OCCURRENCE OF RIFT VALLEY FEVER AND ASSOCIATED RISK FACTORS IN CATTLE IN IJARA DISTRICT, KENYA

By

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

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DEDICATION

This work is dedicated to my wife, Beatrice Adhiambo and daughter, Hadasah Achieng for their overwhelming support with understanding, patience and encouragement which enabled me to carry out this work smoothly to its conclusion. I also dedicate this work to my father, Michael Owange, mother, Gaudensia Arua, and my friends; Rusel Ochieng and Rev Moses Wangila for being wonderful confidants. To God be the Glory, Honour and Power, the one whom I pledge all allegiance.

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ACRONYMS AND ABREVIATIONS

Ab	Antibody
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid
Ag	Antigen
AHA	Animal Health Assistants
BDSL	Biological Diagnostic Supplies Limited
CABHW	Community Based Animal Health Workers
CDC	Centre for Disease Control and Prevention
CI	Confidence Interval
CV	Coefficient of Variation
CVL	Central Veterinary Laboratory
DVS	Department of Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
ENSO	El Nino/Southern Oscillation
GOK	Government of Kenya
GPS	Global Positioning System
ICIPE	International Centre for Insect Physiology and Science
ID	Identification

IDRC	International Development Research Centre
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQC	Internal Quality Control
KII	Key Informant Interview
LCL	Lower Control Limit
NE	North Eastern
MDGs	Millennium Development Goals
DoPH&S	Department of Public Health and Sanitation
NGOs	Non-Governmental Organizations
RNA	Ribonucleic Acid
RT-PCR	Reverse transcriptase polymerase chain reaction
RVF	Rift valley fever
RVFV	Rift Valley Fever virus
SAS	Statistical Analysis Software
SFV	Semiliki forest virus
SPSS	Statistical Package for Social Sciences
UCL	Upper Control Limit

WNV West Nile Virus

ABSTRACT

Ijara district in Kenya was one of the hotspots of Rift Valley Fever (RVF) during the 2006/2007 outbreak which led to human and animal deaths causing huge economic losses. The main constraint in the control and prevention of RVF is inadequate knowledge on its occurrence during the interepidemic period. This study was aimed at understanding the occurrence of RVF and perceived risk factors by pastoralists in cattle in Ijara to enable the development of improved community-based disease surveillance, prediction, control and prevention.

Six herds of 700 to 1000 cattle were identified and one animal tagged with Global Position System (GPS) collar to enable follow up during sero-surveys as well as understanding the herd's movement through various ecological zones. Sixty animals under 3 years from each herd were randomly selected during each sero-survey and sero-surveyed for RVF four times (September 2012, December 2012, February 2013 and May 2013) during the study period. Serum samples collected were subjected to RVF inhibition ELISA test to detect if there was exposure for RVF Virus (RVFV). The positive samples to RVF inhibition ELISA were subjected to IgM ELISA test to determine if the exposures were current (within 14 days). Thirty one key informant interviews were also conducted with relevant stakeholders to determine the local pastoralists' understanding of risk factors and risk pathways of RVF in cattle in Ijara district.

The result of the survey indicated that 13.1% (183/1396) of cattle sero-surveyed had RVFV antibodies under inhibition ELISA test while 1.2% (18/1396) of the cattle was positive when subjected to IgM ELISA. This clearly indicated that RVFV was in circulation in cattle in Ijara

district even during the interepidemic period. On the other hand, the respondents interviewed rated the high presence of mosquitoes, availability of large herds of cattle and once in a while high rainfall leading to floods in the relatively flat land of the region to be the main risk factors. Close contact between wildlife and cattle was suggested to be another main risk factor for occurrence of RVF. The main risk pathways were infected mosquitoes that bite cattle while grazing and at watering points as well as the close contact between domestic animals and wildlife. The likelihood of contamination of the environment due to poor handling of carcasses and aborted foetuses during RVF outbreaks was not considered an important pathway. The mobility of the cattle in search of pasture suggested the likelihood of infection transfer over a wide area.

The findings pointed that low rainfall within Ijara was able to maintain the circulation of RVFV in Ijara region with the ability to become an epidemic if the rainfall increased to cause extensive floods. As a result there is need to carry out regular participatory disease surveillance in domestic animals, vectors, human population and wildlife while carrying out community awareness as well as vaccination campaigns against RVF for preparedness, prevention and control of any possible epizootics. Additionally, monitoring of environmental conditions to detect enhanced rainfall and flooding should be prioritized for preparedness.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background information

Rift valley fever (RFV) is a mosquito-borne viral zoonosis that periodically causes disease outbreaks in humans and livestock and has been endemic in sub-Saharan Africa since 1912 (Peters *et al*. 1994). The disease is caused by rift valley fever virus (RVFV), a member of the genus *Phlebovirus*, family *Bunyaviridae* transmitted to humans through bites from infected mosquitoes and direct contact with tissues and blood of infected animals. Before the 1977 outbreak in Egypt, RVF was considered a disease of livestock with little impact on humans (Meegan *et al*., 1979) but subsequently, periodic outbreaks associated with widespread human infection resulting in acute febrile illness with hemorrhagic syndrome have been reported in many African countries, Saudi Arabia, Yemen and Mauritania (Hoogstraal *et a.l*, 1979; McIntosh *et al*., 1980; Meegan and Bailey, 1988; Ksiazek *et al*., 1989; Morvan *et al*., 1992; Abdo-Salem *et al*., 2006; CDC, 2007).

Outbreaks of RVF in North Eastern (NE) Kenya (Garissa County) have been associated with unusually heavy rainfall that causes extensive flooding of basins and low lying grassland depressions called *dambos*, triggering mass emergence of *Aedes* mosquitoes (Davies *et al*., 1985). In 1997/98 and 2006/07, massive outbreaks of RVF occurred in East Africa, both associated with *El Nino* events (Woods *et al*., 2002; CDC, 2007), with an estimated 27,500 human cases, and more than 600 deaths being reported in 1997/98 in Kenya alone. Historical outbreaks of RVF since the early 1950s have been associated with cyclical patterns of the *El*

Nino/Southern Oscillation (ENSO) phenomenon, which results in elevated and widespread rainfall over the RVF endemic areas of Africa (Anyamba *et al*., 2010). In Garissa, RVFV was first detected in livestock in 1961 and though 21 national outbreaks have been documented since then, only six of these occurred in Garissa district. The two out breaks, 1997/1998 and 2006/2007), were the most notable in terms of public health and socio-economic impact (Murithi *et al*., 2010).

The main economic livelihood for the people living in Garissa and Ijara district is livestock keeping. About 90% of the population is directly dependent on livestock for daily nourishment and as a source of resource. During the last outbreak, a ban on livestock trade and imposition of quarantine resulted in severe economic losses greater than US$9.3 million (Murithi *et al.,* 2010). Understanding disease transmission, spread and outbreaks requires a good understanding of vector ecology in terms of vector distribution and survival in relation to human and animal habitats, climatic conditions, cattle movement and trade. In Ijara district, livestock (cattle) are driven over long distances towards Tana River Delta or into Boni forest passing through various ecosystems. The prevalence of RVF and associated risk factors including increasing human and livestock populations putting pressure on pasture, water for livestock, wild animals, human beings and other public health amenities has not been well understood at the various points within the movement corridors. The study used cattle sero-survey and community participatory approaches to establish the occurrence of RVF and associated risk factors in Ijara along the livestock movement corridors. The information from this study can be used for awareness creation as well as formulating prevention and mitigation measures for the RVF.

1.2 Problem statement and Justification

Kenya's vision 2030 is a programme addressing the *Millennium Development Goals* (MDGs) that aims at transforming Kenya into "*a middle-income country providing a high quality of life to all its citizens by the year 2030*" (GOK, 2007). The programme seeks to, "*improve the overall livelihoods of Kenyans; the country aims to provide an efficient and high quality health care system with best standards*" (GOK, 2007). The Department of Public Health and Sanitation (DoPH&S) in the Ministry of Health is working in line with this vision and seeks to "*establish better health care provision and disease surveillance using modern information technology techniques*".

The State Department of Veterinary Services (DVS) in the Ministry of Agriculture, Livestock and Fisheries is mandated to "*prevent and control animal diseases and pests to safeguard human health, improve animal welfare, increase livestock productivity, ensure high quality livestock and their products and facilitate domestic and international trade"* The DVS vision is to "*promote and facilitate the achievement of optimal animal health, production, welfare and trade to contribute to public health, food security and poverty alleviation*". This study sought to fill important knowledge gaps in maintenance of RVF and associated risk factors in its ecosystem to enable the development of better community-based disease surveillance, prediction and prevention. The target was pastoral communities in NE Kenya who live in one of the most underdeveloped parts of the country with only limited access to healthcare for humans and animals. Apart from this, RFV outbreaks were mainly in these areas.

Garissa and Ijara districts were hotspots during the last RVF outbreaks in the arid/semi arid NE province of Kenya. The nomadic/semi-nomadic pastoralist communities maintain large

livestock herds even in circumstances of limited pasture and water. RVF outbreaks had caused major disruptions to public health and economic mainstay for this population. The movement of these viruses among animals, vectors and occasional involvement of human populations, under the influence of environmental factors required further study to better understand the interplay between the changing ecosystem, climate and the emergence of infections.

This study was part of a bigger project whose overall objective was to bring about a better understanding of the environmental, biotic and socio-economic drivers of emergence of RVF and other *arboviruses* and the viable control options in the arid/semi-arid NE province of Kenya, with focus on Ijara district, a major hotspot of the disease.

1.3 Objectives

1.3.1 Broad Objective

To describe the occurrence of RVFV and its associated risk factors in cattle in Ijara to enable the development of better community-based disease mitigation measures in the district.

1.3.2 Specific Objectives

i. To estimate the occurrence of RVFV in cattle in Ijara district
ii. To describe and map out perceived risk factors of RVF by local pastoralists in Ijara district.
iii. To describe the perceived risk pathways of RVF by local pastoralists in cattle in Ijara district

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition, Aetiolgy and Distribution

The Rift Valley Fever virus was first isolated from a sheep in 1930 during an epizootic at a farm by Lake Naivasha in the Rift Valley Province of Kenya (Daubney *et al*., 1931). Before the 1977 outbreak in Egypt, RVF was considered a disease of livestock with little impact on humans (Meegan *et al*., 1979) but subsequently, periodic outbreaks associated with widespread human infection resulting in acute febrile illness with hemorrhagic syndrome have been reported in many African countries, Saudi Arabia, Mauritania, Yemen and especially in regions of eastern and southern Africa, Egypt, Madagascar, Arabian peninsula, Kenya, Tanzania, and Somalia (Mundel & Gear, 1951; Scott & Heisch, 1959; Imam and Darwish, 1977; Hoogstraal *et al*., 1979; McIntosh *et al*., 1980; Meegan and Bailey, 1988; Ksiazek *et al*., 1989; Morvan *et al.,* 1992; Madani *et al*., 2003; Abdo-Salem *et al*., 2006; CDC, 2007).

In Kenya RVFV has been detected in 34 out of the 47counties including Baringo, Elgeyo Marakwet, Transzoia, Uasin Gishu, Bomet, West Pokot, Isiolo, Kajiado, Laikipia, Nakuru, Samburu, Marsabit, Nyeri, Embu, Nyandarua, Kitui, Machakos, Makueni, Meru, Tharaka Nithi, Garissa, Mandera, Wajir, Kiambu, Kirinyaga, Muranga, Tana River, Lamu, Kilifi, Kwale, Mombasa, Taita Taveta and Nairobi (Murithi *et al*., 2010, Munyua *et al*., 2010).

2.2 Cycles and Risk Factors

The life cycle of RVFV has distinct endemic and epidemic (epizootic) cycles. During the endemic cycle the virus persists within inter-epizootic periods through vertical transmission

in *Aedes* mosquito eggs (Linthicum *et al*., 1985). Flooding of mosquito habitats can introduce RVFV into domestic animal populations by the production of vertically infected *Aedes* mosquitoes. Epizootic/epidemic cycles are driven by the subsequent elevation of various *Culex* mosquito populations, which serve as excellent secondary vectors if immature mosquito habitats remain flooded long enough.

