalo to sheep (Wesonga *et al*., 2001) and the relevance of the species in transmission and maintenance of arboviruses has been well elucidated in Kenya (Sang *et al*., 2006). Thus more attention should be paid to the role of *Am. gemma* ticks in TBP transmission. Further, more tick species and TBPs were isolated from Baringo County ticks, suggesting more complex and higher transmission rates in the region.

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Among samples collected, *Rh. pravus* (Dönitz, 1910) was the most frequent (44.9% in Baringo County, 34.5% in Homa Bay County) tick parasite of domestic ruminants and dogs, followed by *Rh. evertsi evertsi* (14.7% in Baringo County, 16.6% in Homa Bay County) and *Rh. pulchellus* (12.4% in Baringo County, 19.6% in Homa Bay County), the only three tick species to

also parasitize dogs and transmit agents of canine ehrlichiosis (*E. canis*) and canine anaplasmosis (*A. platys*) in this study. Indeed, we only found *A. platys* and *E. canis* bacterial pathogens in *Rh. pravus* ticks, the latter only in Baringo. This tick species has previously been shown in two studies in Kenya to be the most common among tick species that feeds not only on domestic animals, but also on wildlife (Kariuki *et al*., 2012, Wanzala and Okanga 2006). Although *Rh. pravus* has rarely been implicated in pathogen transmission, these findings potentially incriminate *Rh. pravus* in the transmission of both. Further studies to determine its vector competence will be critical to understanding its role in TBP transmission.

Heartwater ehrlichiosis (*Ehrlichia ruminantium*) is an important disease of wildlife and livestock ruminants in SSA, with considerable economic impact (Mukhebi *et al*., 1999). Unlike past studies, which have found *E. ruminantium* to be specific to *Amblomma* tick species (Trout Fryxell and DeBruyn 2016), we also isolated the pathogen from *Rh. evertsi evertsi* in both study locations, though we cannot rule out that this may have come from livestock bloodmeals. *Amblyomma variegatum* (Fabricius, 1794) is the most common and widely distributed *Amblyomma* tick species of livestock in SSA and is a very important vector of the causative micro-organism of heartwater (*E. ruminantium*) in cattle (Kelly *et al*., 2011). In this study, we detected *E. ruminantium* in 58.9% and 35.3% tick pools of *Am. variegatum* sampled from Baringo and Homa Bay Counties, respectively, suggesting very high infection rates.

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Interestingly, *Am. sparsum* was found on both cattle and tortoises in Baringo and, along with *Am. falsimarmoreum and A. nuttalli,* were infected with *E. ruminatium*, The occurrence of *E. ruminantium* has previously been reported among *Am. sparsum* that were sampled from tortoises imported to the United States from Zambia (Burridge *et al*., 2000). *Ehrlichia ruminantium* was also found in all three *Am. nuttalli* ticks sampled from tortoises. *Amblyomma nuttalli* and *Am. falsomarmoreum* have both been associated with tortoises before (Horak *et al*., 2006, Nowak *et al*., 2010), but surprisingly, no report that we are aware of has associated them with harboring or the transmission of agents of heartwater ehrlichiosis. Similarly, we found *E. canis* in *Am. latum*

ticks taken from monitor lizards in Homa Bay County. These pathogen-tick-host associations suggest complex transmission dynamics in the epidemiology of heartwater in Baringo County and canine ehrlichiosis in Homa Bay County that potentially involve cold-blooded vertebrate hosts.

We found *A. bovis* in multiple species of each of the tick genera sampled (*Rhipecephalus, Amblyomma, Hyalomma*) in Baringo County, but none were identified in Homa Bay. The highest rates of *A. bovis* detection occurred in *Rh. praetextatus* (Gerstäcker, 1873), a three-host tick species that were sampled from cattle and goats, also only in Baringo County. *Rhipicephalus praetextatus* from Ngorongoro crater of Tanzania have been implicated in the transmission of *A. marginale*, which is an agent of bovine anaplasmosis (Fyumagwa *et al*., 2009). Similarly, we found *A. ovis* in both *Rhipicephalus* and *Amblyomma* ticks in Baringo, but only in *Rh. appendiculatus* (60%) in Homa Bay County. In contrast, in Baringo County, where no *Rh. appendiculatus* were sampled, *A. ovis* occurred most frequently in *Am.* variegatum. Curiously, none of the *Am.* variegatum sampled in Homa Bay County tested positive for *A. ovis*. These discrepant *Anaplasma* infection patterns are rarely associated with clinical symptoms in livestock, but may affect livestock health synergistically during coinfections with other livestock disease agents (Thumbi *et al*., 2013; Hornok *et al*, 2007), and thus may affect livestock disease ecology, particularly in Baringo County.

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Sequences of spotted fever group (SFG) Rickettsiae (*R. africae*, *R. aeschlimannii*, *R. montanensis*, *R. rhipicephalii* and a novel *Rickettsia* sp.) were identified in tick samples from Baringo County, whereas no *Rickettsia* were identified in Homa Bay County despite the fact that in neighboring Siaya County, a high prevalence of a *R. africae* variant was previously identified among *Am. variegatum* parasitizing domestic ruminants (Maina *et al*., 2014). As to be expected, *R. rhipicephalii*, as well as the novel *Rickettsia* sp., was confined to rhipicephaline (*Rh. pulchellus* and *Rh. eversti evertsi)* tick species. *Rickettsia africae*, the etiological agent of African tick bite fever, was particularly prevalent in *Am. gemma* (73.68%) and *Am. variegatum* (41.02%) ticks, but was also found in *Rh. evertsi evertsi* (4.67%) and *Rh. pulchellus* (5.17%) tick pools and *R. montanensis* was similarly found in *Am. gemma* (31.57%) and *Rh. evertsi evertsi* (4.76%) tick

pools. While these findings confirm higher prevalences of *R. africae* and *R. montanensis*, within specific *Amblyomma* species, their occurrence, perhaps opportunistic, in *Amblomma* species should be considered in the transmission ecology of these TBPs.

In Baringo County, we predominantly detected *R. aeschlimannii* in both *Hyalomma* species sampled (*Hy. truncatum* and *Hy. marginatum rufipes*) (55.0%) and incidentally in *Rh. pulchellus* (3.44%). In one *Hy. truncatum* (Koch, 1844) tick (smooth bont-legged tick) pool that was sampled from cattle, sheep and goats, we detected both *A. bovis* and *R. aeschlimannii*, Separate pools of *Hy. marginatum rufipes* parasitizing livestock and poultry in poultry houses that were next to livestock sheds were also positive for *A. bovis* and *R. aeschlimannii*. *Rickettsia aeschlimannii* has previously been isolated from *Hy. truncatum* parasitizing camels in the Kano area of Nigeria [9]. Though SFG rickettsiosis caused by this TBP has been rarely reported in sub-Saharan Africa, it has been reported in humans in Algeria (Mokrani *et al*., 2008), and has also been found in *Am. variegatum* ticks in Western Kenya (Maina *et al*., 2014). The likely role of *Hyalomma* ticks as reservoirs for *R. aeschlimannii* (Matsumoto *et al*., 2004), makes these ticks of particular importance in the epidemiology of SFG rickettsiosis in East Africa that warrants further attention.

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Additionally, we found both *H. fitzsimonsi* and *Paracoccus* sp. bacteria ticks *Am. falsomarmoreum* and *Am. sparsum* from tortoises in Baringo. In South Africa, tortoises have recently been documented to harbor concurrent parasitic infection with *H. fitzsimonsi* (Cook *et al*., 2010). *Paracoccus* sp. bacteria, also identified in pools of *Am. variegatum* and *Am. gemma* ticks sampled from Baringo County, was first reported in a population of *Am. cajennense* from South America in 2012, but it is still unknown if *Paracoccus* sp. infection in ticks is a group of pathogenic rhodobacteraceae or simply plays a role in tick physiology (Machado-Ferreira *et al*., 2012). Nonetheless, our findings also demonstrate that primers targeting specific *Ehrlichia* 16S rRNA gene fragment could also be used for detection of *Paracoccus* species. *Paracoccus* sp. and *H. fitzsimonsi*.

Surprisingly, we detected no arboviruses in our detection panel but managed to detect one novel *Theileria* sp. sequence in three *Rh. evertsi evertsi* tick pools and one *B. caballi* sequence in eight *Rh. pulcellus* pools, despite the fact that diverse *Theileria* and *Babesia* (Moumouni *et al*., 2015) species have been found in other surveillance studies in different regions in Kenya, and diverse arboviruses are known to be endemic in the focal areas of this study (Lutomiah *et al*., 2014). Nonetheless, *Rh. appendiculatus* (Neumann, 1901), a vector of *Theileria parva* (Odongo *et al*., 2009), was sampled from livestock of Homa Bay County, but not in Baringo. We only sampled 29 ticks of this species over the wet sampling seasons of 2012 and 2013. This was contrary to a previous study done over two decades ago that found *Rh. appendiculatus* to be highly prevalent among Zebu cattle grazing along the shores of Lake Victoria in Rusinga Island (Punyua *et al*., 1991). This change may be an indication of tick control efforts, most likely with acaricides. Although we detected *A. ovis* in three *Rh. appendiculatus* tick pools, we did not find *T. parva*, indicating a possible absence of *T. parva* or extremely low prevalence within our study sites.

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4.3 Arbovirus occurrence among patients with acute febrile illnesses visiting rural health clinics in Homa Bay County

In this study, three cases of Bunyamwera acute infection in two females and one male and two cases of acute Sindbis infections in each a male and a female were identified. Evidence of Bunyamwera virus infection in Kenya has been a common phenomenon among pastoralist communities in North eastern part Kenya (Lwande *et al*., 2013). For Bunyamwera, The two infected females were an elderly housewife and a farmer and the Sindbis infected female was similarly a housewife. Bunyamwera reacting antibodies have previously been shown to correlate with increase in age (Odhiambo *et al*., 2014) suggesting a possible increase risk of infection with age. Sindbis virus causes febrile arthritis and is present throughout Afro-Eurasia, though little is known of the epidemiology of Sindbis fever due to insufficient surveillance in most endemic

countries although outbreak of Sindbis fever has recently been reported in South Africa (Storm *et al*., 2014). Both Bunyamwera and Sindbis were isolated through inoculation on confluent Vero cells culture and by reverse transcription (RT)-PCR using *Orthobunyavirus* and *Alphavirus* genera primers, respectively, suggesting that these are ongoing infections in Mfangano. Sindbis virus has previously been isolated from mosquitoes in surveillance studies around the Lake Victoria region both in Kenya and Uganda (Woodall *et al*., 1964, Ochieng *et al*., 2013). Mfangano Island is isolated 30 km from the mainland shore, at the time of sampling, the island had limited access due to limited transportation to and from the island, making it a unique habitat with abundant diversity of wildlife including migratory birds that thrive from aquatic organisms and thus could act as reservoir hosts for Sindbis virus (Kurkela *et al*., 2008, Jourdain *et al*., 2007), and possibly Bunyamwera. Additionally, mosquito vectors associated with previous isolations of the two viruses, including *Aedes (Ae.) mcintoshi*, *Ae. ochraceus*, *Culex pipiens*, *Anopheles (An.) gambiae*, *An. phareoensis*, , and *Mn. uniformis* are abundant in along the shores of Lake Victoria (Lutomiah *et al*., 2013) and may likely be transmitters of these viruses (Omondi *et al*., 2015).

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Cultural practices among the Luos and Abasuba communities that inhabit Mfangano Island include polygamy and wife inheritance, which have also perpetuated high transmission of HIV to a prevalence of 33.8% with 80% of hospital beds in the area having been occupied by patients with AIDS related illnesses (NASCOP; 2005). AIDS related immunosuppression could lead to opportunistic infections and prolonged viremia of arboviral infection, thus making it easier to detect by PCR. Even though viremia of these viruses is known to be transient and of low magnitude, Bunyamwera (n=3) and Sindbis (n=2) were isolated by cell culture and PCR-HRM from patients with febrile illness in Mfangano Island. Therefore, this study implicates Bunyamwera and Sindbis viruses as possible etiological causes of febrile illness in Mfangano Island. The estimated seroprevalence indicative of previous exposure to Sindbis virus antibodies was 5.4%. The primary reservoir hosts implicated in maintaining the virus are the birds. The abundant supply of fish provided by the lake ensures a constant presence of both migratory and

native birds which use Rusinga Island as their nesting place, bringing the infection nearer to the humans. A relatively similar prevalence rate in humans of 5.2% was observed in Finland between 1999-2003 (Kurkela *et al*., 2008). The reason(s) why more men than women had been previously exposed to Sindbis virus is not presently apparent though environmental factors and increased interaction between reservoirs, humans and mosquito vectors could have been responsible (Hall *et al*., 2012). During 2007-2012 period, Ochieng and colleagues (2013) reported the presence of Sindbis and Sindbis like viruses in *Culex* and *Culiseta* mosquitoes from Kisumu; a town that is 73 km from Rusinga Island. Their presence is indicative of transmission in this region in suitable environmental and socioeconomic conditions. In their study, they did not find Bunyamwera virus in any of the sampled mosquitoes from this lake-side region. Given that night fishing is popular among inhabitants around the lake, it is possible that that there is higher exposure to biting mosquitoes that could be efficient in the transmission of Bunyamwera and Sindbis viruses.

The limitation of this study was that due to limited resources, examination of the plaque reduction and neutralization assays among study participants in Kisegi and Kitare health clinics as well as examination of flaviviruses and other zoonotic alphaviruses was not accomplished. Only one blind passage was done and there was a possibility of missing out of samples with extremely low viral titer. Although viruses isolating from serum is easier, viruses that are strongly associated with red blood cells may have been missed. Further, whole blood can also cause cell line necrosis, which could potentially confound virus induced cytopathology. Given that all patients in the study sought care for unspecified fevers, the potential etiology of these fevers could be diverse and therefore extensive studies that will highlight all the causes of febrile fevers need to be conducted. The use of very sensitive molecular methods that can detect sub-clinical *Plasmodium* parasitemia, and possibly remnant DNA levels of *Plasmodium* (no live parasites) that have no clinical significance, may lead to false positive diagnoses (Noormahomed *et al*., 2012).

The inability of microscopy to detect low parasitemia consequently led to prescription of wrong treatments among the individuals confirmed to have malaria infection by nested PCR, of

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which 56% (20 out of 36) were treated with antibiotics instead of antimalarials. Misdiagnosis has far reaching effects in terms of development of drug resistance, undesirable side effects, improper patient care and the development of gametocytes (in case of malaria) and hence reservoirs of transmission are maintained (Bousema *et al*., 2004). Proper diagnosis of any disease leads to proper treatment and positive prognosis consequently reducing advancement to severe morbidity or even mortality.

Among the true malaria negative individuals (n=56), prescription of antibiotics was highest with 36% (20), followed by antimalarial medication at 29% (16). A prescription consisting of a combination of the above constituted 27% (15), with a small percentage being treated for amoeba and other intestinal based protozoal infections. A study carried out in Tanzania showed that although a total of 201 slides tested negative for malaria, 22% (44) of these patients were treated with antimalarials alone, 34% (68) with antibiotics alone, 26% (52) on a combination of the two and 18% (37) left the centers with no medication (Reyburn *et al*., 2006). Though medical practitioners are limited to whether to diagnose viral infections due to lack of diagnostic tools (LaBaeud *et al*., 2011), the absence of further bacterial based tests in this study to establish whether the fever is as a result of bacterial infections does not justify why more antibiotics were prescribed to patients. Clear policies and guidelines on the management of non- malaria febrile illnesses in Kenya should be developed and implemented to guide clinicians (Nyandigisi *et al*., 2011). Individuals involved in fishing and farming (outdoor) activities were more exposed to being infected with malaria compared to those who work indoors such as shopkeepers, housewives, carpenters, teachers and students. This can be attributed to the fact that while outdoors and especially in areas that have water (artificially made or naturally present) which are breeding sites for mosquitoes, chances of getting bitten are increased (Imbahale *et al*., 2011). Fishermen usually spend nights out fishing and hence are even more prone to being bitten by mosquitoes.