Apart from the mosquito transmission to domestic animals and humans, during the epidemic cycle, aerosols and contact between infected animals and human can transmit the virus (Anyamba *et al*., 2010).The virus is amplified in people and animals. Flat topography, presence of water retaining soil types and dense bush cover are important factors for flooding and or mosquito breeding (Anyangu *et al*., 2010).

2.3 Risk pathways

Risk pathway analysis involves investigation of possibility of entry, release/ exposure and eventual consequences of the disease. It helps establish the routes the disease follows for possible entry, establishment and spread (Breiman *et al*., 2010). It involves estimating the probabilities of occurrence considering the epidemiology of the disease. Factors such as vectors, hosts, animal movement pattern, and the role of wildlife are used in estimating the probability of occurrence of the disease. Many countries have adopted the Risk pathway analysis for emerging and re-emerging diseases in order for them to establish exact areas of target for better control and prevention (Kasari *et al.,* 2008). Breiman *et al.*, (2010) had previously documented that RVF can enter a new area through infected mosquitoes and their eggs, infected livestock, infected wildlife, infected humans and smuggling (terrorism action) of the virus. The virus can

then spread through mosquitoes' bites, contaminated environment by infected carcasses or aborted foetuses and movement of infected animals and humans leading to huge losses of lives, livelihoods and trade.

2.4 Signs and Symptoms

In animals, mass abortion and death of goats, sheep and cattle during heavy rains is an indicative sign. The lambs, kids, calves and pregnant animals are the most affected. In calves, clinical signs of febrile condition, anorexia, diarrhoea with bloody and or foetid character and fatalities 2-8 days after infection are common. Adult cattle manifests as acute or in apparent, fever for 24-96 hrs, anorexia, bloody/foetid diarrhoea, weakness, discharge from cranial mucous membranes (lachrymation, salivation, and nasal discharge), dysgalactia, icterus and abortion (Reininghaus, 2008).

Coetzer (1977) reported massive diffuse necrosis of hepatocytes, bile thrombi and intranuclear inclusions in hepatocytes in new-born lambs infected with RVFV. Lymphoid depletion in lymph nodes and spleen histopathologic findings has also been observed in cattle, calves, and aborted foetuses with RVFV infection (Coetzer, 1982).

2.5 Diagnosis

History of direct contact with sick or dead animals or the animals' products; or direct contact with body fluids of an infected person; or resident in or recent travel to an area where RVF activity in animals or humans was confirmed is paramount towards disease investigation. Clinical signs of abortion and foetid bloody diarrhoea during floods can guide laboratory tests (Reininghaus, 2008).

Laboratory confirmation of RVF is by detection of viral immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA), detection of viral RNA by real-time reverse transcriptase polymerase chain reaction (RT-PCR), detection of viral antigens in biopsy tissues by immunohistochemistry (Meegan *et al*., 1979; Madani *et al*., 2003; Mohamed *et al*., 2010).

2.6 Management, Prevention and Control

There is no curative medication in both animals and humans. It is therefore advisable to target the transmission process to prevent or control disease outbreaks. Control strategies should control mosquitoes which transmit the RVFV (Breiman *et al*., 2010). Public education for transmission risk reduction such as safe animal husbandry and slaughtering practices, safe consumption of livestock products reduces possible contact with infected animals and animal products hence reducing infection during outbreaks (Anyamba *et al*., 2010). Animal and human surveillance including sentinel and entomological surveillance permit very early detection which can be managed leading to minimal impact. Strengthening animal and human health agencies for early detection and response, collaboration of all stakeholders in identifying and mapping risk areas can substantially reduce the losses (Kasari *et al*., 2008)

2.7 Economic Impact

Massive outbreaks of RFV which occurred in East Africa in 2006/2007were associated with *El Nino* events (Woods *et al*., 2002; CDC, 2007). There were an estimated 27,500 human cases, and more than 600 deaths being reported in 1997/1998 outbreak in Kenya alone. There were a total of 121,069 animal deaths in Ijara alone **(**Rich and Wanyoike**,** 2010)**.** Apart from

direct losses resulting from livestock death, there were enormous losses to other sectors, for example, the effects on trade, the impact on human resources leading to diversion of production or activities. Both animal and animal products cannot be exported during the outbreaks. The 2007 outbreak led to cancellation of live animal export to Mauritius (Rich and Wanyoike, 2010).

2.8 Knowledge gap

There is limited information supporting the management of RVF. With cyclic occurrence of the disease, it is not certain where RVF virus is maintained during the inter-epidemic period since much of the work done is based on epidemic periods. Mosquito eggs have been postulated to maintain the RVFV during the inter-epidemic period (Anyamba *et al*., 2010) but the role of domestic and wild vertebrate animals in the maintenance of the virus during inter-epidemic periods has not been done (Robert *et al*., 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

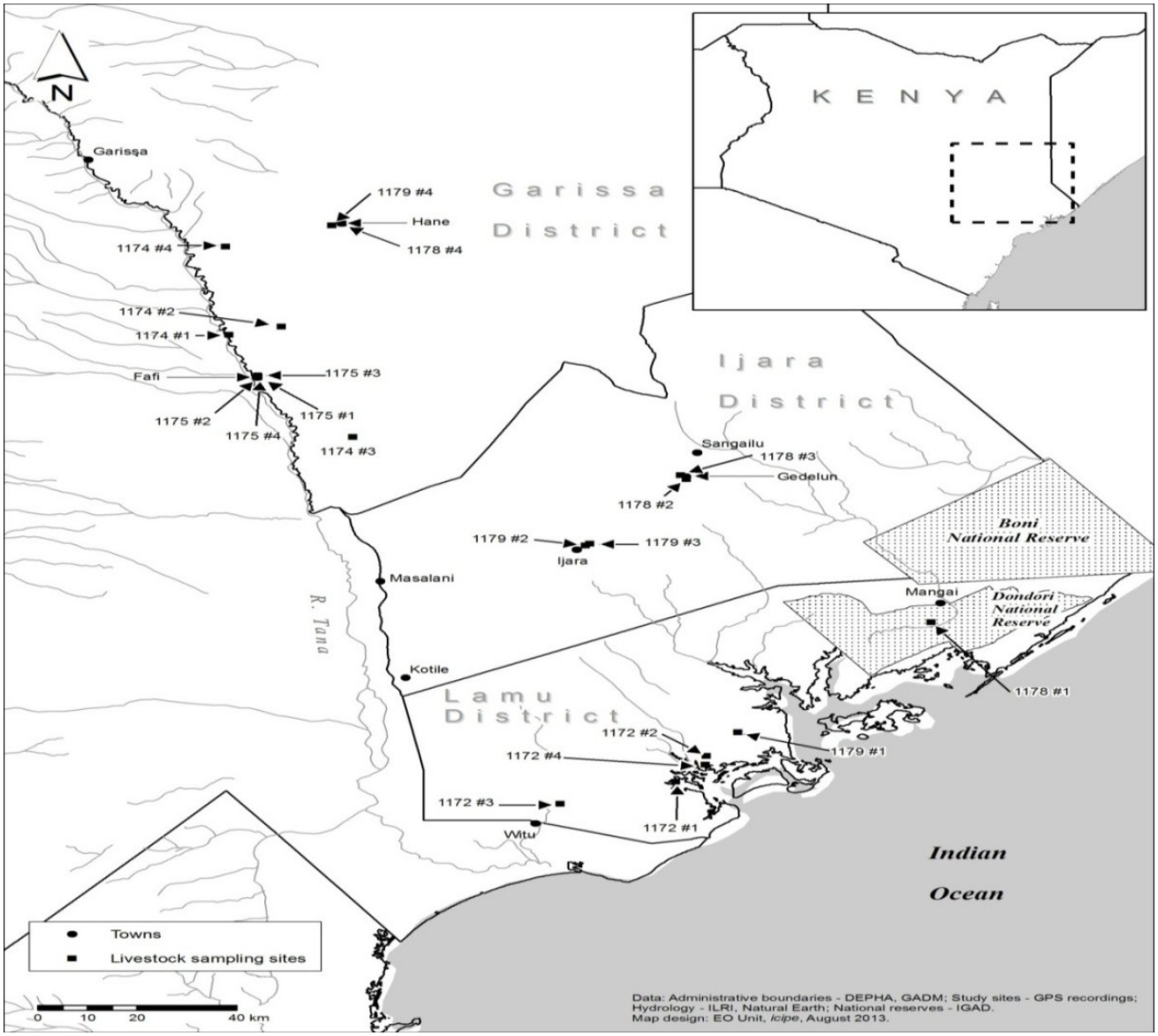

Figure 3.1: Map of study area comprising Garissa, Ijara and Lamu districts where livestock sampling for RVFV sero-survey was carried out.

The study was carried out in the arid and semi arid region of North Eastern Kenya between Garissa and Lamu counties with Ijara district at the centre of the study. However, due to cattle migratory movements, samples were collected while the selected herds moved through Garissa, Fafi, Lamu and Tana River districts as shown in the map of the study area as *Figure 3.1*.

More than 90% of the land in the study area was trust land and title deeds had not been issued. The study area falls in ecological Zone V-IV (arid and semi arid) with a total forest cover of 2,484Km². Boni forest, which is an indigenous open canopy forest that forms part of the Northern Zanzibar-Inhamdare coastal Forest Mosaic, covers a major part of the study area. A section of the forest, the Boni National Reserve is under the management of the Kenya Wildlife Service as a protected conservation area. The soil types are black cotton and alluvial, temperatures ranges between 15ºC – 38ºC, bimodal rainfall range between 700 to 1000mm per annum, average relative humidity of 68mm and altitude ranging between 0-90 meters above sea level.

Migration in the district is occasioned by search for pasture during the dry seasons and involves movement of people and livestock to the Tana River Delta and the Boni forest area where water and pasture are abundant long after the rainy season. Other movements in search of pasture and water are towards Fafi / Garissa districts and the Somali Republic.

3.2 Occurrence of RVF in cattle in Ijara District, Kenya

3.2.1 Sampling method

A longitudinal study of the occurrence of RVF in cattle in Ijara was carried out between August 2012 and June 2013. Six herds, each comprising 700 to 1000 cattle were identified through five focused group discussions at Fafi, Masalani, Ijara, Sangailu and Lamu with technical experts (Entomologists, Epidemiologists, Socio-economists, Anthropologists, Virologists, Veterinarians, Biostatisticians and administrators from International Centre for Insect Physiology and Science (*icipe)*, Department of Veterinary Services and the University of Nairobi, community elders and owners along the cattle movement corridors. In each herd, one of the animals was fitted with a GPS collar (Sweden) for monitoring the movement and tracking the herd for subsequent sero-surveys. The herds were identified by the collar number specific to the herd as 1172, 1174, 1175, 1178, 1179 and 1181. During the study, GPS collar, 1181 got lost and was replaced with a new GPS collar, 1177.

A sample size ($n=60$ per herd) was calculated using the formula $n=Z_{\alpha}^{2}pq/l^{2}$ (where Z_{α} is the ($1-\alpha/2$) percentile of a standard normal distribution). The $Z_{0.05}$required for confidence=95% is 1.96. P is a priori estimate of the proportion (sero-prevalence in ruminants = 20% (Cêtre-Sossah *et al.,* 2012), q is $1-p$ and l is the precision of the estimate (also called the 'allowable error' or 'margin of error') equal to ½ the confidence interval (Dahoo *et al*., 2010)}.

Cattle aged three years old and below were randomly sampled from each herd each time of sero-survey with no traceability to the individual animals sampled. The limitation of sampling by age to three years was meant to block out cattle with possibility of having RVFV

antibodies due to the previous outbreak in 2007. The actual ages were determined by inquiring the ages from the herd owner. In cases where the investigator was in doubt, dentition was used to determine the age. Blood samples were collected in September 2012 (baseline data), December 2012, February 2013 and May 2013. The sero-survey date was scheduled during the rainy season when the mosquitoes' activity was presumed to be highest. Cattle were chosen for this study following the advice from the DVS that no vaccination for RVF had been carried out in the study area in cattle in the region hence no chances of RVFV antibody in cattle due to vaccination.

3.2.2 Blood sample collection

Vacutainers (*BDSL*) or syringe fitted in the needle (*BDSL*) were used to collect 10 mls of blood from the jugular vein of every individual cattle after sterilizing the skin around the injection site with cotton gauze soaked in ethanol. The labelling on the Vacutainers included herd identification number and the sample number, which were also written on the sample collection sheet. A sample of the collection sheet is annexed as *appendix 1*. Serum was extracted from the whole blood sample by allowing the blood in glass containers to clot at room temperature for 1 hour then loosening the clot from the walls of the container to aid retraction. The containers were left at room temperature overnight. The expressed serum samples were collected and centrifuged at 350rpm (1548g) for 15minutes to sediment the erythrocytes (Fisher Centrific, 113mm radius centrifuge). The samples were transferred and stored frozen at -20^0c in solid carbon dioxide (*BOC, Kenya*). Both whole blood in EDTA and serum were stored in cool boxes before transfer to the Central Veterinary Laboratories (CVL) in Nairobi.

3.2.3 Laboratory Sample processing and Analysis

RVF Inhibition ELISA

An inhibition enzyme-linked immunoassay for the detection of antibody to RVFV in cattle was used. The inhibition ELISA kit (*BDSL*) with ability to process 1000 samples per kit was used. The inhibition ELISA is based on the ability of RVF antibodies in the test sera to inhibit the binding of RVF antigen to the capture antibody on the plate. The plates are coated with polyclonal anti-RVF capture antibody and then reacted with the serum/antigen mixture. If test sera contains anti-RVF antibody; this will bind to the RVF antigen in a separate incubation tube. A mouse anti-virus antibody added after the serum/antigen mixture will find few specific binding sites available, and the coloured reaction due to horseradish peroxidase (HRPO)-labelled anti-mouse antibody will be weak. In the absence of anti-RVF antibody, the RVF antigen in the serum/antigen mixture will be free and bound by the anti-RVF capture antibody on the plate, detected with mouse anti-virus antibody and HRPO-labelled anti-mouse antibody, which will result in a strong coloured reaction. The reagents were irradiated to inactivate RVF virus during manufacture hence considered safe for handling in the laboratory as long as safety procedures in the laboratory are adhered to.

The procedure for laboratory analysis adopted was the one adopted by Paweska *et al.,* (2003) and *BDSL* entitled "*IgG sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to RVFV in domestic ruminants*". Preparation of the agents involved dissolving 1 sachet of phosphate-buffered saline (PBS) in 1 litre of sterile distilled water to make 0.01M PBS at PH 7.4, diluting Tween 20 in PBS to a final concentration of 0.1%

to make wash buffer, preparing 2% skimmed milk in PBS to make diluent buffer and preparing 10% skimmed milk in PBS to make blocking buffer. The capture antibody was prepared by rehydrating polyclonal sheep anti-RVF in 200µl of sterile distilled water, control sera (C++, C+ and C-) by rehydrating in 200µl of sterile distilled water, antigens by rehydrating each in 500µl of sterile distilled water and the detection antibody by rehydrating in 100µl of sterile distilled water. The working dilution of capture polyclonal antibody (1:400) was prepared in PBS, working dilutions of control and test sera (1:10), antigens (1:10), detection antibody (1:500), conjugate (1:2000) were prepared in diluent buffer. The substrate was used as supplied whereas the stop solution was dilute 1:10 in distilled water.

For each day's test the required volumes/working dilutions of reagents were freshly prepared from stocks of reagents. However, reconstituted reagents stored at 4°C were also used since sterile procedures and tips were used to remove aliquots. Due to periodic or intervallic collection of blood from the field, sometimes reagents were diluted 1:10 in PBS, aliquoted in small volumes, and stored at -70°C until required, except for the virus and control antigens. In such cases, the dilution factor was accounted for when using reagents that had been diluted before storage.

The plate layout consisted of C++ (High positive control serum), C+ (Low positive control serum), C- (Negative control serum), 1-40 (Test sera), Rows A-D 1-12 (RVFV Ag) and Rows E-H 1-12 (Control Ag) as shown in Table 3.1.

During the test procedure, volumes used were 100µl /well, and all washes were performed 3 times for 15seconds using 300 µl of wash buffer per well. During step 2 test sera and antigen

were mixed in a separate plate or diluting tubes, not the ELISA test plate. Coating of plates was done using 100µl polyclonal sheep anti-RVF capture antibody diluted 1:400 in PBS after which incubated plates covered were with lids at 4°C overnight, then plates washed. 200µl /well blocking buffer were then added and plates incubated for 1hour in moist chamber at 37°C then plates washed. During the blocking stage, 21µl of each undiluted test and control sera was added into diluting wells containing 189µl virus or control antigen pre-diluted 1: 10 in 2% skim milk in PBS. 100µl of test and control sera / virus antigen mixture was then added to rows A-D 1-12 and 100ul of test and control sera / control antigen mixture to rows E-H 1-12 as shown in plate layout (Table 3.1) and incubated for 1hour in moist chamber at 37°C. After washing the plates, 100µl/well of mouse anti-virus diluted 1:500 in diluents buffer was added and incubated for 1hour in moist chamber at 37°C. The plates were washed then added 100µl /well of anti-mouse IgG HRPO-conjugate diluted 1:2000 in diluents buffer and incubate for 1h in moist chamber at 37°C. The plates were then washed 6 times then added 100µl of 2, 2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid (ABTS)/well. The plates were left for 30 min. at room temperature (22-25°C) in dark then 100µl of 1 x concentrated SDS stop solution was added then optical density read at 405nm.

Table 3.1: Plate layout for RVF Inhibition ELISA used at the Kabete Central Veterinary Laboratories

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	C++	1	5	9	13	17	21	25	29	33	37
B	C+	C+	2	6	10	14	18	22	26	30	34	38
C	C-	C-	3	7	11	15	19	23	27	31	35	39
D	C-	C-	4	8	12	16	20	24	28	32	36	40
E	C++	C++	1	5	9	13	17	21	25	29	33	37
F	C+	C+	2	6	10	14	18	22	26	30	34	38
G	C-	C-	3	7	11	15	19	23	27	31	35	39
H	C-	C-	4	8	12	16	20	24	28	31	36	40

A specific activity of each serum (net Optic Difference-OD) was calculated by subtracting the non-specific background OD in the wells with control antigen from the specific OD in wells with virus antigen. The mean OD readings for replicate tests were converted to a percentage inhibition (PI) value using the equation: [(100 – (mean net OD of test sample / mean net OD of negative control) x 100]. The internal quality control (IQC) validity data and the diagnostic

accuracy of RVF inhibition ELISA from the manufacturer shown in table 3.2 and table 3.3 were adopted.

Table 3.2: A table showing Internal Quality control data for RVF ELISA used at Kabete Regional Veterinary Investigation Laboratory (source; BDSL, LCL = Lower control limit, UCL = upper control limit, PI = Percent inhibition, IQC = Internal Quality Control)

	IQC	LCL	UCL
OD	C-	0.65	1.34
PI	C++	94.26	102.8
PI	C+	48.34	79.5
PI	C-	-4.26	4.33

Table 3.3: A table showing diagnostic accuracy of the Rift Valley fever inhibition ELISA (Source; BDSL)

Measure	Cattle
Cut-off	41.9PI
D-se (%)	100
D-sp (%)	99.52

Cut-off values expressed as a PI of an internal negative serum control was optimized at 95% accuracy level by a two-graph receiver operating characteristic (TG-ROC) analysis. D-Se and D-Sp refer to diagnostic sensitivity and specificity (Source, *BDSL*)

RVF IgM ELISA

Only positive samples from Inhibition RVF ELISA were subjected to RVF IgM ELISA which is a capture enzyme-linked immunoassay used for the detection of anti-RVFV IgM antibody in cattle sera. The procedure for laboratory analysis adopted was the one documented by Paweska *et al.,* (2003). One RVF IgM ELISA kit (*BDSL,*) has the ability to process 1000 samples. It is based on a capture format in which the plates are coated with rabbit anti-sheep IgM capture antibody and then reacted with test sera. Anti-sheep capture antibody can be used for detection of IgM in sheep, goats and cattle**.** The captured IgM antibody was reacted with RVFV antigen, and the bounded antigen was then detected with mouse anti-RVFV antibody and anti-mouse HRPO conjugate plus ABTS substrate. The reagents have been irradiated to inactivate RVF virus for safety while handling.

During the preparation of the reagents, PBS, 0.01M, pH 7.4 was reconstituted by dissolving 1 sachet of PBS in 1 litre of distilled water, wash buffer prepared by diluting Tween 20 in PBS to a final concentration of 0.1%, diluent buffer by preparing 2% skimmed milk in PBS and blocking buffer by preparing 10% skimmed milk in PBS. The capture antibody was prepared by rehydrating each Rabbit anti-sheep IgM in 250µl of sterile distilled water, control sera by rehydrating each in 200µl of sterile distilled water, antigens by rehydrating RVFV antigen ,each in 300µl of sterile distilled water and detection antibody by rehydrating Mouse anti-RVFV serum each in 100µl of sterile distilled water. The working dilution of capture antibody (1:500) was prepared in PBS, working dilutions of control and test sera (1:400), antigens (1:200), detection antibody (1:1000) and conjugate (1:5 000) was prepared in diluent buffer while the substrate was used as supplied. The stop solution was prepared by diluting 1:10 in distilled water.

For each day's test the required volumes/working dilutions of reagents were freshly prepared from undiluted stocks. However, reconstituted reagents stored at 4°C were also used since sterile procedures and tips were used to remove aliquots. Due to periodic or intervallic collection of blood from the field, sometimes reagents were diluted 1:10 in PBS, aliquoted in small volumes, and stored at -70°C until required, except for the virus and control antigens. In such cases, the dilution factor was accounted for when using reagents that had been diluted before storage. The plate layout was as shown in Table 3.1.

During the test procedure, volumes used were 100µl/well, and all washes were performed 3 times for 15s using 300 µl of wash buffer per well. Coating of plates was done with

100µl rabbit anti-sheep IgM diluted 1:500 in PBS and then plates incubated covered with lids at 4°C overnight. After washing the plates, 200µl/well blocking buffer was added and incubated for 1hour in moist chamber at 37°C. The plates were then washed and 100µl of test and control sera diluted 1:400 in diluent buffer added into wells as shown in table 3.1 plate layout and incubated for 1hour in moist chamber at 37°C. After washing the plates, 100µl of RVFV Ag and control Ag diluted 1:200 in diluent buffer was added to rows A-D 1-12 and rows E-H 1-12, respectively (see plate layout in table 3.1) and incubated for 1hour in moist chamber at 37°C then plates washed. 100µl/well of mouse anti-RVFV serum diluted 1:1000 in diluent buffer was added and incubated for 1hour in moist chamber at 37°C. The plates were then washed before adding 100µl/well anti-mouse IgG HRPO conjugate diluted 1:5000 in diluent buffer and incubating for 1hour in moist chamber at 37°C. The plates were then washed 6 times before adding 100µl of ABTS/well. The plates were left for 30 minutes at room temperature (22-25°C) in dark before adding 100µl of 1% SDS stop solution and reading optical density at 405nm.

The amount of colour developed was proportional to the amount of anti-RVFV IgM antibody that had been captured. Net optical density (OD) values were first recorded for each serum as the value determined with RVFV Ag minus the value determined with control Ag. Three levels of micro plate acceptance were applied. The results on a test plate fulfilled the first level of internal quality control (IQC) acceptance if at least three of the net OD values recorded for C++ fell within the range 0.8 (lower control limit) to 1.85 (upper control limit); if the results of two or more of the four replicates of C++ fell outside IQC limits then the plate was rejected and repeated. If the plate was accepted, then the two intermediate net OD values of C++ were

used for the calculation of the net mean OD value of C++. This value was then used in subsequent calculations of percentage positivity (PP) of C+, C- and test sera as PP= {Net OD serum (C+,or C-, or Test serum) / Net mean OD C++}X100. [The results obtained on a test plate fulfil the second level of IQC acceptance if the coefficient of variation {CV = (standard deviation of replicates/mean x 100) for PP values of two replicates of C++ (calculated from intermediate net OD values) and two replicates of C+ are less than 15 %}]. Using the thresholds PP values of cattle sera producing PP values ≥14.3PP were considered to be positive, and less than 14.3PP values were considered to be negative. Both replicates of the C+ and C- control sera must fall within the same interpretive group, i.e. positive or negative (third level of IQC acceptance). The same principle is applied for the acceptance of individual test sera if they were assayed in duplicate.