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Analysis of mosquito bloodmeals using both *cyt* b and 16S r RNA genes demonstrates the improved resolution of HRM-based bloodmeal analysis by using two distinct molecular markers, revealing broad opportunistic host feeding patterns among mosquito vectors in arbovirus endemic regions of Kenya. The diverse vertebrate sources of vector bloodmeals and the presence of arboviruses such as Bunyamwera further demonstrate that many of the vectors identified in this study have the potential to transmit Bunyamwera and other pathogens from potential wildlife reservoirs to livestock that may act as amplifying hosts to humans. Possible commercialization of this powerful method for detection of arboviruses in field collected biological sample would be very useful in the endemic areas for surveillance and diagnostics.

Molecular detection of tick borne pathogens along the shores and adjacent islands of Lakes Baringo and Victoria in Kenya demonstrate possible transmission of TBPs in livestock with undetermined impacts on humans. Tick borne pathogens were isolated among ticks sampled from domesticated animals in both areas, implicating a considerable burden to animal health and a possible transmission to humans. Moreover, this study identified new potential tick vectors and vertebrate reservoirs like *Am. sparsum* and free ranging tortoises as important in the transmission of heartwater disease (*Ehrlichia ruminantium*) in both study areas. Improving the capacity of developing countries to carry out extensive TBPs surveillance will be critical in understanding and mapping novel and existing zoonotic pathogens and thus boosting preparedness to eventual disease outbreaks and vector dynamics factoring in effects of recent climate changes.

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Determination of the occurrence of arboviruses among patients with acute undiagnosed febrile illnesses implicates Bunyamwera and Sindbis in Mfangano Island as possible aetiological agents of these fevers. Therefore, improving surveillance and laboratory capacity for diagnosis of

arboviruses in the region will be integral to early detection of such infections and mitigation of disease outbreaks, which is a priority to public health.

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WESTERN CAPE

REFERENCES

Abbassy M. M., Osman M., Marzouk A. S. (1993).West Nile virus (Flaviviridae: Flavivirus) in experimentally infected *Argas* ticks (Acari: *Argasidae*) *Am J Trop Med Hyg*.48(5):726–737.

Afrane, Y. A., Zhou, G., Githeko, A. K., & Yan, G. (2014). Clinical malaria case definition and malaria attributable fraction in the highlands of western Kenya. *Malar J, 13*, 405. doi: 10.1186/1475-2875-13-405

Alacs EA, Georges A, FitzSimmons NN, Robertson J (2010) DNA detective: a review of molecular approaches to wildlife forensics. *Forensic Sci Med Pathol 6*:180–194 doi:10.1007/s12024-009-9131-7.

Alcaide, M., Rico, C., Ruiz, S., Soriguer, R., Munoz, J., & Figuerola, J. (2009). Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. *PLoS One, 4*(9), e7092. doi: 10.1371/journal.pone.0007092

UNIVERSITY of the
WESTERN CAPE

Alleman AR, McSherry LJ, Barbet AF, Breitschwerdt EB, Sorenson HL, Bowie MV, Belanger M. (2001) Recombinant major antigenic protein 2 of *Ehrlichia canis*: a potential diagnostic tool. J Clin Microbiol.;39(7):2494–2499. doi: 10.1128/JCM.39.7.2494-2499.2001.

Allsopp, M. T., Van Strijp, M. F., Faber, E., Josemans, A. I., & Allsopp, B. A. (2007). *Ehrlichia ruminantium* variants which do not cause heartwater found in South Africa. *Vet Microbiol, 120*(1-2), 158-166. doi: 10.1016/j.vetmic.2006.10.026

Alonso A, Albarran C, Martín P, García P, Capilla J, et al. (2006) Usefulness of microchip electrophoresis for the analysis of mitochondrial DNA in forensic and ancient DNA studies. *Electrophoresis 27*:5101–5109 doi:10.1002/elps.200600331.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol, 215*(3), 403-410. doi: 10.1016/s0022-2836(05)80360-2

Animut, A., Mekonnen, Y., Shimelis, D., & Ephraim, E. (2009). Febrile illnesses of different etiology among outpatients in four health centers in Northwestern Ethiopia. *Jpn J Infect Dis, 62*(2), 107-110.

Anyangu AS, Gould LH, Sharif SK, Nguku PM, Omolo JO, Mutonga D, Rao CY, Lederman ER, Schnabel D, Paweska JT (2010). Risk factors for severe Rift Valley fever infection in Kenya, 2007. *Am. J. Trop. Med. Hyg*. *83*(2 Suppl):14–21. doi: 10.4269/ajtmh.2010.09-0293.

Balenghien T., Vazeille M., Grandadam M. (2008) Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. *Vector-Borne and Zoonotic Dis*. 8(5):589–595. doi: 10.1089/vbz.2007.0266.

Barrett, A. D., & Higgs, S. (2007). Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol, 52*, 209-229. doi: 10.1146/annurev.ento.52.110405.091454

Beall MJ, Alleman AR, Breitschwerdt EB, Cohn LA, Couto CG, Dryden MW, Guptill LC, Iazbik C, Kania SA, Lathan P, Little SE, Roy A, Sayler KA, Stillman BA, Welles EG, Wolfson W, Yabsley MJ (2012). Seroprevalence of *Ehrlichia canis*. *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. *Parasit Vectors*.;5:29. doi: 10.1186/1756-3305-5-29.

Beall MJ, Chandrashekar R, Eberts MD, Cyr KE, Diniz PP, Mainville C, Hegarty BC, Crawford JM, Breitschwerdt EB (2008). Serological and molecular prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia* species in dogs from Minnesota. *Vector Borne Zoonotic Dis*.;8(4):455–464. doi: 10.1089/vbz.2007.0236.

Beatty ME, Letson GW, Margolis HS.(2011). Estimating the global burden of dengue. In Abstract Book: Dengue. The Second International Conference on Dengue and Dengue Haemorrhagic Fever Phuket, Thailand. 2008 (As cited in: Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Trop Med Health*. *39* (4):3–11.

UNIVERSITY of the
WESTERN CAPE

Beier JC, Keating J, Githure JI, Macdonald MB, Impoinvil DE, Novak RJ. (2008). Integrated vector management for malaria control. *Malar J*. 7(Suppl 1):S4. doi: 10.1186/1475-2875-7-S1-S4

Beier, J. C., Odago, W. O., Onyango, F. K., Asiago, C. M., Koech, D. K., & Roberts, C. R. (1990). Relative abundance and blood feeding behavior of nocturnally active culicine mosquitoes in western Kenya. *J Am Mosq Control Assoc, 6*(2), 207-212.

Boakye, D. A., Tang, J., Truc, P., Merriweather, A., & Unnasch, T. R. (1999). Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. *Med Vet Entomol, 13*(3), 282-287.

Boko M.I., Niang A., Nyong C., Vogel A., Githeko M., Medany B., Osman-Elasha R.T., Yanda P. (2007). Chapter 9: Africa. In: Parry M.L., Canziani O.F., Palutikof J.P., van der Linden P.J., Hanson C.E., editors. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press; Cambridge, UK: pp. 433–467

UNIVERSITY of the
WESTERN CAPE

Bousema, J. T., Gouagna, L. C., Drakeley, C. J., Meutstege, A. M., Okech, B. A., Akim, I. N., Beier, J. C., Githure, J. I. & Sauerwein, R. W. (2004). *Plasmodium falciparum* gametocyte carriage in asymptomatic children in western Kenya. *Malar J, 3*, 18. doi: 10.1186/1475-2875-3-18

Braks, M., van der Giessen, J., Kretzschmar, M., van Pelt, W., Scholte, E. J., Reusken, C., Zeller, H., Van Bortel, W. & Sprong, H (2011). Towards an integrated approach in surveillance of vector-borne diseases in Europe. *Parasit Vectors, 4*, 192.

Breitschwerdt EB, Maggi RG (2009). Comparative medical features of canine and human bartonellosis. *Clin Microbiol Infect*.15(Suppl 2):106–107.

Burgdorfer, W., Ormsbee, R. A., Schmidt, M. L., & Hoogstraal, H. (1973). A search for the epidemic typhus agent in Ethiopian ticks. *Bull World Health Organ, 48*(5), 563-569.

Burridge, M. J., Simmons, L. A., Simbi, B. H., Peter, T. F., & Mahan, S. M. (2000). Evidence of *Cowdria ruminantium* infection (heartwater) in *Amblyomma sparsum* ticks found on tortoises imported into Florida. *J Parasitol, 86*(5), 1135-1136. doi: 10.1645/0022-3395(2000)086[1135:eocrih]2.0.co;2

Butler, S. E., Muok, E. M., Montgomery, S. P., Odhiambo, K., Mwinzi, P. M., Secor, W. E., & Karanja, D. M. (2012). Mechanism of anemia in *Schistosoma mansoni*-infected school children in Western Kenya. *Am J Trop Med Hyg, 87*(5), 862-867. doi: 10.4269/ajtmh.2012.12-0248

Caminade C., Ndione J.A., Kebe C.M.F., Jones A.E., Danuor S., Tay S., Tourre Y.M., Lacaux J.P., Vignolles C., Duchemin J.B., Jeanne I., Morse A.P. Mapping Rift Valley fever and malaria risk over west Africa using climatic indicators. Atmos. Sci. Lett. 2011;12:96–103. doi: 10.1002/asl.296.

Campbell-Lendrum, D., Molyneux, D., Amerasinghe, F., Davies, C., Fletcher, E., Schofield, C., Fletcher, E., Schofield, C., Hougard, J., Polson, K. & Sinkins, S (2005). Ecosystems and vector-borne disease control. *Ecosystems and human well-being: policy responses, 2*, 353-372.

Cantas, L., & Suer, K. (2014). Review: The Important Bacterial Zoonoses in "One Health" Concept. *Front Public Health, 2*, 1-14

Centers for Disease Control and Prevention. Entomology. 2009, http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm

Chanda, E., J. M. Govere, M. B. Macdonald, R. L. Lako, U. Haque, S. P. Baba, and A. Mnzava. (2013). Integrated vector management: a critical strategy for combating vector-borne diseases in South Sudan. Malar J 12: 369.

Chandrashekar R, Mainville CA, Beall MJ, O'Connor T, Eberts MD, Alleman AR, Gaunt SD, Breitschwerdt EB. Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia*

UNIVERSITY of the
WESTERN CAPE

burgdorferi and *Dirofilaria immitis* antigen in dogs. Am J Vet Res. 2010;71(12):1443–1450. doi: 10.2460/ajvr.71.12.1443.

Cherry NA, Maggi RG, Rossmeisl JH, Hegarty BC, Breitschwerdt EB. (2011). Ecological diversity of *Bartonella* species infection among dogs and their owner in Virginia. *Vector Borne Zoonotic Dis.*11(11):1425–1432. doi: 10.1089/vbz.2010.0201.

Coleman, R. (1998). Disease burden in sub-Saharan Africa. *Lancet, 351*(9110), 1208. doi: 10.1016/s0140-6736(05)79158-3

Coluzzi M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bull World Health Organ.62*:107–113.

Conlon, J. M., & Mechkarska, M. (2014). Host-defense peptides with therapeutic potential from skin secretions of frogs from the family pipidae. *Pharmaceuticals (Basel), 7*(1), 58-77. doi: 10.3390/ph7010058

Cook, C. A., Smit, N. J., & Davies, A. J. (2010). Hemoproteids (Apicomplexa: Haemoproteidae) from South African tortoises (Cryptodira: Testudinidae). *J Parasitol, 96*(6), 1168-1172. doi: 10.1645/ge-2527.1

UNIVERSITY of the
WESTERN CAPE

Crabtree, M., Sang, R., Lutomiah, J., Richardson, J., & Miller, B. (2009). Arbovirus surveillance of mosquitoes collected at sites of active Rift Valley fever virus transmission: Kenya, 2006-2007. *J Med Entomol, 46*(4), 961-964.

Crabtree, M. B., Sang, R., & Miller, B. R. (2009). Kupe virus, a new virus in the family Bunyaviridae, genus Nairovirus, Kenya. *Emerg Infect Dis, 15*(2), 147-154.

Crowder, C. D., Rounds, M. A., Phillipson, C. A., Picuri, J. M., Matthews, H. E., Halverson, J., Schutzer,S. E., Ecker, D. J. & Eshoo, M. W. (2010). Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. *J Med Entomol, 47*(1), 89-94.

Crump, J. A., Morrissey, A. B., Nicholson, W. L., Massung, R. F., Stoddard, R. A., Galloway, R. L., Ooi, E. E., Maro, V. P., Saganda, W., Kinabo, G. D., Muiruri, C. & Bartlett, J. A. (2013).

Etiology of severe non-malaria febrile illness in northern Tanzania: A prospective cohort study. *PLoS Negl Trop Dis, 7*(7), e2324. doi: 10.1371/journal.pntd.0002324

Da Costa PS, Brigatte ME, Greco DB (2005) . Antibodies to *Rickettsia rickettsii*, *Rickettsia typhi*, *Coxiella burnetii*, *Bartonella henselae*, *Bartonella quintana*, and *Ehrlichia chaffeensis* among healthy population in Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz*. 100(8):853–859. doi: 10.1590/S0074-02762005000800006.

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop, 78*(2), 103-116.

Davies, F. G. (1982). Karai virus, a probable arbovirus isolated from sheep and from the tick *Rhipicephalus evertsi* in Kenya. *J Comp Pathol, 92*(1), 9-14.

Davis, S. M., Wiegand, R. E., Mulama, F., Kareko, E. I., Harris, R., Ochola, E., Samuels, A. M., Rawago, F., Mwinzi, P. M., Fox, L. M., Odiere, M. R. & Won, K. Y. (2015). Morbidity associated with schistosomiasis before and after treatment in young children in Rusinga Island, western Kenya. *Am J Trop Med Hyg, 92*(5), 952-958. doi: 10.4269/ajtmh.14-0346

De la Fuente, J., Kocan, K. M., Almazan, C., & Blouin, E. F. (2008). Targeting the tick-pathogen interface for novel control strategies. *Front Biosci, 13*, 6947-6956.

Desquesnes, M., Dargantes, A., Lai, D. H., Lun, Z. R., Holzmuller, P., & Jittapalapong, S. (2013). *Trypanosoma evansi* and Surra: A review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. *Biomed Res Int, 1,* 13-20.

Devaux, C. A. (2012). Emerging and re-emerging viruses: A global challenge illustrated by Chikungunya virus outbreaks. *World J Virol, 1*(1), 11-22. doi: 10.5501/wjv.v1.i1.11

Diallo M, Lochouarn L, Ba K, Sall AA, Mondo M, Girault L, Mathiot C. (2000). First isolation of the Rift Valley fever virus from *Culex poicilipes* (Diptera: Culicidae) in nature. *Am J Trop Med Hyg*. 62(6):702–4.

Diniz PP, Maggi RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty B, Breitschwerdt EB. (2007). Canine bartonellosis: serological and molecular prevalence in Brazil and evidence

UNIVERSITY of the
WESTERN CAPE

of co-infection with *Bartonella henselae* and *Bartonella vinsonii* subsp. *berkhoffii*. *Vet Res*. 38(5):697–710. doi: 10.1051/vetres:2007023.

Dunster, L., Dunster, M., Ofula, V., Beti, D., Kazooba-Voskamp, F., Burt, F., Burt, F., Swanepoel, R. & DeCock, K. M. (2002). First documentation of human Crimean-Congo hemorrhagic fever, Kenya. *Emerg Infect Dis, 8*(9), 1005-1006. doi: 10.3201/eid0809.010510

Dworkin, S. L., Grabe, S., Lu, T., Hatcher, A. M., Kwena, Z., Bukusi, E., & Mwaura-Muiru, E. (2013). Property rights violations as a structural driver of women's HIV risks: a qualitative study in Nyanza and western provinces, Kenya. *Arch Sex Behav, 42*(5), 703-713. doi: 10.1007/s10508-012-0024-6

Ecker DJ, Massire C, Blyn LB, Hofstadler SA, Hannis JC, Eshoo MW, Hall TA, Sampath R. (2009).Molecular genotyping of microbes by multilocus PCR and mass spectrometry: a new tool for hospital infection control and public health surveillance. *Methods Mol Biol*.;551:71–87. doi: 10.1007/978-1-60327-999-4_7.