Molecular Analysis

Though molecular identification of the virus is the sure way of confirming the presence of RVFV, funds were not available for this work. The positive IgM samples are however preserved for possible RVFV isolation once the funds are available.

3.2.4 Data Analysis

The data analysis was done using Statistical Package for Social Sciences (SPSS) version 15.0 and excel (Ms excel 2007) to calculate the percentage detection of RVF antibodies within the herds, time of sampling, by age and overally using both Inhibition and IgM ELISA results. The age of cattle was determined from the owners view point as well as using dentition where the investigator doubted the cattle owners' age suggestion. Confidence intervals for proportion

of detected antibodies were calculated at 95% confidence and level of significance. Chi square ($\chi 2$) was used to detect any association of the antibody detection between the herds, different ages (3 age groups, that is <1, 1-2 and 2-3) and time of collection. Multivariate Analysis was conducted to check if age of cattle, herd location, time of sampling and sex were significant factors in determining the sero-prevalence of RVFV. ArcGIS version 3.1 was used to draw maps from GPS coordinates during sero-sampling, key informant interviews as well as downloaded coordinates from the GPS collars for the herds.

3.3 Perceived Risk factors and risk pathway analysis by local pastoralists for RVF in cattle in Ijara District, Kenya

3.3.1 Sampling Method

Purposive sampling was used to select individuals who were later interviewed in order to describe the perceived risk factors and risk pathways for RVF in cattle as understood by the locals (Chambers, 2010; World Bank, 2004). Targeted stakeholders for key informant interviews included local leader(s) in charge of every selected herd of cattle for sero-survey, veterinary officers, animal health assistants, community based animal health workers, Kenya Wildlife Service personnel, and local administrative officers. The GPS collaring of one cattle in each of the six herds (1172, 1174, 1175, 1178, 1179 and 1181) enabled mapping of cattle movement for RVF in the study area.

3.3.2 Key Informant Interviews

Key informant interviews were used to identify and rank the perceived RVF associated risk factors and risk pathways by the local pastoralists in the study area. Non formal ranking

was used and later cross checked by a more formal pair wise and matrix ranking and scoring. The identified pathways were also qualitatively ranked as high, medium, low or negligible in the study area by the respondents. The check list guide used during the Key Informant Interviews is annexed in this document as *appendix 2*. The categories of people interviewed are also annexed as *appendix 3*.

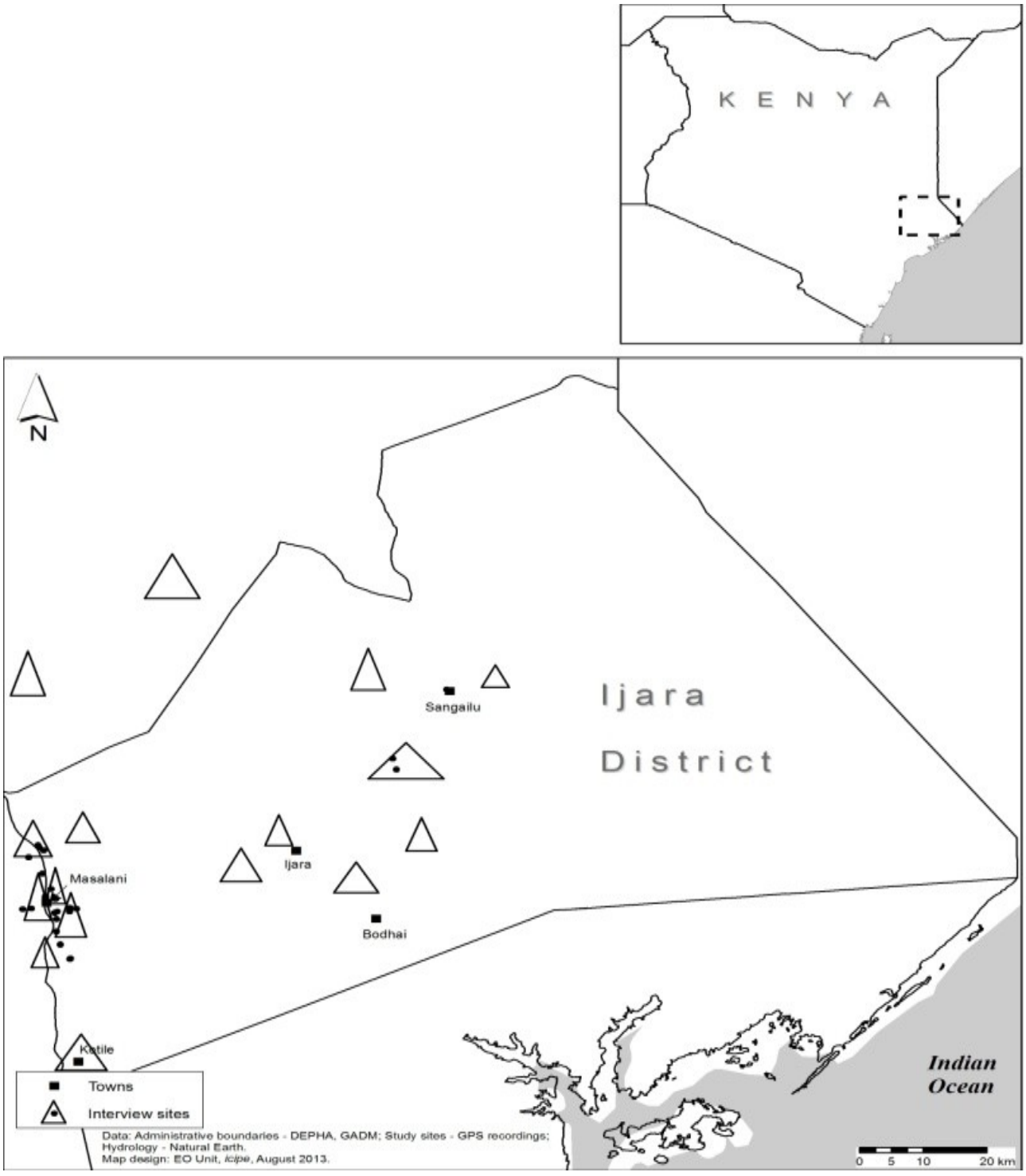

Figure 3.2: Map of study area showing areas where Key Informant Interviews for perception of the pastoralists on risk factors and risk pathways for RVF were carried out

As shown in figure 3.2, most of the key informant interviews were done in Ijara district (the intended district of study). The interviews showing as outside Ijara district were for respondents who were interviewed away from their home district (Ijara) at the time of the interview due to their commitments during the interviewing period.

3.3.3 Data Analysis

Qualitative data analysis was undertaken on a continuous basis and in such a way that subsequent phases benefited from what was foregoing. However, at the end of the data collection process, all information gathered was analyzed for purposes of addressing the objectives of the study. SPSS version 15.0 was used to calculate *Kendall's coefficient of concordance* to understand the degree of agreement between the key informants on perceived risk factors and risk pathways for RVF in cattle in the study area. Additionally, these analyses were preceded by examining, categorizing, tabulating and recombining evidence in order to establish the perceived associated risk factors for RVF to enable the development of better community-based disease surveillance, prediction and prevention in Ijara district. Two dominant techniques were used in the analytical strategy - pattern matching and explanation building (Yin, 1994). Overally, the analysis was driven by the investigator's rigorous thought, along with the sufficient presentation of evidence and careful consideration of alternative interpretations. Important comparisons to rival propositions and threats to the internal validity of any suggested conclusion was explicitly stated for each finding. ArcGIS version 3.1 was also used for mapping the cattle movement during the study period as well as map associated risk factors for understanding the RVF risk pathways.

CHAPTER FOUR

RESULTS

4.1 Occurrence of RVF in cattle in Ijara District, Kenya

4.1.1 Overall Inhibition ELISA results

Blood samples were collected from 1396 cattle. Out of the 1396 blood samples, 183 (13.1%) (95% CI: 11.3, 14.8%) were positive for RVF Inhibition ELISA test. Among the 1396 cattle, 416 (29.8%) (95% CI: 27.4, 32.1%) were less than 1 year old, 510 (36.5%) (95% CI: 33.9, 39.0%) 1-2 years old and 470 (33.7%) (95% CI: 31.2, 36.1%) 2-3 years old. From the whole sample, 1.8% (95% CI: 1.1, 2.4%) of the calves less than one year old, 3.2% (95% CI: 2.2, 4.1%) of 1-2yrs and 8.1% (95% CI: 6.6, 9.5) of 2-3 years old cattle were positive with the RVF inhibition ELISA. There was significant association in antibody detection between cattle aged 2-3 years with those aged 1-2 years and calves <1 year i.e. for every RVF antibody detection in calves <1 year old there would be five detections in 2-3 years of age (χ^2 = 54, RR=0.2, α =0.05, 1 df) while for every three RVF antibody detections in cattle aged 1-2 years old there would be 10 detections in cattle between 2-3 years old (χ^2= 41, RR=0.3, α =0.05, 1 df). The multivariate analysis conducted (R^2=0.052, F (4, 1391) =19.193, p<0.001) showed that age have significant positive regression weights. Herd location, time of sampling and sex were not significant. Figure 4.1 shows a summary of the inhibition ELISA results.

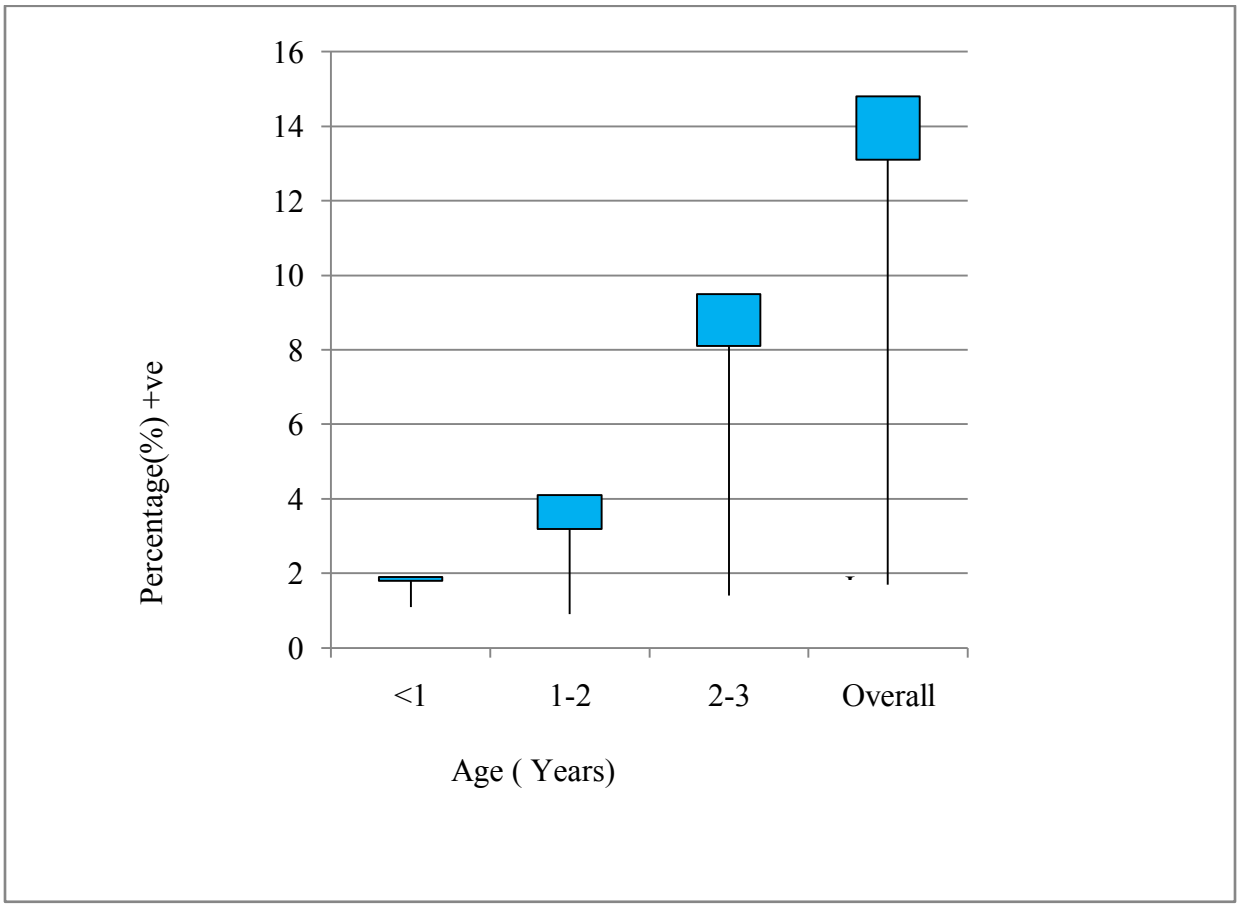

Fig 4.1: A graphical presentation of RVF Inhibition ELISA results for 1936 cattle sero-survey in Ijara district in Kenya from September 2012 to May 2013. (The blue blocks show an area within the upper confidence interval and the actual percent RVFV antibody detection).