Ecker DJ, Sampath R, Massire C, Blyn LB, Hall TA, Eshoo MW, Hofstadler SA. (2008). Ibis T5000: a universal biosensor approach for microbiology. *Nat Rev Microbiol*. 6(7):553–558. doi: 10.1038/nrmicro1918.

UNIVERSITY of the
WESTERN CAPE

Edwards. (1941). *Mosquitoes of the Ethiopian region III. Culicine adults and pupae.* London, UK: : British Museum (Nat. Hist.). pp 1-515

Ellis, B. R., & Wilcox, B. A. (2009). The ecological dimensions of vector-borne disease research and control. *Cadernos de Saúde Pública, 25*, S155-S167.

Eshoo, M. W., Whitehouse, C. A., Zoll, S. T., Massire, C., Pennella, T. T., Blyn, L. B., Sampath, R., Hall, T. A., Ecker, J. A., Desai, A., Wasieloski, L. P., Li, F., Turell, M. J., Schink, A., Rudnick, K., Otero, G., Weaver, S. C., Ludwig, G. V., Hofstadler, S. A. & Ecker, D. J. (2007). Direct broad-range detection of Alphaviruses in mosquito extracts. *Virology, 368*(2), 286-295. doi: 10.1016/j.virol.2007.06.016

Fontenille D, Simard F. (2004). Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. *Comp Immunol Microbiol Infect Dis.*;27:357–375. doi: 10.1016/j.cimid.2004.03.005.

Fontenille D, Traore-Lamizana M, Diallo M, Thonnon J, Digoutte J, Zeller H. (1998). New vectors of Rift Valley fever in West Africa. *Emerg Infect Dis*. 4(2):289–93. doi: 10.3201/eid0402.980218.

Fontenille D, Traore-Lamizana M, Trouillet J, Leclerc A, Mondo M, Ba Y, Digoutte JP, Zeller HG. (1994). First isolations of arboviruses from phlebotomine sand flies in West Africa. *Am J Trop Med Hyg*. 50(5):570–4.

Formosinho P., Santos-Silva M. M. (2006). Experimental infection of *Hyalomma marginatum* ticks with West Nile virus. *Acta Virologica*. *50*(3):175–180.

Fornadel, C. M., Norris, L. C., Franco, V., & Norris, D. E. (2011). Unexpected anthropophily in the potential secondary malaria vectors *Anopheles coustani* s.l. and *Anopheles squamosus* in Macha, Zambia. *Vector Borne Zoonotic Dis, 11*(8), 1173-1179. doi: 10.1089/vbz.2010.0082

UNIVERSITY of the
WESTERN CAPE

Fornadel, C. M., Norris, L. C., Glass, G. E., & Norris, D. E. (2010). Analysis of *Anopheles arabiensis* blood feeding behavior in southern Zambia during the two years after introduction of insecticide-treated bed nets. *Am J Trop Med Hyg, 83*(4), 848-853. doi: 10.4269/ajtmh.2010.10-0242

Fyumagwa, R. D., Simmler, P., Meli, M. L., Hoare, R., Hofmann-Lehmann, R., & Lutz, H. (2009). Prevalence of *Anaplasma marginale* in different tick species from Ngorongoro Crater, Tanzania. *Vet Parasitol, 161*(1-2), 154-157. doi: 10.1016/j.vetpar.2008.12.018

Garcia-Rejon, J. E., Blitvich, B. J., Farfan-Ale, J. A., Lorono-Pino, M. A., Chi Chim, W. A., Flores-Flores, L. F., Rosado-Paredes, E., Baak-Baak, C., Perez-Mutul, J., Suarez-Solis, V., Fernandez-Salas, L. & Beaty, B. J. (2010). Host-feeding preference of the mosquito, *Culex*

quinquefasciatus, in Yucatan State, Mexico. *J Insect Sci, 10*, 32. doi: 10.1673/031.010.3201

Gerrard, S. R., Li, L., Barrett, A. D., & Nichol, S. T. (2004). Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. *J Virol, 78*(16), 8922-8926.

Gillies MT, Coetzee M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara. Vol. 55. Johannesburg: The South African Institute for Medical Research.

Gillies MT, de Meillon B. (1968). The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region) Vol. 54. Johannesburg: The South African Institute for Medical Research

Gray, S. M., & Banerjee, N. (1999). Mechanisms of arthropod transmission of plant and animal viruses. *Microbiol Mol Biol Rev, 63*(1), 128-148.

Greenberg, J. A., DiMenna, M. A., Hanelt, B., & Hofkin, B. V. (2012). Analysis of post-blood meal flight distances in mosquitoes utilizing zoo animal blood meals. *J Vector Ecol, 37*(1), 83-89. doi: 10.1111/j.1948-7134.2012.00203.x

UNIVERSITY of the
WESTERN CAPE

Greenberg, J. A., Lujan, D. A., DiMenna, M. A., Wearing, H. J., & Hofkin, B. V. (2013). Identification of blood meal sources in *Aedes vexans* and *Culex quinquefasciatus* in Bernalillo County, New Mexico. *J Insect Sci, 13*, 75. doi: 10.1673/031.013.7501

Gubbels, J. M., de Vos, A. P., van der Weide, M., Viseras, J., Schouls, L. M., de Vries, E., & Jongejan, F. (1999). Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J Clin Microbiol, 37*(6), 1782-1789.

Gubler, D. J. (2001). Human arbovirus infections worldwide. *Ann N Y Acad Sci, 951*, 13-24.

Gubler, D. J. (2002). The global emergence/resurgence of arboviral diseases as public health problems. *Arch Med Res, 33*(4), 330-342.

Gubler, D. J. (2009). Vector-borne diseases. *Rev Sci Tech, 28*(2), 583-588.

Haddow, A. J., Davies, C. W., & Walker, A. J. (1960). O'nyong-nyong fever: An epidemic virus disease in East Africa. *Trans R Soc Trop Med and Hyg, 54*(6), 517-522. doi: 10.1016/0035-9203(60)90025-0

Hall, R. A., Blitvich, B. J., Johansen, C. A., & Blacksell, S. D. (2012). Advances in arbovirus surveillance, detection and diagnosis. *J Biomed Biotechnol, 2012*, 512969. doi: 10.1155/2012/512969

Hamer, G. L., Kitron, U. D., Goldberg, T. L., Brawn, J. D., Loss, S. R., Ruiz, M. O., Hayes, D. B. & Walker, E. D. (2009). Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg, 80*(2), 268-278.

Harbach RE. (2004). The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bull Entomol Res*. 94:537–553. doi: 10.1079/BER2004321.

Hegarty BC, Bradley JM, Lappin MR, Balakrishnan N, Mascarelli PE, Breitschwerdt EB. (2014). Analysis of Seroreactivity against Cell Culture-Derived *Bartonella* spp. Antigens in Dogs. *J Vet Intern Med*.;28(1):38–41. doi: 10.1111/jvim.12263.

Henderson, B. E., Kirya, G. B., & Hewitt, L. E. (1970). Serological survey for arboviruses in Uganda, 1967-69. *Bull World Health Organ, 42*(5), 797-805.

Holmes, E. C. (2003). Error thresholds and the constraints to RNA virus evolution. *Trends Microbiol, 11*(12), 543-546.

Horak, I. G., McKay, I. J., Henen, B. T., Heyne, H., Hofmeyr, M. D., & De Villiers, A. L. (2006). Parasites of domestic and wild animals in South Africa. XLVII. Ticks of tortoises and other reptiles. *Onderstepoort J Vet Res, 73*(3), 215-227.

Hornok, S., R. Edelhofer, G. Foldvari, A. Joachim, and R. Farkas. (2007). Serological evidence for *Babesia canis* infection of horses and an endemic focus of *B. caballi* in Hungary. *Acta veterinaria Hungarica 55*: 491-500.

UNIVERSITY of the
WESTERN CAPE

Hunt RH, Coetzee M, Fettene M. (1998). The *Anopheles gambiae* complex: a new species from Ethiopia. *Trans R Soc Trop Med Hyg*. 92:231–235. doi: 10.1016/S0035-9203(98)90761-1.

Hutcheson H. J., Gorham C. H., Machain-Williams C. (2005). Experimental transmission of West Nile virus (Flaviviridae: Flavivirus) by *Carios capensis* ticks from North America. *Vector-Borne and Zoonotic Dis.5*(*3*):293–295. doi: 10.1089/vbz.2005.5.293.

Imbahale, S. S., Paaijmans, K. P., Mukabana, W. R., van Lammeren, R., Githeko, A. K., & Takken, W. (2011). A longitudinal study on *Anopheles* mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malar J, 10*, 81. doi: 10.1186/1475-2875-10-81

Jentes, E. S., Robinson, J., Johnson, B. W., Conde, I., Sakouvougui, Y., Iverson, J., Beecher, S., Bah, M. A., Diakite, F., Coulibaly, M. & Bausch, D. G. (2010). Acute arboviral infections in Guinea, West Africa, 2006. *Am J Trop Med Hyg, 83*(2), 388-394.

Jourdain, E., Gauthier-Clerc, M., Bicout, D., & Sabatier, P. (2007). Bird migration routes and risk for pathogen dispersion into western mediterranean wetlands. *Emerg Infect Dis, 13*(3), 365-372.

UNIVERSITY of the
WESTERN CAPE

Jupp P. G. (1974). Laboratory studies on the transmission of West Nile virus by *Culex* (*Culex*) *univittatus* Theobald; factors influencing the transmission rate. *J Med Entomol*. *11*(4):455–458.

Kamani, J., Baneth, G., Apanaskevich, D. A., Mumcuoglu, K. Y., & Harrus, S. (2015). Molecular detection of *Rickettsia aeschlimannii* in *Hyalomma spp*. ticks from camels (*Camelus dromedarius*) in Nigeria, West Africa. *Med Vet Entomol, 29*(2), 205-209. doi: 10.1111/mve.12094

Kariuki, E. K., Penzhorn, B. L., & Horak, I. G. (2012). Ticks (Acari: Ixodidae) infesting cattle and african buffaloes in the Tsavo conservation area, Kenya. *Onderstepoort J Vet Res, 79*(1), E1-4. doi: 10.4102/ojvr.v79i1.437

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acids Res, 30*(14), 3059-3066.

Kawada, H., Dida, G. O., Ohashi, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Maekawa, Y., Mwatele, C., Njenga, S. M., Mwandawiro, C., Minakawa, N. & Takagi, M. (2011). Multimodal pyrethroid resistance in malaria vectors, *Anopheles gambiae* "s.s"., *Anopheles arabiensis*, and *Anopheles funestus* "s.s". in western Kenya. *PLoS One, 6*(8), e22574. doi: 10.1371/journal.pone.0022574

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647-1649.

Kelly, P. J., Lucas, H., Yowell, C., Beati, L., Dame, J., Urdaz-Rodriguez, J., & Mahan, S. (2011). *Ehrlichia ruminantium* in *Amblyomma variegatum* and domestic ruminants in the Caribbean. *J Med Entomol, 48*(2), 485-488.

UNIVERSITY of the
WESTERN CAPE

Kiara, H., Jennings, A., Bronsvoort, B., Handel, I. G., Mwangi, S. T., Mbole-Kariuki, M., Conradie Van Wyk, I., Poole, E. J., Hanotte, O., Coetzer, J. A. W., Woolhouse, M. E. J. & Toye, P. G. (2014). A longitudinal assessment of the serological response to *Theileria parva* and other tick-borne parasites from birth to one year in a cohort of indigenous calves in western Kenya. *Parasitology, 141*(10), 1289-1298.

Kilpatrick, A. M., Meola, M. A., Moudy, R. M., & Kramer, L. D. (2008). Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathog, 4*(6), e1000092. doi: 10.1371/journal.ppat.1000092

Kilpatrick, A. M., & Randolph, S. E. (2012). Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. *Lancet, 380*(9857), 1946-1955.

Kipanga, P. N., Omondi, D., Mireji, P. O., Sawa, P., Masiga, D. K., & Villinger, J. (2014). High-resolution melting analysis reveals low *Plasmodium* parasitaemia infections among

microscopically negative febrile patients in western Kenya. *Malar J, 13*, 429. doi: 10.1186/1475-2875-13-429

Knobel, D. L., Maina, A. N., Cutler, S. J., Ogola, E., Feikin, D. R., Junghae, M., Halliday, J. E., Richards, A. L., Breiman, R. F., Cleaveland, S. & Njenga, M. K. (2013). *Coxiella burnetii* in humans, domestic ruminants, and ticks in rural western Kenya. *Am J Trop Med Hyg, 88*(3), 513-518. doi: 10.4269/ajtmh.12-0169

Knobloch J, Solano L, Alvarez O, Delgado E (1985). Antibodies to *Bartonella bacilliformis* as determined by fluorescence antibody test, indirect haemagglutination and ELISA. *Trop Med Parasitol*.;36(4):183–185.

Konishi, E. (1989). Size of blood meals of *Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae) feeding on an unrestrained dog infected with *Dirofilaria immitis* (Spirurida: Filariidae). *J Med Entomol, 26*(6), 535-538.

Kumsa, B., Signorini, M., Teshale, S., Tessarin, C., Duguma, R., Ayana, D., Martini, M. & Cassini, R. (2014). Molecular detection of piroplasms in ixodid ticks infesting cattle and sheep in western Oromia, Ethiopia. *Trop Anim Health Prod, 46*(1), 27-31. doi: 10.1007/s11250-013-0442-z

Kumsa, B., Socolovschi, L. Almeras, D. Raoult, and P. Parola. (2015). Occurrence and Genotyping of *Coxiella burnetii* in Ixodid Ticks in Oromia, Ethiopia. *Am J Trop Med Hyg 93*: 1074-1081.

Kuno, G., & Chang, G. J. J. (2005). Biological Transmission of Arboviruses: Reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. *Clin Microbiol Rev, 18*(4), 608-637.

Kurkela, S., Rätti, O., Huhtamo, E., Uzcátegui, N. Y., Nuorti, J. P., Laakkonen, J., Manni, T., Helle, P., Vaheri, A. & Vapalahti, O. (2008). Sindbis virus infection in resident birds, migratory birds, and humans, Finland. *Emerg Infect Dis, 14*(1), 41-47.

UNIVERSITY of the
WESTERN CAPE

Kyle JL, Harris E. (2008). Global spread and persistence of dengue. *Ann Rev Microbiol*. *62*:71–92. doi: 10.1146/annurev.micro.62.081307.163005.

LaBeaud, A. D., Sutherland, L. J., Muiruri, S., Muchiri, E. M., Gray, L. R., Zimmerman, P. A., Hise, A. & King, C. H. (2011). Arbovirus prevalence in mosquitoes, Kenya. *Emerg Infect Dis, 17*(2), 233-241. doi: 10.3201/eid1702.091666

Lambert, A. J., & Lanciotti, R. S. (2009). Consensus amplification and novel multiplex sequencing method for S segment species identification of 47 viruses of the Orthobunyavirus, Phlebovirus, and Nairovirus genera of the family Bunyaviridae. *J Clin Microbiol, 47*(8), 2398-2404. doi: 10.1128/jcm.00182-09

Lawrie C. H., Uzcátegui N. Y., Gould E. A., Nuttall P. A. (2004). Ixodid and argasid tick species and west nile virus. *Emerg Infect Dis*. *10*(4):653–657. doi: 10.3201/eid1004.030517

Lengeler C. (2004). Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database *Syst Rev*.;2:CD000363.

Lewis A, P. D. B. R. (1989). A bird atlas of Kenya. CRC press, Taylor & Fransis group, pp. 56

Linthicum K.J., Anyamba A., Tucker C.J., Kelley P.W., Myers M.F., Peters C.J. (1999). Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science*. 285:397–400. doi: 10.1126/science.285.5426.397.

Linthicum K,J Davies F, Bailey C, Kairo A. (1984). Mosquito species encountered in a flooded grassland dambo in Kenya. *Mosq News*. 44(2):228–32.

Linthicum K, Davies F, Kairo A, Bailey C. (1985). Rift Valley fever virus (family Bunyaviridae, genus Phlebovirus): isolations from Diptera collected during an inter-epizootic period in Kenya. *J Hyg*. 95(01):197–209. doi: 10.1017/S0022172400062434.