4.1.2 Overall IgM ELISA results

One point two percent (95% CI: 0.7, 1.7%) of the 1396 samples were positive for IgM ELISA. Out of the total, 0.2% (95% CI: 0, 0.4%) were calves less than 1 year old, 0.3% (95%

CI: 0, 0.6) 1-2years and 0.7% (95% CI: 0.3, 1.1%) 2-3 years old. The association between the IgM ELISA results by age was not statistically significant. Figure 4.2 shows details of IgM ELISA results.

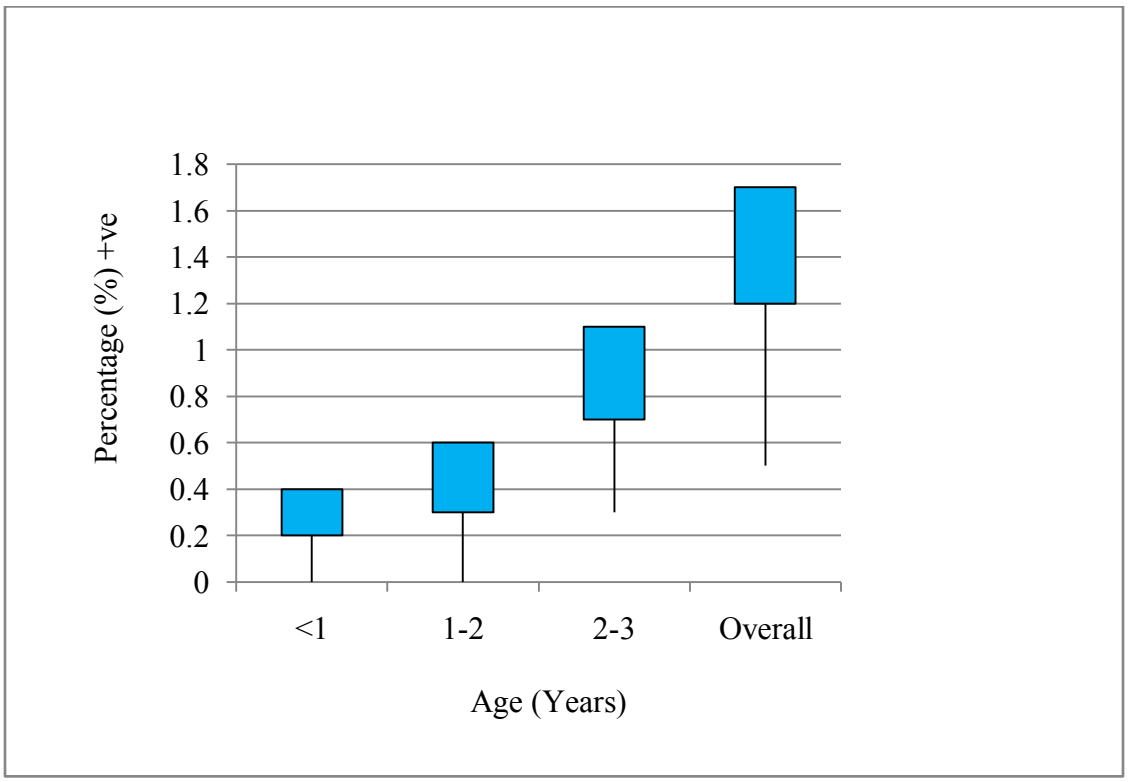

Figure 4.2: A graphical presentation of RVF IgM ELISA results for 1396 cattle sero-surveyed in Ijara district in Kenya from September 2012 to May 2013. (The blue blocks show an area within the upper confidence interval and the actual percent RVFV antibody detection).

4.1.3 Antibody detection results based on period of sero-survey

Overall antibody detection based on inhibition ELISA and IgM ELISA for different periods of sero-survey in September 2012, December 2012, February 2013 and May 2013 were 9.2% (95% CI: 6.4, 12.1%), 15.7% (95% CI: 12.0, 19.5%), 7.5% (95% CI: 4.9, 10.1%) and

13.9% (95% CI: 10.2, 17.2%) and 0.5% (95% CI: -0.1, 1.1%), 1.3% (95% CI: 0.1, 2.5%), 0% (95% CI: 0, 0) and 2.6% (95% CI: 1.0, 4.1%) respectively. Figure 4.3 shows a graphical presentation of the results. The results in figure 4.3 show increased antibody detection in December 2012 and May 2013 and low detection in February 2013.

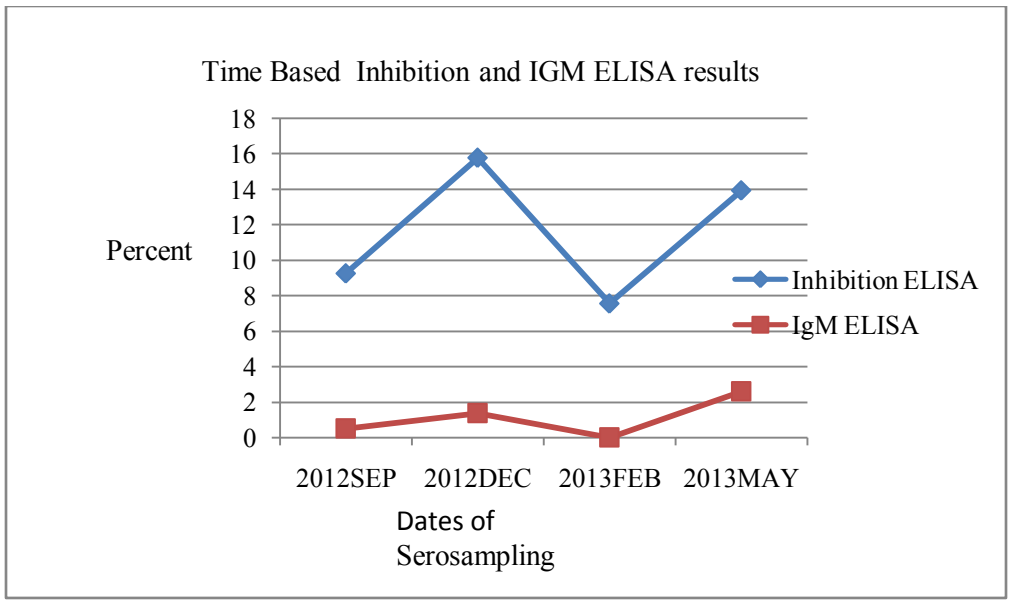

Figure 4.3 Line graph showing period based RVF Inhibition ELISA and IgM ELISA results for cattle sero-survey in Ijara district-Kenya, between September 2012 to May 2013

4.1.4 Period based antibody detection and rainfall pattern

The results of increased RVF antibody detection in December 2012 and May 2013 and low detection in February 2013 can be attributed to normal rainfall in October, November and September 2012 as well as in March, April and May 2013 with low rainfall (dry season) in September 2012 and January 2013 as reported by the Kenya Meteorology Department (Figure 4.4)

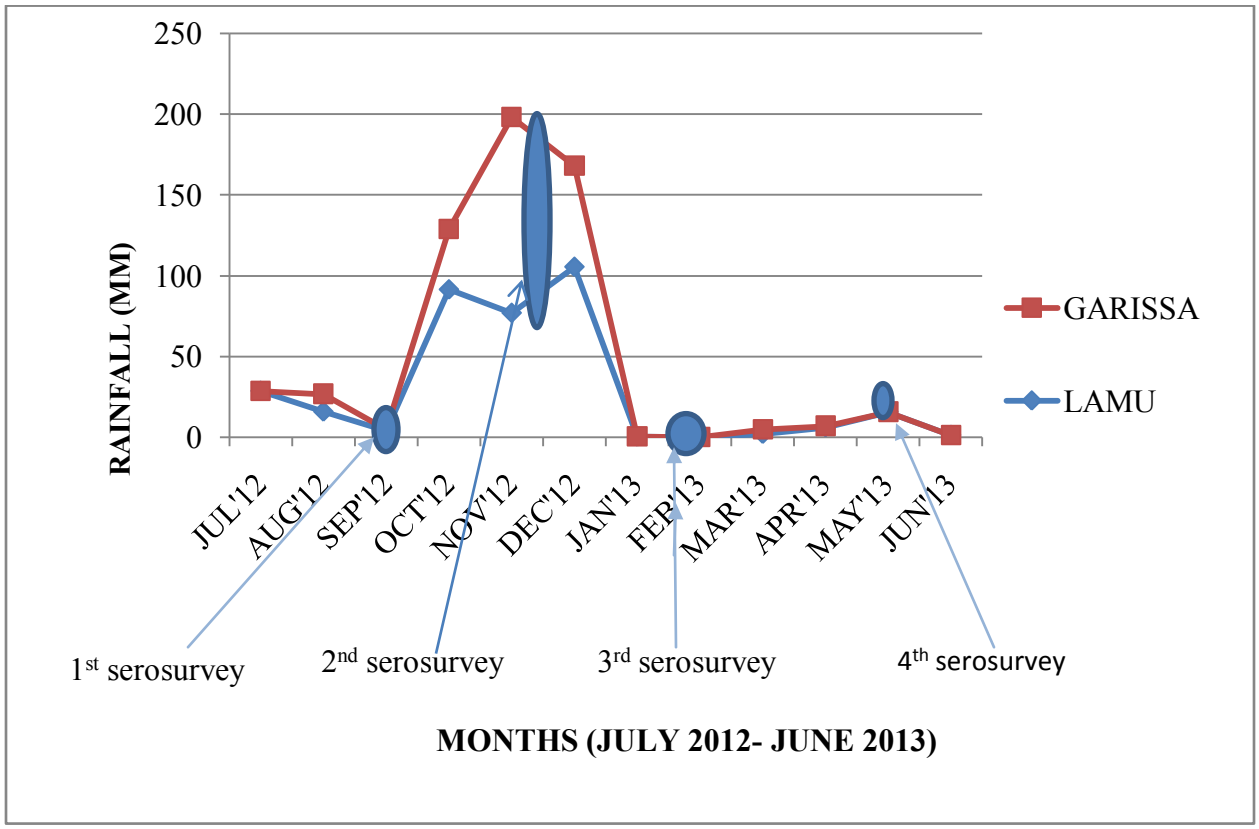

Figure 4.4 *Line graph showing period based RVFV antibody detection and rainfall pattern results for cattle sero-survey in Ijara district, Kenya, from July 2012 to may 2013 (Data used for drawing this graph came from Garissa and Lamu stations of Kenya Meteorological Department and the RFV livestock sero-survey)*

4.1.5 Herd based Antibody detection results

Overall herd antibody detection based on inhibition ELISA and IGM ELISA for herds 1172, 1174, 1175, 1178, 1179 and 1181 are 16.9% (95% CI: 12.1, 21.6%), 4.6% (95% CI: 1.9, 7.2%), 12.8% (95% CI: 8.5, 17.0%), 18% (95% CI: 13.1, 22.8%), 16.8% (95% CI: 12.1, 21.4%) and 8.2% (95% CI: 4.2, 12.1%) and 0.8% (95% CI: -0.3, 1.9%), 0.8% (95% CI: -0.3, 1.9%),

0.4% (95% CI:-0.3, 1.2%), 1.6% (95% CI: 0.04, 3.2%), 2.4% (95% CI: 0.5, 4.4%) and 1.6% (95% CI: -0.2, 3.4%) respectively. Figure 4.5 shows a graphical presentation of the results.