Lutomiah, J., Bast, J., Clark, J., Richardson, J., Yalwala, S., Oullo, D., Mutisya, J., Mulwa, F., Musila, L., Khamadi, S., Schnabel, D., Wurapa, E. & Sang, R. (2013). Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya:

UNIVERSITY of the
WESTERN CAPE

public health implications. *J Vector Ecol, 38*(1), 134-142. doi: 10.1111/j.1948-7134.2013.12019.x

Lutomiah, J., Musila, L., Makio, A., Ochieng, C., Koka, H., Chepkorir, E., Mutisya, J., Mulwa, F., Khamadi, S., Miller, B. R., Bast, J., Schnabel, D., Wurapa, E. K. & Sang, R. (2014a). Ticks and tick-borne viruses from livestock hosts in arid and semiarid regions of the eastern and northeastern parts of Kenya. *J Med Entomol,* 51(1), 269-277.

Lutomiah, J., Omondi, D., Masiga, D., Mutai, C., Mireji, P. O., Ongus, J., Linthicum, K. J. & Sang, R. (2014b). Blood meal analysis and virus detection in blood-fed mosquitoes collected during the 2006-2007 Rift Valley fever outbreak in Kenya. *Vector Borne Zoonotic Dis, 14*(9), 656-664. doi: 10.1089/vbz.2013.1564

Lwande, O. W., Lutomiah, J., Obanda, V., Gakuya, F., Mutisya, J., Mulwa, F., Michuki, G., Chepkorir, E., Fischer, A., Venter, M. & Sang, R. (2013). Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. *Vector Borne Zoonotic Dis, 13*(9), 637-642.

Lwande, O. W., Venter, M., Lutomiah, J., Michuki, G., Rumberia, C., Gakuya, F., Obanda, V., Tigoi, C., Odhiambo, C., Nindo, F., Symekher, S. & Sang, R. (2014). Whole genome phylogenetic investigation of a West Nile virus strain isolated from a tick sampled from livestock in north eastern Kenya. *Parasit Vectors, 7*, 542. doi: 10.1186/s13071-014-0542-2

Mableson, H. E., Okello, A., Picozzi, K., & Welburn, S. C. (2014). Neglected zoonotic diseases-the long and winding road to advocacy. *PLoS Negl Trop Dis, 8*(6), e2800. doi: 10.1371/journal.pntd.0002800

Machado-Ferreira, E., Piesman, J., Zeidner, N. S., & Soares, C. A. (2012). A prevalent alpha-proteobacterium *Paracoccus* sp. in a population of the Cayenne ticks (*Amblyomma cajennense*) from Rio de Janeiro, Brazil. *Genet Mol Biol, 35*(4), 862-867. doi: 10.1590/s1415-47572012005000067

UNIVERSITY of the
WESTERN CAPE

Mackenzie J. S., Lindsay M. D., Coelen R. J., Broom A. K., Hall R. A., Smith D. W. (1994). Arboviruses causing human disease in the Australasian zoogeographic region. *Arch Virol*. 136(3-4):447–467. doi: 10.1007/BF01321074

Maggi RG, Breitschwerdt EB (2005). Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. *J Clin Microbiol*. 43(3):1171–1176. doi: 10.1128/JCM.43.3.1171-1176.2005.

Maggi RG, Duncan AW, Breitschwerdt EB. (2005). Novel chemically modified liquid medium that will support the growth of seven *Bartonella* species. *J Clin Microbiol*. 43(6):2651–2655. doi: 10.1128/JCM.43.6.2651-2655.2005.

Maina, A. N., Jiang, J., Omulo, S. A., Cutler, S. J., Ade, F., Ogola, E., Feikin, D. R., Njenga, M. K., Cleaveland, S., Mpoke, S., Ng'ang'a, Z., Breiman, R. F., Knobel, D. L & Richards, A. L. (2014). High prevalence of *Rickettsia africae* variants in *Amblyomma variegatum* ticks from domestic mammals in rural western Kenya: implications for human health. *Vector Borne Zoonotic Dis, 14*(10), 693-702. doi: 10.1089/vbz.2014.1578

Maina, A. N., Knobel, D. L., Jiang, J., Halliday, J., Feikin, D. R., Cleaveland, S., Ng'ang'a, Z., Junghae, M., Breiman, R. F., Richards, A. L. & Njenga, M. K (2012). *Rickettsia felis* infection in febrile patients, western Kenya, 2007-2010. *Emerg Infect Dis, 18*(2), 328-331. doi: 10.3201/eid1802.111372

Mala, A. O., Irungu, L. W., Shililu, J. I., Muturi, E. J., Mbogo, C. M., Njagi, J. K., Mukabana, W. R. & Githure, J. I. (2011). *Plasmodium falciparum* transmission and aridity: a Kenyan experience from the dry lands of Baringo and its implications for *Anopheles arabiensis* control. *Malar J, 10*, 121. doi: 10.1186/1475-2875-10-121

Mangold KA, Manson RU, Koay ESC, Stephens L, Regner MA, Thomson RB, Jr, Peterson LR, Kaul KL. (2005). Real-time PCR for detection and identification of *Plasmodium* spp. J *Clin Microbiol*. *43*:2435–2440. doi: 10.1128/JCM.43.5.2435-2440.2005.

UNIVERSITY of the
WESTERN CAPE

Mann P, Nye F, Williams G, Walker A, Amadi (2003). A. From trench fever to endocarditis. *Postgrad Med J*. 79(937):655–656. doi: 10.1136/pmj.79.937.655.

Marks, J. (1986). BMA annual report of council 1985-6. *Br Med J* (*Clin Res Ed*), 292(6524), 1-42.

Matsumoto, K., P. Parola, P. Brouqui, and D. Raoult. (2004). *Rickettsia aeschlimannii* in *Hyalomma* ticks from Corsica. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology 23: 732-734.

Mattingly PF. (1957). Genetical aspects of the *Aedes aegypti* problem I. Taxonomy and bionomics. *Ann. Trop. Med. Parasit*. *51*:392–408.

Maudlin, I., Eisler, M. C., & Welburn, S. C. (2009). Neglected and endemic zoonoses. *Philos Trans R Soc Lond B Biol Sci, 364*(1530), 2777-2787.

McIntosh B, Jupp P. (1981). Proceedings Rift Valley fever workshop. Epidemiological aspects of Rift Valley fever in South Africa with reference to vectors; pp. 92–9.

Mease, L. E., Coldren, R. L., Musila, L. A., Prosser, T., Ogolla, F., Ofula, V. O., Schoepp, R. J., Rossi, C. A. & Adungo, N. (2011). Seroprevalence and distribution of arboviral infections among rural Kenyan adults: A cross-sectional study. *Virol J, 8*, 371-378.

Mediannikov, O., G. Diatta, F. Fenollar, C. Sokhna, J. F. Trape, and D. Raoult. (2010). Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl Trop Dis 4*. 17

Medlock, J., & Jameson, L. (2010). Ecological approaches to informing public health policy and risk assessments on emerging vector-borne zoonoses. *Emerg Health Threats J, 3,* 46-53.

Metzker, M. L. (2005). Emerging technologies in DNA sequencing. *Genome Res, 15*(12), 1767-1776. doi: 10.1101/gr.3770505

Minakawa, N., Dida, G. O., Sonye, G. O., Futami, K., & Njenga, S. M. (2012). Malaria vectors in Lake Victoria and adjacent habitats in western Kenya. *PLoS One, 7*, 3-16.

UNIVERSITY of the
WESTERN CAPE

Mokrani, N., P. Parola, S. Tebbal, M. Dalichaouche, A. Aouati, and D. Raoult. (2008). *Rickettsia aeschlimannii* infection, Algeria. *Emerg. Infect. Dis. 14*: 1814-1815.

Montgomery J, Wittwer CT, Palais R, Zhou L (2007) Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. *Nat Protoc 2*:59–66 doi:10.1038/nprot.2007.10

Moudy, R. M., Meola, M. A., Morin, L. L., Ebel, G. D., & Kramer, L. D. (2007). A newly emergent genotype of West Nile virus is transmitted earlier and more efficiently by *Culex* mosquitoes. *Am J Trop Med Hyg, 77*(2), 365-370.

Moumouni, P. F., G. O. Aboge, M. A. Terkawi, T. Masatani, S. Cao, K. Kamyingkird, C. Jirapattharasate, M. Zhou, G. Wang, M. Liu, A. Iguchi, P. Vudriko, A. P. Ybanez, H. Inokuma, R. Shirafuji-Umemiya, H. Suzuki, and X. Xuan. (2015). Molecular detection and characterization of *Babesia bovis*, *Babesia bigemina*, *Theileria* species and *Anaplasma marginale* isolated from cattle in Kenya. *Parasit Vectors 8*: 496.

Mukabana, W. R., Kannady, K., Kiama, G. M., Ijumba, J. N., Mathenge, E. M., Kiche, I., Nkwengulila, G., Mboera, L., Mtasiwa, D., Yamagata, Y., Van Schayk, I., Knols, B. G., Lindsay, S. W., Caldas De Castro, M., Mshinda, H., Tanner, M., Fillinger, U. & Killeen, G. F. (2006). Ecologists can enable communities to implement malaria vector control in Africa. *Malar J, 5*, 9. doi: 10.1186/1475-2875-5-9

Mukhebi, A. W., T. Chamboko, C. J. O'Callaghan, T. F. Peter, R. L. Kruska, G. F. Medley, S. M. Mahan, and B. D. Perry. (1999). An assessment of the economic impact of heartwater (*Cowdria ruminantium* infection) and its control in Zimbabwe. *Prev. Vet. Med. 39*: 173-189.

Muñoz J., Ruiz S., Soriguer R. (2012). Feeding patterns of potential West Nile virus vectors in South-West Spain. *PLoS ONE.*;7 doi: 10.1371/journal.pone.0039549.

Munyua, P., Murithi, R. M., Wainwright, S., Githinji, J., Hightower, A., Mutonga, D., Macharia, J., Ithondeka, P. M., Musaa, J., Breiman, R. F., Bloland, P. & Njenga, M. K (2010). Rift

UNIVERSITY of the
WESTERN CAPE

Valley fever outbreak in livestock in Kenya, 2006-2007. *Am J Trop Med Hyg, 83*(2 Suppl), 58-64. doi: 10.4269/ajtmh.2010.09-0292

Musuva, R. M., Awiti, A., Omedo, M., Ogutu, M., Secor, W. E., Montgomery, S. P., Alaii, J. & Mwinzi, P. N. M. (2014). Community knowledge, attitudes and practices on schistosomiasis in western Kenya-The SCORE project. *Am J Trop Med Hyg, 90*(4), 646-652.

Muturi, C. N., Ouma, J. O., Malele, II, Ngure, R. M., Rutto, J. J., Mithofer, K. M., Enyaru, J. & Masiga, D. K. (2011). Tracking the feeding patterns of tsetse flies (*Glossina* genus) by analysis of bloodmeals using mitochondrial cytochromes genes. *PLoS One, 6*(2), e17284. doi: 10.1371/journal.pone.0017284

Mwangangi, J. M., Mbogo, C. M., Orindi, B. O., Muturi, E. J., Midega, J. T., Nzovu, J., Gatakaa, H., Githure, J., Borgemeister, C., Keating, J. & Beier, J. C. (2013). Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. *Malar J, 12*, 13-13. doi: 10.1186/1475-2875-12-13

National AIDS and STI Control Programme, Ministry of Health, Kenya. AIDS in Kenya, 7th ed. Nairobi: NASCOP; 2005 pp 1-77

Ngumi, P. N., Rumberia, R. M., Williamson, S. M., Sumption, K. J., Lesan, A. C., & Kariuki, D. P. (1997). Isolation of the causative agent of heartwater (*Cowdria ruminantium*) from three *Amblyomma* species in eight Districts of Kenya. *Vet Rec, 140*(1), 13-16.

Nichol. (2001). Bunyaviruses. In H. P. Fields BN, Griffin DE, Lamb RA, Martin MA, (Ed.), Philadelphia: Lippincott Williams & Wilkins Publishers. 4(null) ed ed., pp. p. 24.

Nilsson K, Lukinius A, Pahlson C, Moron C, Hajem N, Olsson B, Lindquist O. (2005). Evidence of *Rickettsia* spp. infection in Sweden: a clinical, ultrastructural and serological study. *APMIS*. 113(2):126–134. doi: 10.1111/j.1600-0463.2005.apm1130206.x.

Noormahomed, E. V., M. Orlov, V. do Rosario, B. W. Petersen, C. Guthrie, R. Badaro, and R. T. Schooley. (2012). A cross-sectional study of sub-clinical *Plasmodium falciparum* infection

UNIVERSITY of the
WESTERN CAPE

in HIV-1 infected and uninfected populations in Mozambique, South-Eastern Africa. *Malar J* 11: 252.

Nowak, M. (2010). The international trade in reptiles (Reptilia)--the cause of the transfer of exotic ticks (Acari: Ixodida) to Poland. *Vet Parasitol, 169*(3-4), 373-381. doi: 10.1016/j.vetpar.2010.01.006

Nyandigisi, A., Memusi, D., Mbithi, A., Ang'wa, N., Shieshia, M., Muturi, A., Sudoi, R., Githinji, S., Juma, E. & Zurovac, D. (2011). Malaria case-management following change of policy to universal parasitological diagnosis and targeted artemisinin-based combination therapy in Kenya. *PLoS One, 6*(9), e24781. doi: 10.1371/journal.pone.0024781

O'Guinn, M. L., & Turell, M. J. (2002). Effect of triethylamine on the recovery of selected South American alphaviruses, flaviviruses, and bunyaviruses from mosquito (Diptera: Culicidae) pools. *J Med Entomol, 39*(5), 806-808.

O'Meara, W. P., Mott, J. A., Laktabai, J., Wamburu, K., Fields, B., Armstrong, J., Taylor, S. M., Macintyre, C., Sen, R., Menya, D., Pan, W., Nicholson, B. P., Woods, C. W. & Holland, T. L. (2015). Etiology of pediatric fever in western Kenya: a case-control study of *Falciparum* malaria, respiratory viruses, and streptococcal pharyngitis. *Am J Trop Med Hyg, 92*(5), 1030-1037. doi: 10.4269/ajtmh.14-0560

Ochieng, C., Lutomiah, J., Makio, A., Koka, H., Chepkorir, E., Yalwala, S., Mutisya, J., Musila, L., Khamadi, S., Richardson, J., Bast, J., Schnabel, D., Wurapa, E. & Sang, R. (2013). Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007 – 2012. *Virol J, 10*, 140-147

Odhiambo, C., Venter, M., Limbaso, K., Swanepoel, R., & Sang, R. (2014). Genome sequence analysis of in vitro and in vivo phenotypes of Bunyamwera and Ngari virus isolates from Northern Kenya. *PLoS One, 9*,8-14.

Odongo, D. O., M. W. Ueti, S. N. Mwaura, D. P. Knowles, R. P. Bishop, and G. A. Scoles. (2009). Quantification of *Theileria parva* in *Rhipicephalus appendiculatus* (Acari:

UNIVERSITY of the
WESTERN CAPE

Ixodidae) confirms differences in infection between selected tick strains. *J. Med Entomol*. 46: 888-894.

Okiro, E. A., Alegana, V. A., Noor, A. M., Mutheu, J. J., Juma, E., & Snow, R. W. (2009). Malaria paediatric hospitalization between 1999 and 2008 across Kenya. *BMC Med, 7*, 75. doi: 10.1186/1741-7015-7-75

Okumu FO, Moore SJ. (2011). Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. *Malar J*. 10:208. doi: 10.1186/1475-2875-10-208.