From the RVF Inhibition ELISA results, the herd antibody detection increased between September and December 2012 and between February 2013 and May 2012. Except for herd 1174 that the antibody detection increased between December 2012 and February 2013, all the other herds the antibody detection decreased. Herds 1178 and 1197 that were the most mobile showed the highest viral activity with respect to inhibition ELISA compared to the less mobile herds 1172, 1174, 1175 and 1181 (see figure 4.5 and 4.6)

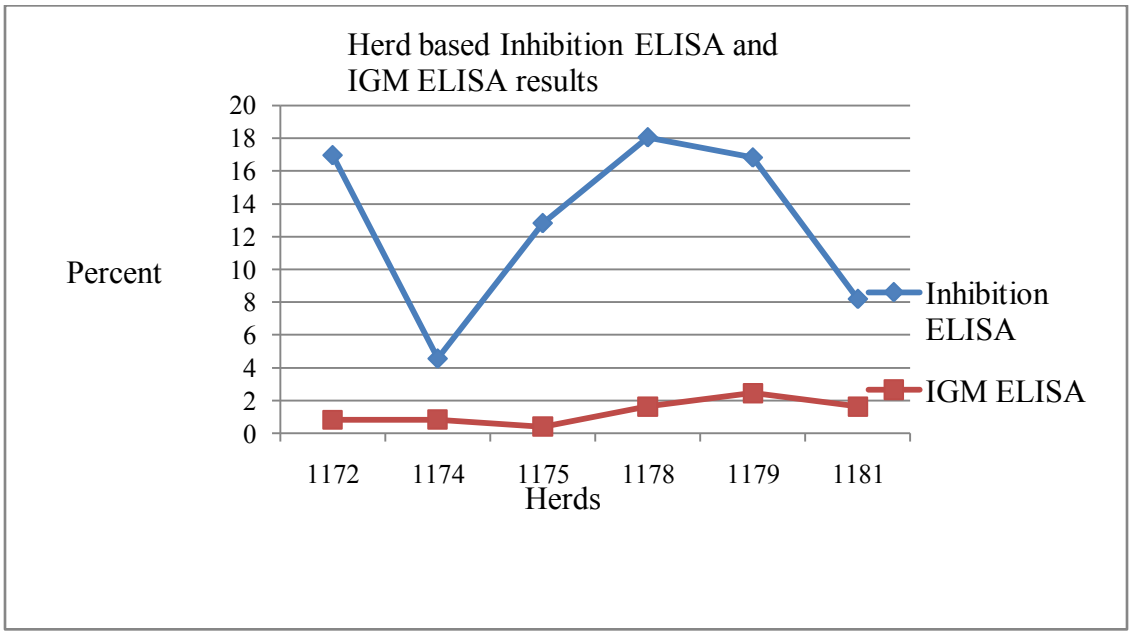

Figure 4.5: Herd based RVF Inhibition ELISA and IgM ELISA results for cattle serosurvey in Ijara district-Kenya, from September 2012 to May 2013

4.1.6 Cattle movement results

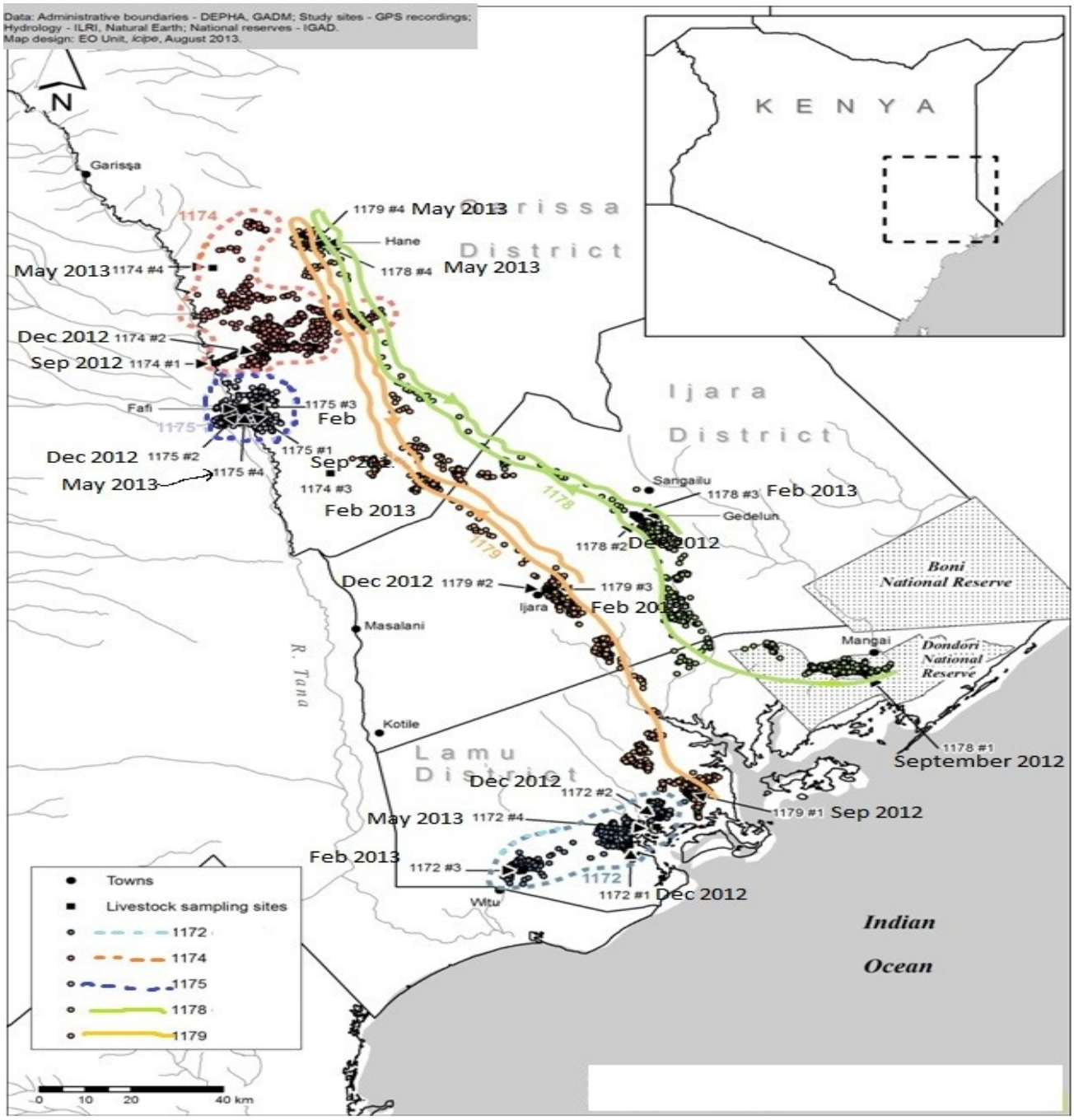

Fig 4.6: Map of study area showing cattle movement pattern and livestock sampling locations for RVF between September 2012 and May 2013 in Ijara study area

Referring to figure 4.6, herds 1178 (continuous lime/orange line) and 1179 (continuous gold line) were the most mobile herds moving from Lamu to Ijara through the thick Boni forest between the first collections in September 2012 at Lamu to the second collection in December 2012 in Ijara, a distance of about 100km. These two herds (1178&1179) moved from Ijara towards Garissa from February 2013 to May 2013 through the thick forest, a distance of about 120km. High RVFV antibody titters were detected after these movements. Between December 2012 and February 2013, these two herds were grazing around the homesteads in Ijara and showed decreased RVF antibody detection in the herds by February 2013. Herds 1172 (interrupted light turquoise/blue line), 1174 (interrupted orange line) and 1175 (interrupted indigo line) maintained a closer oscillation around their grazing areas and showed lower antibody detection compared to the mobile herds 1178 and 1179 (Figure 4.5 and 4.6).

4.2 Perceptions of pastoralists on RVF risk factors in cattle in Ijara District, Kenya

4.2.1 Pair wise ranking of domestic food animals in Ijara by the key informants

Cattle were considered the most important livestock followed by goats, sheep, donkey and poultry respectively (Table 4.1). Camel was not present in Ijara hence could not be ranked.

Table 4.1: Summarized results for pair wise ranking of livestock species to determine perceived importance by the pastoralists' key informants in Ijara, Kenya

Name of Livestock spp	**Fraction scoring**	**Score***	**Kendall's coefficient of concordance (W)**, p<0.05**
Cattle	18/31	1	0.146
	13/31	2	
Goats	12/31	1	0.129
	19/31	2	
Sheep	1/31	1	0.148
	12/31	2	
	18/31	3	
Poultry	5/31	4	0.17
	26/31	5	
Donkey	29/31	4	0.39
	2/31	5	
Camel	31/31	0	1

KEY: Number of key informants was 31.

* Score of 1, 2, 3, 4, and 5 show perceived decreasing degree of importance whereas 0 means not applicable.

** W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement

4.2.2 Pair wise ranking of livestock diseases affecting cattle in Ijara by the key informants

According to the respondents (Table 4.2), RVF was considered third most important disease of cattle after Trypanosomiasis and Contagious Bovine Pleura Pneumonia (CBPP). Other diseases which were also mentioned as important were Black quarter, Tick Borne

Diseases, Anthrax, Lumpy Skin Disease (LSD), Foot and Mouth Disease (FMD) and Helminthiasis.

Table 4.2.: A table showing summarized results for pair wise ranking of cattle diseases to determine perceived importance by the pastoralists' key informants in Ijara, Kenya

Disease	**Fraction Scoring**	**Score[a]**	**Kendall's coefficient of concordance (W)[b], p<0.05**
Rift Valley Fever (*Sandik*)[c]	1/31	1	0.146
	4/31	2	
	13/31	3	
	6/31	4	
	6/31	5	
	1/31	6	
Trypanosomiasis (*Gendi*)[c]	25/31	1	0.126
	5/31	2	
	1/31	3	
Black Quarter (*Bashasha*)[c]	3/31	2	0.1999
	9/31	3	
	15/31	4	
	4/31	5	
Contagious Bovine Pleuropreumonia (*Sanab*)[c]	5/31	1	0
	18/31	2	
	8/31	3	
Tick Bone Diseases (*Qanda*)[c]	1/31	2	0.275
	1/31	3	
	4/31	4	
	20/31	5	
	1/31	6	
	4/31	8	
Lumpy Skin Disease (*Kuskus*)[c]	20/31	6	0.0153
	9/31	7	
	2/31	0	
Anthrax (*Kut*)[c]	3/31	4	0.065

	1/31	5	
	3/31	6	
	7/31	7	
	12/31	8	
	5/31	0	
Helminthosis (*Gorian*)[c]	1/31	4	0.169
	6/31	6	
	7/31	7	
	9/31	8	
	8/31	0	
Foot and Mouth Disease (*Abeb*)[c]	1/31	4	0.079
	3/31	7	
	8/31	8	
	19/31	0	
Rabies	1/31	8	0.134
	30/31	0	

KEY: Number of key informants was 31.

[a] Score of 1, 2, 3, 4, 5, 6, 7 and 8 show perceived decreasing degree of importance whereas 0 means not mentioned.

[b] W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement.

[c] Somali name of the disease

4.2.3 Pair wise ranking of perceived RVF risk factors by the pastoralists in Ijara by the key informants

According to the respondents (table 4.3), availability of vectors (W=1, P<0.05), large number of cattle (W=O.146, P<0.05) and high rainfall (W=0.08, P<0.05) are rated number 1 and or 2 (most important and or important) risk factors associated with RVF in Ijara. There was varied low agreement perception on soil types (W=0.074, P<0.05), dambos (W=0.403, P<0.05), bushy vegetation (W=0.132, P<0.05), wildlife (W=0.156,

P<0.05) and flat topography (W=0.063, P=0.05) ranging from 2 (important) to 4 (not important) risk factors. All the respondents rated drought as not important (W=1, P<0.05) risk factor associated with RVF. High temperature was also rated as less important to not important risk factor. High temperature was perceived as less important or not important risk factor of RVF.

Table 4.3: Summarized results for pair wise ranking of RVF risk factors in cattle as perceived by the pastoralists' key informants in Ijara, Kenya

Risk Factor	Fraction Scoring	Score*	Kendall's coefficient of concordance (W)**, p<0.05
Vector (Mosquitoes)	31/31	1	1
Rainfall	12/31	1	0.08
	19/31	2	
Drought	31/31	4	1
Floods	27/31	1	0.116
	4/31	2	
Dambos	2/31	1	0.403
	9/31	2	
	18/31	3	
	2/31	4	
Soil type	4/31	2	0.074
	25/31	3	
	2/31	4	
Bushy vegetation	15/31	2	0.132
	16/31	3	
Wild life	4/31	1	0.156
	18/31	2	
	8/31	3	
	1/31	4	
Flat topography	1/31	2	0.063
	24/31	3	
	6/31	4	
Cattle	29/31	1	0.146
	2/31	2	
High temperature	18/31	3	0.485
	13/31	4	

KEY: Number of key informants was 31.
* Score of 1 = Most important, 2 = Important, 3 = Less important and 4 = Not important show perceived degree of importance of RVF risk factor
** W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement.

4.3 Perceptions of pastoralists on RVF Risk pathways in cattle in Ijara District

4.3.1 Qualitative ranking of perceived RVF entry pathways by the pastoralists key informants in Ijara, Kenya

As shown in table 4.4, the perceived entry risk pathways for RVF in Ijara district according to the key informants were infected mosquitoes, infected domestic animals, infected aborted foetuses and fluids and infected wild animals. The respondents perceived the most likely routes of RVF entry in Ijara to be through infected mosquitoes, infected domestic and wild animals. Key informants rated infected aborted foetuses and fluids to having very low chance of RVF entry in the study area. Virus smuggling from the neighbouring Somali country was perceived to be a negligible means of virus entry in Ijara.

Table 4.4: Summarized results for qualitative ranking of RVF entry risk pathway in cattle as perceived by the pastoralists' key informants in Ijara, Kenya

RVF entry pathway	Fraction Scoring	Score*	Kendall's coefficient of concordance (W)**, p<0.05
Infected mosquitoes	31/31	1	1
Infected domestic animals	30/31	1	0.051
	1/31	2	
Infected aborted foetuses and fluids	3/31	1	0.115
	17/31	2	
	11/31	3	
Infected wild animals	10/31	1	0.011
	18/31	2	
	3/31	3	
Virus smuggling	1/31	3	0.051
	30/31	4	

KEY: Number of key informants was 31.
* Score of 1 = High, 2= Medium, 3= Low and 4= Negligible, shows perceived degree of importance of the RVF entry pathway
** W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement

4.3.2 Qualitative ranking of perceived RVF exposure / spread pathways by the pastoralists key informants in Ijara, Kenya

The exposure/ spread risk pathway were due to bites from infected mosquitoes at the livestock watering points, around cattle bomas, in bushy environments coming in contact with cattle as well as the exposure to contaminated pasture and environment by infected aborted foetuses and fluids. Spread of RVFV through mosquitoes' bite was perceived to be the most possible form of spread while environmental contamination by infected aborted foetuses and fluids was categorized as low risk pathway (table 4.5).

Table 4.5: Summarized results for qualitative ranking of RVF exposure risk pathway in cattle as perceived by the pastoralists' key informants in Ijara, Kenya

RVF exposure pathway	Scoring	Score*	Kendall's coefficient of concordance (W)**, p<0.05
Infected mosquitoes around watering points	30/31	1	0.152
	1/31	2	
Infected mosquitoes in the bomas	30/31	1	0.254
	1/31	2	
Infected mosquitoes in bushy areas	15/31	1	0.233
	16/31	2	
Infected mosquitoes in contact with cattle	29/31	1	0.158
	2/31	2	
Contamination of environment by infected materials	1/31	3	0.093
	16/31	2	
	14/31	3	

KEY: Number of key informants was 31.
* Score of 1 = High, 2= Medium and 3= Low show perceived degree of importance of the RVF exposure pathway
** W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement

4.3.3 Qualitative ranking of perceived RVF outbreak consequences by the pastoralists key informants in Ijara, Kenya

The consequences resulting from RVF entry, exposure and or outbreak were suggested as high morbidity, abortion and low mortality leading to reduced production. The outbreaks also trigger imposition of quarantine and ban in trade (table 4.6 and 4.7, figure 4.7).

Table 4.6: Summarized results for qualitative ranking of RVF outbreak consequences in cattle as perceived by the pastoralists' key informants in Ijara, Kenya

Consequences of RVF outbreak	Fraction Scoring	Score*	Kendall's coefficient of concordance (W)**, p<0.05
Morbidity	30/31	1	0.119
	1/31	2	
Abortion	10/31	1	0.006
	20/31	2	
	1/31	4	
Mortality	23/31	1	0.148
	5/31	2	
	3/31	3	
Loss of appetite	14/31	1	0.216
	17/31	2	
Reduced production	13/31	1	0.018
	18/31	2	
Quarantine	31/31	1	1
Control (Vaccination)	26/31	1	0.011
	5/31	2	
Ban on trade	31/31	1	1

KEY: Number of key informants was 31.
* Score of 1 = High, 2= Medium, 3= Low and 4= Negligible, show perceived degree of importance of the RVF consequence pathway
** W values vary from 0 to 1.0 with positive values showing agreement while negative values showing disagreement between key informants. The higher the value, the higher the level of agreement or disagreement

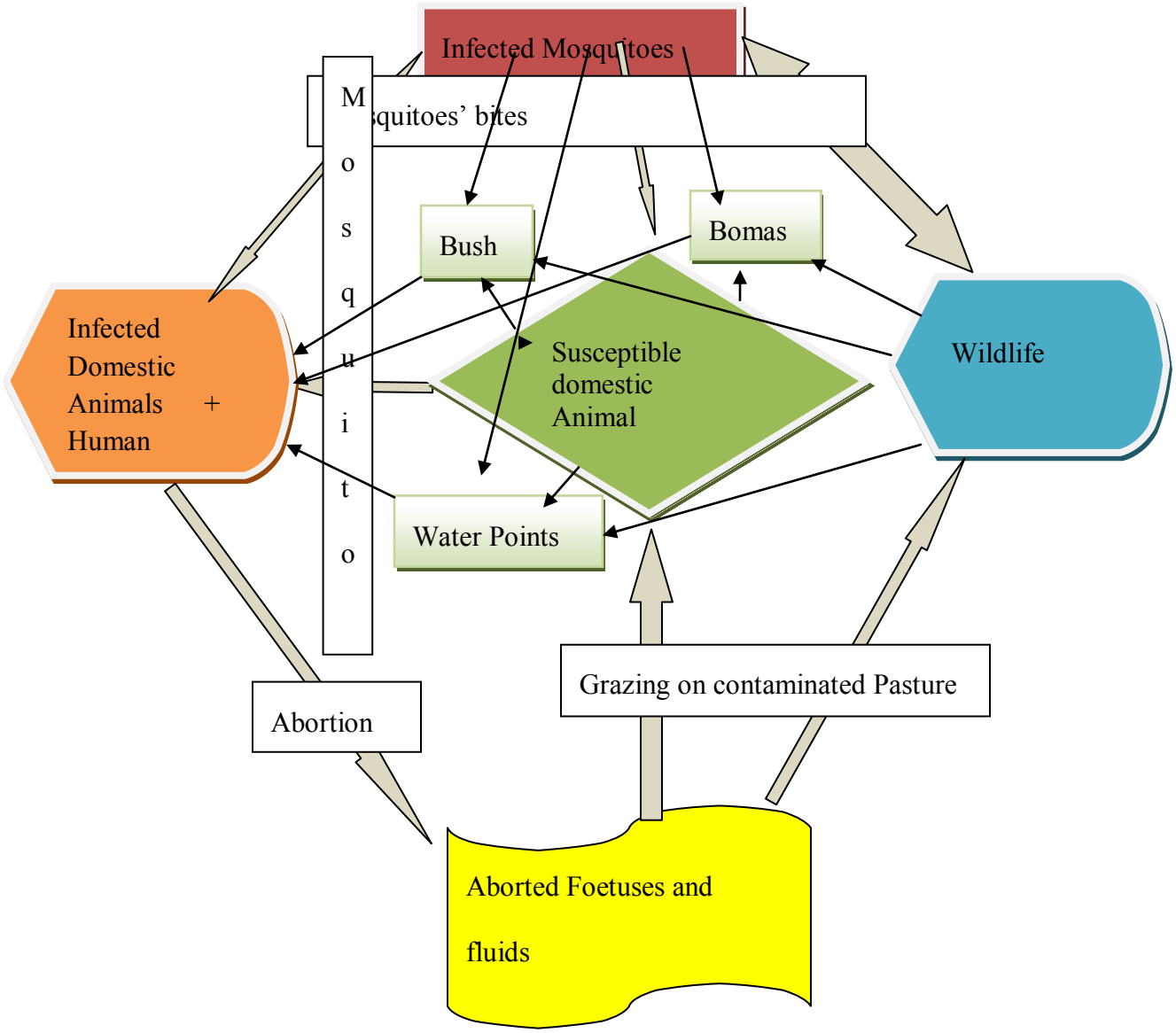

Figure 4.7: Diagrammatic presentation summarising the perceived relationship between RVF release and exposure by pastoralists in cattle in Ijara, Kenya

Table 4.7: A summary table of Risk pathway analysis for RVF in cattle in Ijara, Kenya

Risk Pathway	Factors for risk estimation consideration	Risk Level	Risk reduction Measure
Infected Mosquitoes in cattle grazing areas	Mosquitoes abundance, Livestock abundance, Livestock movement pattern, Occurrence of RVF, RVF risk factors	H	Quarantine, Use of mosquitoes' repellents, Optimum cattle stocking, Draining water in dambos, Clearing bushy vegetation
Infected mosquitoes in bushy areas in contact with cattle	Mosquitoes' abundance, Vegetation type and cover, Livestock abundance, Occurrence of RVF, Livestock movement pattern	H	Avoid grazing in bushy areas, Use of mosquitoes repellents, Proper livestock stocking
Infected mosquitoes in water points where cattle drink water	Mosquitoes' abundance at watering points, Abundance and distribution of watering points, Cattle abundance and distribution at watering points, Livestock movement pattern	H	Use of mosquitoes' repellents at watering points, Optimum cattle at watering points
Infected	Mosquitoes' abundance at	H	Use of mosquitoes repellents,

mosquitoes in cattle Bomas	cattle bomas, Cattle density at the bomas, Cattle movement pattern, Permanency of the cattle bomas		Proper stocking of livestock, Quarantine, Lighting of fire at the cattle bomas to drive away mosquitoes
Infected domestic animals in contact with mosquitoes	Occurrence of RVF in cattle, Mosquitoes' abundance, Cattle movement pattern	M	Quarantine, Use of mosquitoes repellents, Surveillance and control of RVF, Awareness creation and reporting of sick animal, Isolation of sick animal, Ring vaccination
Environmental contamination	Abortion rates in domestic and wild animals, Disposal of dead carcasses, Cattle movement, Wildlife abundance and distribution	L	Proper disposal of carcasses by burning and burying, Reducing/Avoiding contact between cattle and wildlife, Quarantine, Awareness creation on RVF prevention and control
Infected wildlife in contact with Cattle	Abundance and distribution of wildlife, Abundance and distribution of Cattle, Cattle movement pattern, Occurrence of RVF in wildlife and cattle	H	Reducing/Avoid contact between wildlife and cattle, Quarantine' Awareness creation on the risks of cattle coming in contact with wildlife