Olanga, E. A., Okombo, L., Irungu, L. W., & Mukabana, W. R. (2015). Parasites and vectors of malaria on Rusinga Island, Western Kenya. *Parasit Vectors, 8*, 250. doi: 10.1186/s13071-015-0860-z

Omondi, D., Masiga, D. K., Ajamma, Y. U., Fielding, B. C., Njoroge, L., & Villinger, J. (2015). Unraveling host-vector-arbovirus interactions by two-gene high resolution melting mosquito bloodmeal analysis in a Kenyan wildlife-livestock interface. *PLoS One, 10*, 1-23

Onchiri, F. M., Pavlinac, P. B., Singa, B. O., Naulikha, J. M., Odundo, E. A., Farquhar, C., Richardson, B. A., John-Stewart, G. & Walson, J. L. (2015). Low bacteremia prevalence among febrile children in areas of differing malaria transmission in rural Kenya: A cross-sectional study. *J Pediatric Infect Dis Soc*. doi: 10.1093/jpids/piv043

Opiyo, P., Mukabana, W. R., Kiche, I., Mathenge, E., Killeen, G. F., & Fillinger, U. (2007). An exploratory study of community factors relevant for participatory malaria control on Rusinga Island, western Kenya. *Malar J, 6*, 48. doi: 10.1186/1475-2875-6-48

Pappalardo BL, Correa MT, York CC, Peat CY, Breitschwerdt EB (1997). Epidemiologic evaluation of the risk factors associated with exposure and seroreactivity to *Bartonella vinsonii* in dogs. *Am J Vet Res*.;58(5):467–471.

Parola, P. (2006). Rickettsioses in sub-Saharan Africa. *Ann N Y Acad Sci, 1078*, 42-47. doi: 10.1196/annals.1374.005

UNIVERSITY of the
WESTERN CAPE

Parola, P., Paddock, C. D., & Raoult, D. (2005). Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev, 18*(4), 719-756. doi: 10.1128/cmr.18.4.719-756.2005

Pena, V. H., Fernandez, G. J., Gomez-Palacio, A. M., Mejia-Jaramillo, A. M., Cantillo, O., & Triana-Chavez, O. (2012). High-resolution melting (HRM) of the cytochrome B gene: a powerful approach to identify blood-meal sources in Chagas disease vectors. *PLoS Negl Trop Dis, 6*(2), e1530. doi: 10.1371/journal.pntd.0001530

Perez C, Maggi RG, Diniz PP, Breitschwerdt EB. (2011). Molecular and serological diagnosis of *Bartonella* infection in 61 dogs from the United States. *J Vet Intern Med*. 25(4):805–810. doi: 10.1111/j.1939-1676.2011.0736.x.

Pluess B, Tanser FC, Lengeler C, Sharp B. (2010). Indoor residual spraying for preventing malaria. Cochrane Database *Syst Rev*. *4*:CD006657.

Punyua, D. K., Latif, A. A., Nokoe, S., & Capstick, P. B. (1991). Tick (Acari: Ixodidae) infestations on Zebu cattle in western Kenya: seasonal dynamics of four species of ticks on traditionally managed cattle. *J Med Entomol, 28*(5), 630-636.

Rappole, J. H., Derrickson, S. R., & Hubalek, Z. (2000). Migratory birds and spread of West Nile virus in the western hemisphere. *Emerg Infect Dis, 6*(4), 319-328. doi: 10.3201/eid0604.000401

Rappole, J. H., & Hubalek, Z. (2003). Migratory birds and West Nile virus. *J Appl Microbiol, 94 Suppl*, 47S-58S.

Ratnasingham, S. & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355-364. DOI: 10.1111/j.1471-8286.2006.01678.x

Ravnik U, Tozon N, Smrdel KS, Zupanc TA. (2011). Anaplasmosis in dogs: the relation of haematological, biochemical and clinical alterations to antibody titre and PCR confirmed infection. *Vet Microbiol*. 149(1–2):172–176.

UNIVERSITY of the
WESTERN CAPE

Rawlins ML, Gerstner C, Hill HR, Litwin CM. (2005). Evaluation of a western blot method for the detection of *Yersinia* antibodies: evidence of serological cross-reactivity between *Yersinia* outer membrane proteins and *Borrelia burgdorferi*. *Clin Diagn Lab Immunol*.;12(11):1269–1274.

RBM. Key facts, figures and strategies: the Global Malaria Action Plan. Roll Back Malaria Partnership; 2008.

Reed GH, Kent JO, Wittwer CT (2007). High-resolution DNA melting analysis for simple and efficient molecular diagnostics. *Pharmacogenomics* 8:597–608 doi:10.2217/14622416.8.6.597.

Reyburn, H., Mbatia, R., Drakeley, C., Carneiro, I., Mwakasungula, E., Mwerinde, O., Saganda, K., Shao, J., Kitua, A., Olomi, R., Greenwood, B. M. & Whitty, C. J. M. (2004). Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *BMJ, 329*(7476), 1212.

Reyburn, H., Ruanda, J., Mwerinde, O., & Drakeley, C. (2006). The contribution of microscopy to targeting antimalarial treatment in a low transmission area of Tanzania. *Malaria J, 5*, 4. doi: 10.1186/1475-2875-5-4

Rosenstein, M. (1976). Paralysis in chickens caused by larvae of the poultry tick, Argas persicus. *Avian Dis, 20*(2), 407-409.

Rossi, S. L., T. M. Ross, and J. D. Evans. (2010). West Nile Virus. *Clin. Lab. Med. 30*: 47-65.

Rozendaal JA. (1997). Vector control: Methods for use by individuals and communities. World Health Organization.

Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G., Vaarst, M., Kabagambe, E. K., Mwayi, W., Musunga, D. & Wandukwa, W. (2004). The prevalence of serum antibodies to tick-borne infections in Mbale District, Uganda: The effect of agro-ecological zone, grazing management and age of cattle. *J Insect Sci, 4*,8-16

UNIVERSITY of the
WESTERN CAPE

Sambou, M., Faye, N., Bassene, H., Diatta, G., Raoult, D., & Mediannikov, O. (2014). Identification of rickettsial pathogens in ixodid ticks in northern Senegal. *Ticks Tick Borne Dis, 5*(5), 552-556. doi: 10.1016/j.ttbdis.2014.04.002

Sampath R, Hall TA, Massire C, Li F, Blyn LB, Eshoo MW, Hofstadler SA, Ecker DJ. (2007). Rapid identification of emerging infectious agents using PCR and electrospray ionization mass spectrometry. *Ann N Y Acad Sci.*;1102:109–120. doi: 10.1196/annals.1408.008.

Sander A, Posselt M, Oberle K, Bredt W. (1998). Seroprevalence of antibodies to *Bartonella henselae* in patients with cat scratch disease and in healthy controls: evaluation and comparison of two commercial serological tests. *Clin Diagn Lab Immunol*.;5(4):486–490.

Sang, R., Lutomiah, J., Koka, H., Makio, A., Chepkorir, E., Ochieng, C., Yalwala, S., Mutisya, J., Musila, L., Richardson, J. H., Miller, B. R. & Schnabel, D. (2011). Crimean-Congo hemorrhagic fever virus in *Hyalommid* ticks, northeastern Kenya. *Emerg Infect Dis, 17*(8), 1502-1505. doi: 10.3201/eid1708.102064

Sang, R., Onyango, C., Gachoya, J., Mabinda, E., Konongoi, S., Ofula, V., Dunster, L., Okoth, F., Coldren, R.,Tesh, R., Travassos Da Rosa, A., Finkbeiner, S., Wang, D., Crabtree, M. & Miller, B. (2006). Tickborne arbovirus surveillance in market livestock, Nairobi, Kenya. *Emerg Infect Dis, 12*(7), 1074-1080.

Simpson, J. E., Folsom-O'Keefe, C. M., Childs, J. E., Simons, L. E., Andreadis, T. G., & Diuk-Wasser, M. A. (2009). Avian host-selection by *Culex pipiens* in experimental trials. *PLoS One, 4*(11), e7861. doi: 10.1371/journal.pone.0007861

Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, Thaithong S, Brown KN. (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol Biochem Parasitol*. *61*:315–320. doi: 10.1016/0166-6851(93)90077-B.

UNIVERSITY of the
WESTERN CAPE

Soghaier, M. A., Hagar, A., Abbas, M. A., Elmangory, M. M., Eltahir, K. M., & Sall, A. A. (2013). Yellow fever outbreak in Darfur, Sudan in October 2012; the initial outbreak investigation report. *J Infect Public Health, 6*(5), 370-376. doi: 10.1016/j.jiph.2013.04.007

Solano-Gallego L, Bradley J, Hegarty B, Sigmon B, Breitschwerdt E. (2004). *Bartonella henselae* IgG antibodies are prevalent in dogs from southeastern USA. *Vet Res.*;35(5):585–595. doi: 10.1051/vetres:2004034.

Storm, N., Weyer, J., Markotter, W., Kemp, A., Leman, P. A., Dermaux-Msimang, V., Nel, l. H. & Paweska, J. T. (2014). Human cases of Sindbis fever in South Africa, 2006-2010. *Epidemiol Infect, 142*(2), 234-238. doi: 10.1017/s0950268813000964

Tabachnick WJ. (1991). Evolutionary genetics and insect borne disease. The yellow fever mosquito, *Aedes aegypti*. *Am. Entomol*. *37*:14–24.

Tabue, R. N., Nem, T., Atangana, J., Bigoga, J. D., Patchoke, S., Tchouine, F., Fodjo, B. Y., Leke, R. G. & Fondjo, E. (2014). *Anopheles ziemanni* a locally important malaria vector in Ndop health district, north west region of Cameroon. *Parasit Vectors, 7*, 262. doi: 10.1186/1756-3305-7-262

UNIVERSITY of the
WESTERN CAPE

Tappe, D., Kapaun, A., Emmerich, P., Campos, R. M., Cadar, D., Günther, S., & Schmidt-Chanasit, J. (2014). O'nyong-nyong virus infection imported to Europe from Kenya by a traveler. *Emerg Infect Dis, 20*(10), 1766-1767.

Tesh, R.B., B.N.Chaniotis, and K.M.John-son. (1972).Vesicular stomatitis virus (Indiana serotype): transovarial transmission by phlebo-tomi sandflies. *Science 175*:1477-1479.

Thumbi, S. M., C. B. B. M. de, E. J. Poole, H. Kiara, P. Toye, M. Ndila, I. Conradie, A. Jennings, I. G. Handel, J. A. Coetzer, O. Hanotte, and M. E. Woolhouse. (2013). Parasite co-infections show synergistic and antagonistic interactions on growth performance of East African zebu cattle under one year. *Parasitology. 140*: 1789-1798.

Tigoi, C., Lwande, O., Orindi, B., Irura, Z., Ongus, J., & Sang, R. (2015). Seroepidemiology of selected arboviruses in febrile patients visiting selected health facilities in the lake/river

basin areas of Lake Baringo, Lake Naivasha, and Tana River, Kenya. *Vector Borne and Zoonotic Diseases, 15*(2), 124-132. doi: 10.1089/vbz.2014.1686

Thomson M.C., Doblas-Reyes F.J., Mason S.J., Hagedorn R., Connor S.J., Phindela T., Morse A.P., Palmer T.N. (2006). Malaria early warnings based on seasonal climate forecasts from multi-model ensembles. *Nature*. *439*:576–579. doi: 10.1038/nature04503.

Tokarz, R., Kapoor, V., Samuel, J. E., Bouyer, D. H., Briese, T., & Lipkin, W. I. (2009). Detection of tick-borne pathogens by MassTag polymerase chain reaction. *Vector Borne Zoonotic Dis, 9*(2), 147-152. doi: 10.1089/vbz.2008.0088

Townson H, Nathan MB, Zaim M, Guillet P, Manga L, Bos R, Kindhauser M. (2005). Exploiting the potential of vector control for disease prevention. *Bull World Health Organ*.;*83*:942–947.

Traore-Lamizana M, Fontenille D, Diallo M, Bâ Y, Zeller HG, Mondo M, Adam F, Thonon J, Maïga A. (2001). Arbovirus surveillance from 1990 to 1995 in the Barkedji area (Ferlo) of Senegal, a possible natural focus of Rift Valley fever virus. *J Med Entomol*. 38(4):480–92. doi: 10.1603/0022-2585-38.4.480.

UNIVERSITY of the
WESTERN CAPE

Trout Fryxell, R. T., and J. M. DeBruyn. (2016). Correction: The Microbiome of *Ehrlichia*-Infected and Uninfected Lone Star Ticks (*Amblyomma americanum). PLoS ONE 11*: e0155559.

Turell M. J., Dohm D. J., Sardelis M. R., O'Guinn M. L., Andreadis T. G., Blow J. A. (2005). An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol*. *42*(1):57–62. doi: 10.1603/0022-2585(2005)042[0057:AUOTPO]2.0.CO;2.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG. (2012). Primer3-new capabilities and interfaces. *Nucleic Acids Res*. 40:e115. doi: 10.1093/nar/gks596.

Varanat M, Maggi RG, Linder KE, Breitschwerdt EB. (2011). Molecular prevalence of *Bartonella*, *Babesia*, and hemotropic *Mycoplasma* sp. in dogs with splenic disease. *J Vet Intern Med*. 25(6):1284–1291. doi: 10.1111/j.1939-1676.2011.00811.x.

Vazquez, A., Sanchez-Seco, M. P., Palacios, G., Molero, F., Reyes, N., Ruiz, S., Aranda, C., Marques, E., Escosa, R., Moreno, J., Figuerola, J. & Tenorio, A. (2012). Novel flaviviruses detected in different species of mosquitoes in Spain. *Vector Borne Zoonotic Dis, 12*(3), 223-229. doi: 10.1089/vbz.2011.0687

Vermeulen MJ, Verbakel H, Notermans DW, Reimerink JH, Peeters MF. (2010). Evaluation of sensitivity, specificity and cross-reactivity in *Bartonella henselae* serology. *J Med Microbiol*. 59(Pt 6):743–745.

Villinger J, Mbaya MK, Ouso DO, Kipanga PN, Lutomiah J & Masiga DK. (2016). Arbovirus and insect-specific virus discovery in Kenya by novel six genera multiplex high resolution melting analysis. *Mol. Ecol. Resour*. In Press.

Walker AR, Bouattour, A., Camicas, J-L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G. & Preston, P.M: (2003).Ticks of domestic animals in Africa: a guide to identification of species. In Book Ticks of domestic animals in Africa: a guide to identification of species (Editor ed.^eds.). City. International Consortium on Ticks and Tick-borne Diseases (ICTTD)

Walker, J. B., & Olwage, A. (1987). The tick vectors of *Cowdria ruminantium* (Ixodoidea, Ixodidae, genus *Amblyomma*) and their distribution. *Onderstepoort J Vet Res, 54*(3), 353-379.

Wanzala, W., & Okanga, S. (2006). Ticks (Acari: Ixodidae) associated with wildlife and vegetation of Haller park along the Kenyan coastline. *J Med Entomol, 43*(5), 789-794.

Wesonga, F. D., Mukolwe, S. W., & Grootenhuis, J. (2001). Transmission of *Cowdria ruminantium* by *Amblyomma gemma* from infected African buffalo (*Syncerus caffer*) and eland (*Taurotragus oryx*) to sheep. *Trop Anim Health Prod, 33*(5), 379-390.

UNIVERSITY of the
WESTERN CAPE

Wesonga, F. D., Mukolwe, S. W., & Rurangirwa, F. (1993). *Cowdria ruminantium* identified in *Amblyomma gemma* using a DNA probe pCS20. *Rev Elev Med Vet Pays Trop, 46*(1-2), 179-181.

White GB. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Trans Roy Soc Trop Med Hyg*. *68*:278–302. doi: 10.1016/0035-9203(74)90035-2

WHO Technical Report Series No. 719 (1985). Arthropod-borne and Rodent-borne Viral Diseases. Geneva, Switzerland, Report of WHO Scientific Group, pp 10-110

WHO. Handbook for Integrated Vector Management WHO/HTM/NTD/VEM/2012.3. Geneva: World Health Organization; 2012. http://whqlibdoc.who.int/publications/2012/9789241502801_eng.pdf.

WHO. Guidance on policy-making for Integrated Vector Management. Geneva: World Health Organization; 2012.

WHO. Global Strategic Framework For Integrated Vector Management. WHO/CDS/CPE/PVC/2004.10. Geneva: World Health Organization; 2004. http://whqlibdoc.who.int/hq/2004/WHO_CDS_CPE_PVC_2004_10.pdf.

WESTERN CAPE

WHO. Public health pesticide registration and management practices by WHO Member States. (WHO/HTM/NTD/WHOPES/2011.3) Geneva: World Health Organization; 2011.

WHO. Monitoring and Evaluation indicators for Integrated Vector Management. Geneva: World Health Organization; 2012. http://www.who.int/iris/bitstream/10665/76504/1/9789241504027_eng.pdf.

WHO. Malaria vector control and personal protection: report of a WHO study group. WHO technical report series 936. Geneva: World Health Organization; 2006.

WHO. A global strategy for malaria control. Geneva: World Health Organization; 1993.

WHO. Global Malaria Action Plan. World Health Organization; 2009

WHO. Vector control for malaria and other mosquito-borne diseases. Report of a WHO Study Group. WHO Technical Report Series 857. World Health Organization; 1995.

WHO. Global strategic framework for integrated vector management. World Health Organization; 2004.

WHO. WHO position statement on integrated vector management. World Health Organization; 2008

World Health Organization . Dengue and Severe Dengue. Switzerland, Geneva: World Health Organization; 2012. p. 117.

World Health Organization . Dengue. Switzerland, Geneva: World Health Organization; 2009. Guidelines for Diagnosis, Treatment, Prevention and Control; p. 3.

Williams, C. R., Smith, B. P., Best, S. M., & Tyler, M. J. (2006). Mosquito repellents in frog skin. *Biol Lett, 2*(2), 242-245. doi: 10.1098/rsbl.2006.0448

Woodall, J. P., Williams, M. C., Corbet, P. S., & Haddow, A. J. (1964). The isolation of Sindbis virus from the mosquito *Mansonia* (Coquillettidia) *fuscopennata* (Theobald) in Uganda. *Ann Trop Med Parasitol, 58*, 383-389.

Woods CW, Karpati AM, Grein T, McCarthy N, Gaturuku P, Muchiri E, Dunster L, Henderson A, Khan AS, Swanepoel R. (2002). An outbreak of Rift Valley fever in northeastern Kenya, 1997–98. *Emerg Infect Dis*. 8(2):138–44. doi: 10.3201/eid0802.010023.

Worrall, E., & Fillinger, U. (2011). Large-scale use of mosquito larval source management for malaria control in Africa: a cost analysis. *Malar J, 10*, 338. doi: 10.1186/1475-2875-10-338

Yanase, T., Kato, T., Yamakawa, M., Takayoshi, K., Nakamura, K., Kokuba, T., & Tsuda, T. (2006). Genetic characterization of Batai virus indicates a genomic reassortment between Orthobunyaviruses in nature. *Arch Virol, 151*(11), 2253-2260. doi: 10.1007/s00705-006-0808-x

Zhu, Y., Fournier, P.-E., Ogata, H., & Raoult, D. (2005). Multispacer typing of *Rickettsia prowazekii* enabling epidemiological studies of epidemic typhus. *J Clin Microbiol, 43*(9), 4708-4712. doi: 10.1128/JCM.43.9.4708-4712.2005

UNIVERSITY of the
WESTERN CAPE

Zirkel, F., Kurth, A., Quan, P. L., Briese, T., Ellerbrok, H., Pauli, G., Leendertz, F. H., Lipkin, W. I., Ziebuhr,J., Drosten, C. & Junglen, S. (2011). An insect Nidovirus emerging from a primary tropical rainforest. *MBio, 2*(3), e00077-00011. doi: 10.1128/mBio.00077-11

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APPENDIX 1

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                                                                                  1          10        20        30        40        50        60
                                                                                  |          |         |         |         |         |         |
                           Bufo regularis (African Toad) bloodmeal (sample B70)   ----------------------ACATTTTAATTTT-----TTTAAG-TAGTCTGACTATA
                                                     Bufo regularis (AF220889)   ----------------------ACATTTTAATTTT-----TTTAAG-TAGTCTGACTATA
 Ptychadena anchietae (Anchieta's Ridged Frog) bloodmeal (Sample B79)   -----------------------CATGGTAATCTT---GGCTTT---TA-TCTA----TT
                                                Ptychadena anchietae (GQ183598)   -----------------------CATGGTAATCTT---GGCTTT---TA-TCTA----TT
                    Ptychadena nilotica (Grass frog) bloodmeal (Sample B31)   -----------------------CA--CTAACCTTACAGACTCTAG-AA-TCTACTTGTT
                                                 Ptychadena nilotica (DQ525928)   -----------------------CA--CTAACCTTACAGACTCTAG-AA-TCTACTTGTT
                       Hirundo rustica (Barn swallow) bloodmeal (Sample B21)   ---------CA---CCTCGGGCTTACTAACT-CACG-GGTGACTGGCTCGCA--------
                                                    Hirundo rustica (AB042382)   ---------CA---CCTCGGGCTTACTAACT-CACG-GGTGACTGGCTCGCA--------
   Urocolius macrourus (mousebird) bloodmeal (Sample B11,22,B20,B229)   ------CCCTC---CACCGGACCTATTAATT--GTA-CACTCCTGGCTA-AA--------
                                                 Urocolius macrourus (AF173589)   ------CCCTC---CACCGGACCTATTAATT--GTA-CACTCCTGGCTA-AA--------
                           Bubulcus ibis (Cattle Egret) bloodmeal (sample B180)   ------CCCTA---CT--GGATCTACTACC---CTA-AAACGCTGGC-CCGC--------
                                                       Bubulcus ibis (KJ190945)   ------CCCTA---CT--GGATCTACTACC---CTA-AAACGCTGGC-CCGC--------
                                 Gallus gallus (Chicken) bloodmeal (Sample B19)   ------------------GGGTCCACCCACA-CATA-AACCCCTGG-TCGAC--------
                                                       Gallus gallus (AY236430)   ------CCCCA---CT--GGGTCCACCCACA-CATA-AACCCCTGG-TCGAC--------
                         Gallus gallus variant (Chicken) bloodmeal (Sample B15)   ----------------------CTACCCGCA-CA-A-AGTCATTGG-TCGAT--------
                                              Francolinus pintadeanus (EU165707)   ------------------GGATTCACCCATA-CA-A-AGCCCTTGG-TCGAC--------
                               Streptopelia turtur(Dove) bloodmeal (Sample B5)   ----------------A--GGCCCACCACTA--GCA-AGATGCTGGCTC-GC--------
                                                 Streptopelia turtur (KC984248)   ------CCC-A---CTA--GGCCCACCACTA--GCA-AGATGTTGGCTC-AC--------
                            Cairina moschata (Muscovy duck) bloodmeal (Sample B6)   --------------CT--GGGGCCACTACTAATCGC-AAGGCATGGC-CGAC--------
                                                    Cairina moschata (EU755254)   --------------CT--GGGGCCACTACTAATCGC-AAGGCATGGC-CGAC--------
                             Ardea cinerea (Grey heron) bloodmeal (Sample B21)   --------------------ATCTACTACCC---TA-AAACGCTGGCTC-GC--------
                                                       Ardea cinerea (KJ190947)   ------CCCTA---CT--GGATCTACTACCC---TA-AAACGCTGGCTC-GC--------
                          Pseudonestor sp. (Passerine) bloodmeal (Sample B19)   ------CCC-A---CT--GGGTTCACTGCTA-CATA-AGCGACTGGT-CTGT--------
                                             Pseudonestor xanthophrys (KM078809)   ------CCC-A---CT--GGGTTCACTGTTA-CATA-AGACACTGGT-CTGC--------
                                           Small bird (Passerine) (Sample B152)   ------CCC-A---CT--GGGTATACGCACA-CACT-AACCGCTGGC-CTGC--------
                                                       Ficedula parva (FJ465224)   ------CCC-A---CT--GGGCCCACTTACA-CATA-AGCCACTGGC-CTGC--------
           Weaver bird (Ploceus baglaflecht-like) bloodmeal (Sample B221)   ------CCC-A---CT--GGGTTCACTGCCACA-TA-AGTTACTGG-TCCGT--------
                           Small bird (Weaver bird) bloodmeal (Sample B209)   ------CCC-A---CT--GGGTTCACTGCCACA-TA-AGCTACTGG-TTCGT--------
                                                 Ploceus baglaflecht (AY283898)   ------CCC-A---CT--GGGTTCACTGCCGCA-TA-AGCCACTGG-TCCGT--------
                                               Black bird bloodmeal (Sample B228)   ------CCC-A---TC--GGGCCCACTCCCCTAACA-AGTCATTGG-TCCAC--------
                                                      Curaeus curaeus (JX516070)   ------CCC-A---CT--GGGTTCACTGACACA-TA-AGACTTTGG-TCTAC--------
                    Micropteropus pusillus (Fruit bat) bloodmeal (Sample B34)   ---------------------AAA--TA--A--ACC--AACTTTTACT-GG---ACTAGC
                                              Micropteropus pusillus (JN398183)   --------------------AAAA--TA--A--ACC--AACTTTTACT-GG---ACTAGC
                                        Bos taurus (Cow) bloodmeal (Sample B25A)   --------------TTAAGGAA----TA--AC-A-----ACAATCTCCATG--AGTTGGT
                                        Bos taurus (Cow) bloodmeal (Sample B146)   --------------TTAAGGAA----TA--AC-A-----ACAATCTCCATG--AGTTGGT
                                                          Bos taurus (KJ789953)   --------------TTAAGGAA----TA--AC-A-----ACAATCTCCATG--AGTTGGT
                                       Canis lupus (Dog) bloodmeal (Sample B105)   -----------------AGGCA----TA--AC-ATA-ACACCATTATTATG--AGTTAGC
                                                         Canis lupus (KJ789955)   -----------------AGGCA----TA--AC-ATA-ACACCATTATTATG--AGTTAGC
                                  Equus asinus (Donkey) bloodmeal (Sample B93)   -----AACC-----CTCAGGGA----CA--AC-A---AAACTTTTGAT-TG--AATCAGC
                                                        Equus asinus (KM881681)   -----AACC-----CTCAGGGA----CA--AC-A---AAACTTTTGAT-TG--AATCAGC
                                                        Equus asinus (AP012271)   -----AACC-----CTCAGGGA----CA--AC-A---AAACTTTTGAT-TG--AATCAGC
                                    Capra hircus (Goat) bloodmeal (Sample B217)   ----------------AAGGGA----TA--AC-A-----ACATCCTTTATG--GACTAGC
                                                        Capra hircus (KP195268)   ----------------AAGGGA----TA--AC-A-----ACATCCTTTATG--GACTAGC
                                   Homo sapiens (Human) bloodmeal (Sample B148)   -----AACC-----CACAGGTCCT--AA--AC-T-----ACCA--AACCTG--CATTAAA
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                                              Homo sapiens (KP218948)   -----AACC-----CACAGGTCCT--AA--AC-T-----ACCA--AACCTG--CATTAAA
                   Arvicanthis niloticus (Rat) bloodmeal (Sample B36)   TAACTTAAC-----CTAATGGGAC--CA--AC-AT----A-AAAAAAAATA--AGCTAGT
                                     Arvicanthis niloticus (AF141228)   TAATATAAC-----CTAATGGGCC---A--AC-AT----A-AAAAAAAATA--AGCTAGA
                         Ovis aries (Sheep) bloodmeal (Sample B183)     --------------CCAAGGGA----TA--AC-A-----ACACTCCTTATG--AGTTAAC
                                                Ovis aries (KF312238)   --------------CCAAGGGA----TA--AC-A-----ACACTCCTTATG--AGTTAAC


                                                                        61       70        80        90        100       110       120
                                                                        |        |         |         |         |         |         |
           Bufo regularis (African Toad) bloodmeal (sample B70)         AGTTTTTGGTTGGGGTGACCGCGGAGCATAACATAACCTCC-----AT----GCTGA--A
                                            Bufo regularis (AF220889)   AGTTTTTGGTTGGGGTGACCGCGGAGCATAACATAACCTCC-----AT----GCTGA--A
Ptychadena anchietae (Anchieta's Ridged Frog) bloodmeal (Sample B79)    AGTTTTGGGTTGGGGTGACCGCGGAGAATAACCTAACCTCC-----GC----AATGAAAA
                                      Ptychadena anchietae (GQ183598)   AGTTTTGGGTTGGGGTGACCGCGGAGAATAACCTAACCTCC-----GC----AATGAAAA
        Ptychadena nilotica (Grass frog) bloodmeal (Sample B31)         AGCTTTAGGTTGGGGTGACCGCGGAGAAAAACTTAACCTCC-----AT----AATGAAAA
                                       Ptychadena nilotica (DQ525928)   AGCTTTAGGTTGGGGTGACCGCGGAGAAAAACTTAACCTCC-----AT----AATGAAAA
          Hirundo rustica (Barn swallow) bloodmeal (Sample B21)         TTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AACACT-A
                                           Hirundo rustica (AB042382)   TTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AACACT-A
  Urocolius macrourus (mousebird) bloodmeal (Sample B11,22,B20,B229)    ATTTTTCGGTTGGGGCGACCTTGGAGTAAAACAGATCCTCC-----A-----AAAACA-A
                                       Urocolius macrourus (AF173589)   ATTTTTCGGTTGGGGCGACCTTGGAGTAAAACAGATCCTCC-----A-----AAAACA-A
             Bubulcus ibis (Cattle Egret) bloodmeal (sample B180)       ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAAATCCTCC-----A-----AAAACA-A
                                             Bubulcus ibis (KJ190945)   ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAAATCCTCC-----A-----AAAACA-A
                  Gallus gallus (Chicken) bloodmeal (Sample B19)        ATTTTTCGGTTGGGGCGACCTTGGAGAAAAAAAAATCCTCC-----A-----AACCCACA
                                             Gallus gallus (AY236430)   ATTTTTCGGTTGGGGCGACCTTGGAGAAAAAAAAATCCTCC-----A-----AACCCACA
          Gallus gallus variant (Chicken) bloodmeal (Sample B15)        ATTTTTTGGTTGGGGCGACCTTGGAGAAAAGAAAATCCTCC-----A-----AACCTACA
                               Francolinus pintadeanus (EU165707)       ATTTTTCGGTTGGGGCGACCTTGGAGAAAAGAAAATCCTCC-----A-----AACATAAA
                Streptopelia turtur(Dove) bloodmeal (Sample B5)         ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAAATCCTCC-----A-----AAAACA-A
                                       Streptopelia turtur (KC984248)   ATTTTTCGGTTGGGGCGACCTTGGAGAAAAGTAAATCCTCC-----A-----AAAACA-A
         Cairina moschata (Muscovy duck) bloodmeal (Sample B6)          ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAGACCCTCC-----A-----AAAACA-A
                                          Cairina moschata (EU755254)   ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAGACCCTCC-----A-----AAAACA-A
                 Ardea cinerea (Grey heron) bloodmeal (Sample B21)      ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACGAATCCTCC-----A-----AAGACA-A
                                             Ardea cinerea (KJ190947)   ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACGAATCCTCC-----A-----AAGACA-A
            Pseudonestor sp. (Passerine) bloodmeal (Sample B19)         GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AAAATT-A
                                Pseudonestor xanthophrys (KM078809)     GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAGAACCTCC-----A-----AAAATT-A
                                Small bird (Passerine) (Sample B152)    ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAAATCCTCC-----A-----AAAATT-A
                                              Ficedula parva (FJ465224) ATTTTTCGGTTGGGGCGACCTTGGAGAAAAACAAATCCTCC-----A-----AAAACT-A
    Weaver bird (Ploceus baglaflecht-like) bloodmeal (Sample B221)      GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AAAATT-G
                 Small bird (Weaver bird) bloodmeal (Sample B209)       GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AAAATT-G
                                       Ploceus baglaflecht (AY283898)   GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AAAATT-G
                               Black bird bloodmeal (Sample B228)       GTTTTTCGGTTGGGGCGACCTTGGAGCAAAGCAAAACCTCC-----A-----AAAATT-G
                                            Curaeus curaeus (JX516070)  GTTTTTCGGTTGGGGCGACCTTGGAGCAAAACAAAACCTCC-----A-----AAAATT-G
        Micropteropus pusillus (Fruit bat) bloodmeal (Sample B34)       AA-TTTAGGTTGGGGTGACCTCGGAATATAAAACAACTCCCGAGTGA-C--ACAGTCTA-
                                   Micropteropus pusillus (JN398183)    AA-TTTAGGTTGGGGTGACCTCGGAATATAAAACAACTCCCGAGTGA-C--ACAGTCTA-
                            Bos taurus (Cow) bloodmeal (Sample B25A)    AG-TTTCGGTTGGGGTGACCTCGGAGAATAAAAAATCCTCCGAGCGATTTTAAAGACT-A
                            Bos taurus (Cow) bloodmeal (Sample B146)    AG-TTTCGGTTGGGGTGACCTCGGAGAATAAAAAATCCTCCGAGCGATTTTAAAGACT-A
                                               Bos taurus (KJ789953)    AG-TTTCGGTTGGGGTGACCTCGGAGAATAAAAAATCCTCCGAGCGATTTTAAAGACT-A
                           Canis lupus (Dog) bloodmeal (Sample B105)    AA-TTTAGGTTGGGGTGACCTCGGAATATAAAAAAACTCCCGAGTGAT--TAAAATTTA-
                                               Canis lupus (KJ789955)   AA-TTTAGGTTGGGGTGACCTCGGAATATAAAAAAACTCCCGAGTGAT--TAAAATTTA-
                      Equus asinus (Donkey) bloodmeal (Sample B93)      AA-TTTCGGTTGGGGTGACCTCGGAGAACAAAACAACCTCCGAGTGATTT--AAATCTA-
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                                            Equus asinus (KM881681)   AA-TTTCGGTTGGGGTGACCTCGGAGAACAAAACAACCTCCGAGTGATTT--AAATCTA-
                                            Equus asinus (AP012271)   AA-TTTCGGTTGGGGTGACCTCGGAGAACAAAACAACCTCCGAGTGATTT--AAATCTA-
                          Capra hircus (Goat) bloodmeal (Sample B217)   AG-TTTTGGTTGGGGTGACCTCGGAGAACAAGAGATCCTCCGAGCGATTTTAAAGACTA-
                                            Capra hircus (KP195268)   AG-TTTTGGTTGGGGTGACCTCGGAGAACAAGAGATCCTCCGAGCGATTTTAAAGACTA-
                         Homo sapiens (Human) bloodmeal (Sample B148)   AA-TTTCGGTTGGGGCGACCTCGGAGCAGAACCCAACCTCCGAGCAGT---ACATGCTAA
                                            Homo sapiens (KP218948)   AA-TTTCGGTTGGGGCGACCTCGGAGCAGAACCCAACCTCCGAGCAG---TACATGCTAA
                  Arvicanthis niloticus (Rat) bloodmeal (Sample B36)   AA-TTTCGGTTGGGGTGACCTCGGAGAATAAAAAATCCTCCGAATGATTAT--AGTTCA-
                                   Arvicanthis niloticus (AF141228)   AA-TTTCGGTTGGGGTGACCTCGGAGAATAAAAAATCCTCCGAATGATTTT--AGCATA-
                          Ovis aries (Sheep) bloodmeal (Sample B183)   AG-TTTCGGTTGGGGTGACCTCGGAGAACAGAAAATCCTCCGAGCGATTTTAAAGACTA-
                                              Ovis aries (KF312238)   AG-TTTCGGTTGGGGTGACCTCGGAGAACAGAAAATCCTCCGAGCGATTTTAAAGACTA-
                                                                     121       130       140       150       160       170       180
                                                                      |         |         |         |         |         |         |
               Bufo regularis (African Toad) bloodmeal (sample B70)   GGATTTAA-TT-CTAAGCTGAGACCTACGCCTCTAAGCATCAGCACACTGACATTAATTG
                                          Bufo regularis (AF220889)   GGATTTAA-TT-CTAAGCTGAGACCTACGCCTCTAAGCATCAGCACACTGACATTAATTG
Ptychadena anchietae (Anchieta's Ridged Frog) bloodmeal (Sample B79)   GAATAATA-TC-CTAATCTAAGAGGGACACCTCTAAGAATTAATAAATTAACGTATGATG
                                    Ptychadena anchietae (GQ183598)   GAATAATA-TC-CTAATCTAAGAGGGACACCTCTAAGAATTAATAAATTAACGTATGATG
            Ptychadena nilotica (Grass frog) bloodmeal (Sample B31)   GAATAAAA-TC-CTAATCTATGAGCCACACCTCTAAGAATCAACAAATTGACATAAAATG
                                     Ptychadena nilotica (DQ525928)   GAATAAAA-TC-CTAATCTATGAGCCACACCTCTAAGAATCAACAAATTGACATAAAATG
            Hirundo rustica (Barn swallow) bloodmeal (Sample B21)   GACCACAC--ATCTAGACCAAGAGCTACAACTC----GACGTGCAAATAGC-ACCCA--G
                                         Hirundo rustica (AB042382)   GACCACAC--ATCTAGACCAAGAGCTACGACTC----GACGTGCAAATAGC-ACCCA--G
  Urocolius macrourus (mousebird) bloodmeal (Sample B11,22,B20,B229)   GACCT-AC-CCTCTTAATCAAGAGCCACCTCTC----AACATGCCAATAGCCA-CCA--G
                                     Urocolius macrourus (AF173589)   GACCT-AC-CCTCTTAATCAAGAGCCACCTCTC----AACATGCCAATAGCCA-CCA--G
               Bubulcus ibis (Cattle Egret) bloodmeal (sample B180)   GACCACAC--CTCTTAACCAAGAGCGACATCTC----TACGTGCTAACAGTAA-CCA--G
                                           Bubulcus ibis (KJ190945)   GACCACAC--CTCTTAACCAAGAGCGACATCTC----TACGTGCTAACAGTAA-CCA--G
                  Gallus gallus (Chicken) bloodmeal (Sample B19)   GACCACAA--CTCTTCACTAAGACCAACTCCTC----AAAGTACCAACAGTAA-CCA--G
                                           Gallus gallus (AY236430)   GACCACAA--CTCTTCACTAAGACCAACTCCTC----AAAGTACCAACAGTAA-CCA--G
          Gallus gallus variant (Chicken) bloodmeal (Sample B15)   GACCACAA--CTCTTCACTAAGACCAACCCTTC----AAAGTACAAATAGTAA-CTA--G
                                   Francolinus pintadeanus (EU165707)   GACCACAA--CTCTTTACCAAGACCAACACCTC----AAAGTACTAATAGTAATTTA--G
              Streptopelia turtur(Dove) bloodmeal (Sample B5)   GACCACCC-CCTCTTAACTAAGAGCAACCCCTC----AACGTACTAATAGTAA-CCA--G
                                     Streptopelia turtur (KC984248)   GACCACTC-CCTCTTAACTAAGAGCAACCCCTC----AACGTACTAACAGTAA-CCA--G
            Cairina moschata (Muscovy duck) bloodmeal (Sample B6)   GACCACAC--CTCTTTACTTAGAGCCACCCCTC----AAAGTGCTAATAGCGA-CCA--G
                                       Cairina moschata (EU755254)   GACCACAC--CTCTTTACTTAGAGCCACCCCTC----AAAGTGCTAATAGCGA-CCA--G
                  Ardea cinerea (Grey heron) bloodmeal (Sample B21)   GACCACAC--CTCTTAACCAAGAGCAACATCTC----TACGTGCTAATAGTAA-CCA--G
                                           Ardea cinerea (KJ190947)   GACCACAC--CTCTTAACCAAGAGCAACATCTC----TACGTGCTAATAGTAA-CCA--G
            Pseudonestor sp. (Passerine) bloodmeal (Sample B19)   GACCACAC--CTCTAGACTAAGAGCAACCCCTC----AACGTGCTAATAGCAA-CCA--G
                               Pseudonestor xanthophrys (KM078809)   GACCACAC--CTCTAGACTGAGAGCAACCCCTC----AACGTGCTAATAGCAC-CCA--G
                             Small bird (Passerine) (Sample B152)   GACCACAC--CTCTAGACTAAGAGCTACCCCTC----AACGTGCTAATAGCAA-CCA--G
                                          Ficedula parva (FJ465224)   GACCACAC--CTCTAGACTAAGAGCAACCCCTC----AACGTGCTAATAGCAA-CCA--G
   Weaver bird (Ploceus baglaflecht-like) bloodmeal (Sample B221)   GACCATAC--CTCCAGACCAAGAGCAACCCCTC----AACGTGCTAATAGC-ATCCA--G
                  Small bird (Weaver bird) bloodmeal (Sample B209)   GACCACAA--CTCCAGACCAAGAGCAACCTCTC----AACGTGCTAATAGC-ATCCA--G
                                     Ploceus baglaflecht (AY283898)   GATCACAC--CTCCAGACCAAGAGCAACCCCTC----AACGTGCTAATAGC-ATCCA--G
                              Black bird bloodmeal (Sample B228)   GACCACAC--CTCCAGACCAAGAGCAACCACTC----AACGTGCTAATAGC-AACCA--G
                                          Curaeus curaeus (JX516070)   GACCATAC--CTCTAGACTAAGAGCAACCTCTC----AACGTGCTAATAGC-AACCA--G
     Micropteropus pusillus (Fruit bat) bloodmeal (Sample B34)   GACT-AACAAGTCGAAAC--CCT-CTATCA--TT----------CA-GTG--ATCCAA--
                                Micropteropus pusillus (JN398183)   GACT-AACAAGTCGAAAC--CCT-CTATCA--TT----------CA-GTG--ATCCAA--
                         Bos taurus (Cow) bloodmeal (Sample B25A)   GACC-CACAAGTCAAATC--ACT-CTATCGCTC-------------ATTG--ATCCAA--
                         Bos taurus (Cow) bloodmeal (Sample B146)   GACC-CACAAGTCAAATT--ACT-CTATCGCTC-------------ATTG--ATCCAA--
                                            Bos taurus (KJ789953)   GACC-CACAAGTCAAATC--ACT-CTATCGCTC-------------ATTG--ATCCAA--
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                    Canis lupus (Dog) bloodmeal (Sample B105)   GACC-CACAAGTCAAAAT-A-CA-ACATCA--CT----------T-ATTG--ATCCAA--
                                       Canis lupus (KJ789955)   GACC-CACAAGTCAAAAT--ACA-ACATCA--CT----------T-ATTG--ATCCAA--
                 Equus asinus (Donkey) bloodmeal (Sample B93)   GACT-AACCAGTCAAAAT--ACA-TAATCA--CT----------TA-TTG--ATCCAA--
                                      Equus asinus (KM881681)   GACT-AACCAGTCAAAAT--ACA-TAATCA--CT----------TA-TTG--ATCCAA--
                                      Equus asinus (AP012271)   GACT-AACCAGTCAAAAT--ACA-TAATCA--CT----------TA-TTG--ATCCAA--
                  Capra hircus (Goat) bloodmeal (Sample B217)   GACTT-ACAAGTCAAATC-AA-A-TTATCG--CT----------TA-TTG--ATCCAA--
                                      Capra hircus (KP195268)   GACTT-ACAAGTCAAATC-AA-A-TTATCG--CT----------TA-TTG--ATCCAA--
                 Homo sapiens (Human) bloodmeal (Sample B148)   GACTTCACCAGTCAAAGC-GA-A-CTACTATACT----------CAATTG--ATCCAA--
                                      Homo sapiens (KP218948)   GACTTCACCAGTCAAAGC-GA-A-CTACTATACT----------CAATTG--ATCCAA--
            Arvicanthis niloticus (Rat) bloodmeal (Sample B36)   GACC-AACAAGTCAAAAC-AACA-CTTTAAATCT----------TA-TTG--ATCCAA--
                             Arvicanthis niloticus (AF141228)   GACT-AACAAGTCAAAGC-AACA-TTACAAATCT----------TA-TTG--ACCCAA--
                   Ovis aries (Sheep) bloodmeal (Sample B183)   GACT-AACAAGTCAAACC-AA-A-CCATCG--CT----------TA-TTG--ATCCAA--
                                        Ovis aries (KF312238)   GACT-AACAAGTCAAACC-AA-A-CCATCG--CT----------TA-TTG--ATCCAA--
                                                               181      190       200       210
                                                                |        |         |         |
          Bufo regularis (Leopard Toad) bloodmeal (sample B70)   ACCCAATAC----AATTGAGCAACGAACCAAGTTACCC
                                    Bufo regularis (AF220889)   ACCCAATAC----AATTGAGCAACGAACCAAGTTACCC
Ptychadena anchietae (Anchieta's Ridged Frog) bloodmeal (Sample B79)   ATCCAATACTTATATTTGATCAATGAACCAAGTTACCC
                              Ptychadena anchietae (GQ183598)   ATCCAATACTTATATTTGATCAATGAACCAAGTTACCC
        Ptychadena nilotica (Grass frog) bloodmeal (Sample B31)   ACCCGATA-----ATTCGATCAATGAACCAAGTTACCC
                               Ptychadena nilotica (DQ525928)   ACCCGATA-----ATTCGATCAATGAACCAAGTTACCC
          Hirundo rustica (Barn swallow) bloodmeal (Sample B21)   ACCCAATAA----AATTGATCAATGGACCAAGCTACCC
                                   Hirundo rustica (AB042382)   ACCCAATAA----AATTGATCAATGGACCAAGCTACCC
 Urocolius macrourus (mousebird) bloodmeal (Sample B11,22,B20,B229)   ACCCAATAC----ACTTGATTAATGGACCAAGCTACCC
                               Urocolius macrourus (AF173589)   ACCCAATAC----ACTTGATTAATGGACCAAGCTACCC
           Bubulcus ibis (Cattle Egret) bloodmeal (sample B180)   ACCCAATAT----AATTGATTAATGAACCAAGCTACCC
                                     Bubulcus ibis (KJ190945)   ACCCAATAT----AATTGATTAATGAACCAAGCTACCC
               Gallus gallus (Chicken) bloodmeal (Sample B19)   ACCCAATAT----AATTGAGCAATGGACCAAGCTACCC
                                     Gallus gallus (AY236430)   ACCCAATAT----AATTGAGCAATGGACCAAGCTACCC
       Gallus gallus variant (Chicken) bloodmeal (Sample B15)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
                            Francolinus pintadeanus (EU165707)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
              Streptopelia turtur(Dove) bloodmeal (Sample B5)   ACCCAATAC----AATTGACCAATGGACCAAGCTACCC
                               Streptopelia turtur (KC984248)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
          Cairina moschata (Muscovy duck) bloodmeal (Sample B6)   ACCCAATAT----AATTGATTAATGGACCAAGCTACCC
                                  Cairina moschata (EU755254)   ACCCAATAT----AATTGATTAATGGACCAAGCTACCC
              Ardea cinerea (Grey heron) bloodmeal (Sample B21)   ACCCAATAC----AATTGATTAATGAACCAAGCTACCC
                                     Ardea cinerea (KJ190947)   ACCCAATAC----AATTGATTAATGAACCAAGCTACCC
         Pseudonestor sp. (Passerine) bloodmeal (Sample B19)   ACCCAATAT----AATTGATCAATGGACCAAGCTACCC
                          Pseudonestor xanthophrys (KM078809)   ACCCAATAT----AATTGATCAATGGACCAAGCTACCC
                            Small bird (Passerine) (Sample B152)   ACCCAATAT----AATTGATCAATGGACCAAGCTACCC
                                    Ficedula parva (FJ465224)   ACCCAATAT----AATTGATCAATGGACCAAGCTACCC
   Weaver bird (Ploceus baglaflecht-like) bloodmeal (Sample B221)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
              Small bird (Weaver bird) bloodmeal (Sample B209)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
                               Ploceus baglaflecht (AY283898)   ACCCAATAC----AATTGACCAATGGACCAAGCTACCC
                          Black bird bloodmeal (Sample B228)   ATCCAATAT----AATTGATCAATGGACCAAGCTACCC
                                  Curaeus curaeus (JX516070)   ACCCAATAC----AATTGATCAATGGACCAAGCTACCC
      Micropteropus pusillus (Fruit bat) bloodmeal (Sample B34)   ----TTT-CTT----TTGATCAACGGAACAAGTTACCC
                         Micropteropus pusillus (JN398183)   ----TTT-CTT----TTGATCAACGGAACAAGT-----
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            Bos taurus (Cow) bloodmeal (Sample B25A)   ----AAA---C----TTGATCAACGGAACAAGT-----
            Bos taurus (Cow) bloodmeal (Sample B146)   ----AAA---C----TTGATCAACGGAACAAGT-----
                               Bos taurus (KJ789953)   ----AAA---C----TTGATCAACGGAACAAGT-----
           Canis lupus (Dog) bloodmeal (Sample B105)   ----TAA-TTT----TTGATCAACGGAACAAGT-----
                              Canis lupus (KJ789955)   ----TAA-TTT----TTGATCAACGGAACAAGTT----
        Equus asinus (Donkey) bloodmeal (Sample B93)   ----ACC---T----TTGATCAACGGAATAAGT-----
                             Equus asinus (KM881681)   ----ACC---T----TTGATCAACGGAACAAGT-----
                             Equus asinus (AP012271)   ----ACC---T----TTGATCAACGGAACAAGT-----
          Capra hircus (Goat) bloodmeal (Sample B217)  ----AAA--AC----TTGATCAACGGAACAAGT-----
                             Capra hircus (KP195268)   ----AAA--AC----TTGATCAACGGAACAAGT-----
        Homo sapiens (Human) bloodmeal (Sample B148)   ----TAA-C------TTGACCAACGGAACAAGT-----
                             Homo sapiens (KP218948)   ----TAA-C------TTGACCAACGGAACAAGT-----
Arvicanthis niloticus (Rat) bloodmeal (Sample B36)     ----AACTA-T----TTGATCAACGGACCAAGTTACCC
                    Arvicanthis niloticus (AF141228)   ----ATCTATT----TTGATCAACGGACCAAGTTACCC
           Ovis aries (Sheep) bloodmeal (Sample B183)  ----AAA---C----TTGATCAACGGAACAAGT-----
                               Ovis aries (KF312238)   ----AAA---C----TTGATCAACGGAACA
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Appendix 1

INFORMED CONSENT AGREEMENT

What is the study called: **An investigation of arbovirus prevalence and ecology in Suba District**

What is this study about: Sometimes adults and children have illnesses that have fever, but this is not because of common infections like malaria. In this study we are trying to find out if some of these illnesses are caused by viruses that are transmitted by mosquitoes and other vectors (carriers of germs). We will use new methods that allow us to test for the presence of germs that are difficult to find by common methods, even when they are only present in very small amounts.

Who is running the study: The study is being run by Dr. Daniel Masiga, Jandouwe Villinger and, Dr. Patrick Sawa (Head, St. Jude's Clinic, Thomas Odhiambo Campus, icipe). We will collaborate with other scientists at icipe, and MoH.

Do I have to participate: Participation in this study is voluntary. There is no penalty for refusing to participate. If you start the study you (your child) may discontinue your (your child's) participation at any time. The principal investigators and co-investigators from ICIPE and MOH may decide to withdraw you (your child) from the study if we are unable to obtain a blood sample from you (your child).

What will happen to me if I participate in the study: You will be asked some questions about where you live, your illness and any medications you may have taken recently. Then about a tablespoon of blood will be taken from a vein in your arm. The blood will be put into small tubes so we can test for germs that may be causing your illness. We will not test for HIV.

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Are there any risks if I participate in the study: There is the possibility of mild discomfort, bruising and very rarely infection at the site where the blood is taken. But, should you (your child) be injured as a direct result of participating in this research project, you (your child) will be provided medical care, at no cost to you (your child), for that injury. You (your child) will not receive any injury compensation, only medical care. You (your child) should also understand that this is not a waiver or release of your (your child's) legal rights. You should discuss this issue thoroughly with the principal investigator before you (your child) enroll in this study.

✓

Appendix 2

ASSENT FORM FOR INDIVIDUALS 12 THROUGH 17 YEARS OF AGE

What is the study called: An investigation of arbovirus prevalence and ecology in Suba District

What is this study about: We are interested in finding out what germs cause fever without signs of infection (like when you get sick with malaria). We want to draw a small amount of blood and test it in the lab to see if we can find the germ that is causing your illness.

Who is running the study: The study is being run by Dr Daniel Masiga, Dr. Jandouwe Villinger and Dr. Patrick Sawa.

Do I have to be in the study: No you do not have to be in the study. There is no penalty if you do not participate. Even if you start the study you may stop at any time.

What will happen to me if I participate in the study: You and the adult you are with will be asked some questions about where you live, your illness and any medications you may have taken recently. Then about a tablespoon of blood will be taken from a vein in your arm. The blood will be put into small tubes so we can test for germs that may be causing your illness. We will not test for HIV.

Are there any risks if I participate in the study: Having your blood drawn may hurt a little. You may have some bleeding or a bruise after the blood is drawn.

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Are there any benefits from participating in the study: The study can help us improve medical care in Kenya. You may help us learn about some new diseases.

Will I get anything for being in the study: No, you do not receive anything for being in the study.

How long does the study last: Answering the questions and the blood draw will take about 30 minutes.

Who can be in this study: Anyone can be in the study if you have a fever without a source after evaluation by the clinician.

Who will be able to see my information or lab results: Only the people will be involved in the study. Your name will be removed from everything that anyone else sees.

What will happen to my blood: Your blood will be tested for things that could cause your fever. Your blood will NOT be tested for HIV. A sample of your blood will be kept frozen in case we want to do more testing on it in the future.

Who can I contact if I need information on the conduct of the study:
If you have any question you or your parent should contact:

Dr. Daniel Masiga
Molecular Biology and Bioinformatics Unit
International Centre of Insect Physiology and Ecology (ICIPE)
Duduville, Kasarani
Off Thika Road
P.O. Box 30772
Nairobi 00100
Kenya

or

Dr. Patrick Sawa
St. Jude's Clinic
Thomas Odhiambo Campus, icipe - African Insect Science for Food and Health PO Box 30, *Mbita* Point, Kenya

Who should I contact if I have questions on my rights as a volunteer in this research study: If you have any question on your rights as a volunteer, you or your parent should contact.

The Secretary, Ethical Review Committee
C/o Kenya Medical Research Institute
P.O. Box 54840, Nairobi, Kenya
Tel. 254-20-2722541

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Appendix 3

Interviewer	Study Number
________	________

Date of Collection: ________________ (dd/MON/yr)

Sex: Male Female Age: : ______years

Where is your (your child's) current residence:

Village: ________________ District: ________________

Province: ________________

How long have you (your child) been living in this district? ______years ______months

During the past five days, where have you (your child) been mostly (check one)?

- ☐ Village of Residence
- ☐ In the country, but not in residence, Where? ________________
- ☐ Out of the country, Where? ________________

How many times have you (your child) traveled outside of your district in the last two months: ____

How long ago: <2 weeks 2-4 weeks 1-2 months Where: ________________

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IF THERE IS ANY PORTION OF THIS CONSENT AGREEMENT THAT YOU DO NOT UNDERSTAND, PLEASE ASK STUDY TEAM BEFORE SIGNING.

Subject's or Guardian's Signature: ________________________ Date: ________

Permanent Address: ____________________________________

Witness's Name: ____

(Must be literate in case of an illiterate participant)

Witness's Signature: ________________________ **Date:** _______

Study Number: ______________

Thumbprint of Volunteer or Volunteer's Parent/Guardian if Unable to Sign

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Person Administering Consent:

Name: ____

Signature: ____

How long ago: <2 weeks 2-4 weeks 1-2 months Where:______________

How long ago: <2 weeks 2-4 weeks 1-2 months Where:______________

Have you (your child) ever received a yellow fever vaccine? Yes No Unknown

Date of vaccination (if known) ____________(dd/MON/yy)

If adult: What is your occupation: ______________________

If child: Where do you go to school: ______________________

Do you have contact with any of the following species of animals?

Bats Geese Ducks Chickens Other birds:

Goats Cows Donkeys Camels Monkeys ---Animals: ______________

Others types

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For each species checked above:

1) list the species

2) describe the contact, e.g., trapping, farming, slaughter, food preparation, veterinary work, casual contact (e.g., a neighbor keeps chickens, there is a slaughterhouse nearby), eating raw fowl products or drinking blood

3. Were the animals showing signs of illness?

Appendix 4

DHOLUO TRANSLATION

OBOKE MAR YIERUOK

THUON NONRONI: Arbovirus Prevalence and Ecology in Suba District

KAR THIETH MA ITIME: St. Judes Clinic ICIPE ,Mbita-Kenya

JOLONY MA OCHUNG' NE NONRONI: Laktar Patrick Sawa. Laktar Daniel Masiga kod Laktar Jandouwe Villinger (ICIPE, Mbita, Nairobi- KENYA).

LONY MATUT KUOM JOTIM NONRO: Patrick Sawa nikod rang'iny mamalo mar thieth kendo laktar maduong' e ma ICIPE- Mbita, Laktar Daniel Masiga nikod lony gi rang'iny mamalo e weche mag nonro.(PhD) kod Laktar Jandouwe Villinger bende nikod lony matut e weche mag nonro.

WACH MA ONEGO ONG'E E BEDO E NONRONI

Wabiro kwayo in kod nyathini ma osekalo higa apar kod ariyo mondo ing'e moko kuom weche madongo ma otenore kod nonroni;

1. Yie ebedo e nonroni en mana chiwruok
2. Oyiene ng'ato mondo owuog e nonro saa moro amora ma ohero; kendo wuok e nonro ok bi ng'iko thieth mari.
3. Bang' ka isesomo ler mar nonroni, okwayi ni bed thuolo mondo ipenj penjo moro amora ma otenore kod nonroni mondo mi ing'e matut kedo maler.
4. Watimo nonroni mana koluwore kod rang'iny ma itime e nonro e piny mangima (International Standards of Medical Research)

WACH MOTELO KATA THOR WACH

Sura kod kute moko mafuyo (arthropods) iparo ni kelo kata lando kute moko mag tuo. Kutegi iwacho ni kelo tuoche ma ranysi gi nenore mana kaka touché mamoko; kendo ma nyalo kelo ng'ikruok kata mana duoko maok ber a sache ma idwa thiedh touché mamoko kaka midhusi (malaria). Ma emomiyo wadwaro timo nonro ma touché mamoko gi , kaka gi landore e gwenge mag Suba. Nikech wadwaro nono ma eyo matut, wabiro kawo remo matin to watero kar rango touché(laboratory) mondo warang gi masinde mondo mi wang'e ni remo bende oting'o kute makelo touché gi. E yo machielo wabiro pimo gigo maloso dend kutegi (genetic profile). Ng'e ni ok wpim tuo mar AYAKI.

CHENRO MA ONEGO OLUW

Wabiro penji sigand tuo ma isebedogo, kaka otimi kendo ranyisi mage. Bang'e wabiro gwero lueti matin mondo wakaw remo matin. To ka opo ni nyathini oyudi kod malaria obiro yudo thieth mar nono to kod yadhe makueyo del mahore (paracetamol).

HINYRUOK MATIN KATA NG'AW

Onge hinyruok moro malich ma nyathini biro winjo mak mana gwarruok matin a sache ma ikawo remo matin.

NDALO MAG CHIWRUOK

Odiechieng' achiel

BER MA IYUDO

Joma ochiwore biro yudo thieth mar malaria nono maonge chudo a sache mag chiwruok.

NAMBA MA IDWARO MONDO OCHIWRE

380 bang' dweche ariyo.

ADIERA MA NITIE E KANO DWOKO MAR NONRO

En adiera ni ok bi ti kod nying ng'ato e chiwo duoko mar nonroni to ibiro kane kama opondo.

OKENGE MA INYALO GOL GODO NYATHI E NONRO

Inyalo mana gol kata chung nyathini e nonro ka opo ni;

1. Ngima ne ng'aw kendo nyalo kelo ne hinyruok a sache mag nonro.
2. Okenge ma nyalo sieko ma miyo ngima nyathi bedo marach.

OKANG' MA IKAWO E SACHE MAG HINYRUOK KATA TUO

E sache mag hinyruok kata tuo, ibiro kaw ne nyathini okang' mar thieth nono maonge chudo kokalo kuom jolony mag thieth.

PENJO KATA MANG'ENY MA IDWARO NG'EYO KA OLUWORE KOD NONRONI

Ka opo ni idwa ng'eyo mang'eny ka oluwore kod nonroni to inyalo tudori kod Laktar Patrick Sawa, St.Judes Clinic. ICIPE P.O BOX 30 Mbita, Suba District- Kenya.

Kata;

Jagoro, KEMRI Ethics Review Committee, P.o Box 54840-00200 Nairobi, Namba sime 0202722541/0722205901/0733400003.

Ka opo ni ni nitie kamoro amora kata tie andiko moro ma ok iwinjo maber to pod inyalo penjo jachung' ne nonroni ka pok iketo koki kata sei.

Nying' nyathi..

Sei mar janyuol/jant ....Tarik...............................

✓

Appendix 6

Kare mar Adek

Japenjo	**Namba mar Nonro**
______	______
______	______

Tarik mar choko nonro ______ (tarik, dwe, higa)

Chwech: Dichwo ______ Miyo ______ Higa mar nyuol ______

Kanye ma in kata nyathini odake sani?

Gweng:______ Distrikt______ Province ______

In kata nyathini osedak e distrikt ni marom nade?

______ higa ______ dweche

Kuom higni abich ma osekalo, in kata nyathini usebedo ka udak mang'eny kanye? Rang achiel

Gweng' ma idake ______

Piny ma idake ______

Oko mar piny (Kenya) ______

In kod nyathini usebedo ga ka uwuotho kata uwuok oko mar district ni marom nade kuom dweche ariyo ma osekalo?

Bang' jumbe ariyo ______

Bang' jumbe 2 – 4 ______

Bang' dweche 1 -2 ______

Kanye? ______

Be in kata nyathini useyudo chanjo mar tuo mar midhusi maloko del maratong' (yellow fever)?

Ee ______ Ooyo ______

Ka ee, to tarik mane mane ochanjue (ka poni ing'eyo) ______

Tiji makelo ni konyruok en mane ______

Be isebedo machiegni kata mulo moko kuom gigi;

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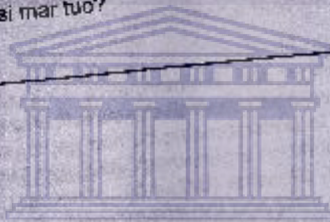
Winy; Oliktiga Mbata Atudo Gweno Mamoko

Le; Diel Dhiang' Punda Ngamia Ong'er Mamoko

Kidienje mamoko

Kuom kidieny kata mana kido mar chwech mamalogi;

1. Chan ane kanyakla mar kido gi e chal margi e yor chwech
2. Ler ane malut e yo mane mane ibedo kod gik mamalogi machiegni kata mana mulogi, ne imakogi ka iketo negi obadho, ne ipuro kodgi, ne iyang'ogi, ne imiyogi chiemo, ne ithiedhogi, mulogi (kuom jirani ka opidho gwen machiegni, kata kar yeng'o machiegni), ne ichamo ring gweno ma pok otedi kata mana ne imadho remo menumu
3. Be le gi ne nigi ranyisi mar tuo?



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Individual informed consent agreement

I, ________________________(name of parent/legal guardian) being the legal representative of (name of the participant)________________________, certify giving hereby my consent for the child to participate in the research project titled: Arboviruses in Suba. I understand that I may feel some discomfort during the procedure and I understand that although the risks are minimal I am aware that there will be no benefit apart from those described in the first part of this form to either my child or myself personally.

I have read the foregoing information, or it has been read to me. I have been given the opportunity to ask questions concerning this project. Any such questions have been answered to my full satisfaction. I have been provided with the name of the Project Coordinator who I may contact if any further questions arise concerning the rights of the child.

I consent voluntarily to participate in this study and I understand that I have the right to withdraw from the study at any time without penalty or loss of benefits.

Print Name and Signature ________________________

Date________________

Day/month/year

If illiterate

I have witnessed the accurate reading of the informed consent form to the parent/legal guardian of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

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Print name and Signature of Witness________________________ **Thumb print of parent**

Date________________

Day/month/year

I have accurately read or witnessed the accurate reading of the informed consent form to the parent/legal guardian of the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Researcher________________ **Signature** ________________