Smuggling of virus across the border	Border control measures, Likelihood of terrorist attacks, Movement of cattle and people across border	N	Screening of cattle and people across border points, Security measures against terrorist attacks, Awareness creation, surveillance and prevention measures
Livestock markets	Likelihood of trade on infected cattle, Trade routes, Implementation of movement of livestock regulations	L	Screening of livestock before allowed to the market centres, Formulation and implementation of cattle movement regulation, Awareness creation on risks of trading in infected cattle, Ban of cattle trade during outbreaks, Quarantine

KEY: H=High, M=Medium, L=Low, N=Negligible (According to the respondents interviewed)

CHAPTER FIVE

DISCUSSIONS

5.1 Occurrence of RVF in cattle in Ijara District, Kenya

5.1.1: Inhibition ELISA

Rift Valley fever inhibition ELISA is a non-specific test determining the presence or absence of both IgG and IgM. The positive inhibition ELISA indicates that the cattle in Ijara have been exposed RVFV. Given that the cattle selected for this study were three years old and less, their exposure to RVFV occurred after the 2006/2007 RVF outbreak. According to the contingency plan (ILRI/FAO, 2009), cattle within Kenya have never been vaccinated against RVF. As a result, the presence of RVF antibodies in cattle can only be attributed to RVF infection and not vaccination. The inhibition ELISA results of increasing antibody detection by age can be attributed to the fact that older cattle have a longer duration of the likelihood of exposure to RVF. Consequently, there was lowered RVFV antibody detection in younger cattle than older ones. Furthermore, a significant positive regression weights for age indicate that older cattle are more likely to test sero-positive than calves with inhibition ELISA. However, calves are usually left at temporary bomas and provided with feed or graze nearby while the other animals move around to look for grass. This practice may provide some level of protection to the younger animals. On the same note, calves normally sleep closer to herdsmen where a fire is lit to scare animals which may also protect them from mosquitoes.

5.1.2: IgM ELISA

As opposed to the inhibition ELISA, RVF IgM ELISA is a specific test which detects IgM only. IgM are short lived and can only be detected within 14 days of exposure to the antigen (Paweska *et al*., 2003). Detection of RVFV IgM in cattle in this study is an indication that RVFV was actively in circulation within the herds of cattle in Ijara. However, the short half life (approximately 14 days) of IgM in blood explains why the statistical association between the age categories (<1 yr, 1-2yrs and 2-3yrs) in cattle was not significant.

During the period when the detectable RVF antibodies increased through inhibition ELISA, the number of IgM positive samples also increased suggesting the likelihood of new infections being responsible for the increased detectable antibodies using inhibition ELISA which detects both IgM and IgG antibodies.

5.1.3 Period based antibody detection and rainfall pattern

It is quite important to note that the increased antibody detection in December 2012 and May 2013 and low detection in February 2013 can be attributed to the increased rainfall in October, November and December 2012 as well as in March, April and May 2013 within low rainfall (dry season) in September 2012 and January 2013 as reported by the Kenya Meteorology Department (KMD/FCST/5-2013/SO/06 and KMD/FCST/5-2013/SO/01). The increased rainfall may have led to increased carrier mosquito activity leading to the observed increased antibody detected. This finding is in line with those of previous authors (Davies *et al*., 1985; Anyamba *et al*., 2010) who recorded increased mosquito and viral activity during increased rainfall activity in RVF endemic areas with normally below average rainfall.

Consequently, such viral activity enables the maintenance of RVF during the interepidemic periods. The absence of detectable IgM antibodies in February 2013 sampling, a time also after the dry spell is a clear indication that rainfall pattern is an important determinant in circulation of RVF. However, the increased rainfalls mentioned were considered normal rains for the region. As a result, in this study, it can be argued that the normal rains in Ijara were responsible for maintenance of RVF viral circulation without causing an outbreak.

5.1.4: Herd based antibody detection and cattle movements

Apart from the increased rainfall (wet season) during September 2012 to December 2012 (movement between Lamu and Ijara in bushy environment) and February 2013 to May 2013 (movement between Ijara towards Garissa in bushy environment), the bushy vegetation might have been a hide out of mosquitoes. Additionally, contact with wildlife and other herds might have contributed to cross infection which led to high RVF antibody detection in herds 1178 and 1179 compared to less mobile herds 1172, 1174 and 1175. This may be relevant considering the findings of Evans *et al*., (2007) of high antibody titters in wild ruminants in Garissa, opening a window for recognition of their being relevant in the maintenance of RVF virus. Between December 2012 and February 2013, herds 1178 and 1179 were grazing around the homesteads in Ijara with less contact with wildlife and less bushy environment leading to the decreased RVF antibody detected in the herds by February 2013. However, the low antibody detection in February 2013 may also be attributed to drought which occurred during the same period. Herds 1172, 1174 and 1175 maintained a closer oscillation around their grazing area and had increased antibody detection during the rainy season but lower compared to herds 1178 and

1179 which were mobile. As a result, it can be argued that movement of cattle puts them at risk of exposure to RVF as they come in contact with different infective environments.

5.2 Perceptions of pastoralists on RVF risk factors in cattle in Ijara District, Kenya

Cattle were considered the most important livestock followed by goats, sheep, donkey and poultry respectively. Camel did not come into the mind of locals in Ijara while discussing the domestic animals due to the trypanosomosis menace in this region. According to the respondents, RVF was considered third most important disease of cattle after Trypanosomosis and Contagious Bovine Pleural Pneumonia. Other diseases which were also important are Black quarter, Tick Borne Diseases, Anthrax, Lumpy Skin Disease, Foot and Mouth Disease and Helminthosis.

Additionally, the informants highly rated availability of vectors, large number of cattle, and high rainfall leading to floods as perceived risk factors associated with RVF in Ijara. These findings were supported by those of Anyangu *et al.,* (2010) who showed strong association between severe infections of RVF and handling of large number of animals, closeness to water sources and mosquitoes in the 2006/2007 outbreak. The low agreement on the role of wildlife on RVF transmission by the respondents does not match with the findings of Evans *et al*., (2007) who detected RVF antibodies in Warthogs, Gerenuk, waterbucks and Buffalo. The respondents considered drought as an irrelevant risk factor in the occurrence of Rift Valley fever since previous outbreaks occurred during the rainy season. Conversely, the respondents did not consider the soil type as important in occurrence of RVF in the region. However, the soil type might have been confounded by topography and the more likely clay soil which traverses

nearly the whole region giving no alternative for comparison. The respondents considered drought as an irrelevant risk factor in the occurrence of Rift Valley fever since previous outbreaks occurred during the rainy season. This finding was supported by the sero-survey data which detected no IgM antibodies in the February 2013 sero-sampling after a dry season in September 2012 and January 2013, suggesting no recent viral activity.

5.3 Perceptions of pastoralists on RVF risk pathways in cattle in Ijara District, Kenya

The risk pathway analysis was based on three possible stages; assessment (Entry, Exposure and Consequence), communication and management. There were basically three pathways in consideration for the above analysis in relation to the possible entry of the RVF virus into study area, transmission and spread of the virus and release of the virus to neighbouring areas.

The entry risk pathways for RVF in Ijara district mentioned by the pastoralists were through infected mosquitoes, infected domestic animals, infected aborted foetuses and fluids, and infected wild animals were corroborated by the studies carried out by Robert *et al.,* (2010). The perceived exposure/ spread risk pathways of infected mosquitoes at the livestock watering points, around cattle *bomas*, in bushy environments coming in contact with cattle as well as the exposure to contaminated pasture and environment by infected aborted foetuses and fluids were clear indication of the communities understanding of the risk factors associated with RVF.

It is also important to note that the respondents considered aborted foetuses as a less important pathway in the entry and spread of RVF, a factor which contradicts the findings of Anyangu *et al*., (2010) in which aborted foetuses was the single most factor having direct

association with severe RVF infections in humans during the 2006/2007 outbreak compared to presence of mosquitoes, water bodies, contact with livestock which were jointly associated.

CHAPTER SIX:

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

6.1.1 Occurrence of RVF in cattle in Ijara District, Kenya

All the six herds of cattle sero-surveyed had detectable RVFV antibodies by both inhibition and IgM ELISA tests proving that virus was actively in circulation in cattle in Ijara district even during the interepidemic period. The high antibodies detected after every rainy season was a clear indication of the likely central role played by mosquitoes in maintaining endemic infections in cattle in Ijara during the interepidemic period.

On the other hand, it is important to note that the most mobile cattle herds in Ijara showed more viral activity than the less mobile herds. Additionally, there was an indication of more viral activity after the cattle herds pass through thick forests with wildlife than when they were close to the homesteads.

6.1.2 Perceived Risk factors of RVF by pastoralists in cattle in Ijara District, Kenya

Availability of the RVFV vectors (mosquitoes) and hosts (cattle) as well as rainfall causing flooding were the main risk factors understood by the locals enabling epidemics of RVF in Ijara. Additionally, the locals in Ijara believe that their livestock were infected when they come in contact with wildlife while sharing grazing areas and water points in the thick forested areas.

6.1.3 Perceived Risk pathways for RVF by pastoralists in cattle in Ijara District, Kenya

The main perceived routes of entry, exposure and spread of RVF in Ijara were infected mosquitoes, infected domestic and wild animals and environmental contamination from poor disposal of infected carcasses. However, the locals did not consider transmissions from contaminated environment important.

Rift Valley fever vaccination of cattle in Ijara district had never been carried out. Though the sero-survey carried out during this study indicated exposure of RVF virus to some of the cattle, most of the cattle were free of RVF antibodies. As a result, any serious outbreak could lead to huge losses to the locals in Ijara whose main economic activity has been pastoralism.

The cattle in Ijara were very mobile traversing between Lamu in the coast to Garissa to the north and Tana River to the west. Ijara was also bordering Somali hence the likelihood of the cattle herds coming in contact with the neighbours given the owners were kinsmen as well as the ongoing trade. There was therefore a huge chance of infected herd infecting the whole region since the region was not a closed one.

The close association between cattle and wildlife was perceived to be one of the main pathways of RVF transmission in the study area. The areas of contact with wildlife were bushy grazing areas and watering points. As a result, there could have been cross transmission at these domestic-wildlife interfaces.

6.2 Recommendations

6.2.1 Occurrence of RVF in cattle in Ijara District, Kenya

Due to the on-going circulation of RVFV in cattle in Ijara, there is need for awareness creation to the pastoralists even during the interepidemic period as well as enabling measures for preparedness. There is need for planned grazing of cattle to limit the mobility of cattle in bushy areas and wildlife inhabitants hence reducing the risk of RVF transmission in such environments. Strategic vaccination of cattle can also protect the cattle during the interepidemic period.

There is need to sero-survey cattle herds in Ijara before they get into the forest, while inside the forest and after they come out of the forest both during the dry and rainy seasons in order to vividly ascertain the relevance of bushy environment to maintenance of RVF during both dry and rainy seasons, or else rainfall can confound the impact of bushy environment. On the same note, sero-survey of wildlife in Ijara for RVF during the same period of doing livestock, wildlife, human and mosquitoes' RVF screening would go a long way in understanding RVFV maintenance during the interepidemic period in all the hosts at the same time.

6.2.2 Risk factors of RVF in cattle in Ijara District, Kenya

The lack of preparedness by the locals in dealing with outbreaks calls for community awareness sessions on the state of RVF in Ijara in cattle. The aim of this awareness would be to expose all the risk factors and the direction towards community participation in prevention and control. More so, management of cattle carcasses would cause a big loss to the pastoralists in Ijara in

cases of RVF outbreak given their current perception of it being a low risk factor. There is also need for awareness on controlling mosquitoes' bites in humans given that RVFV is in circulation in cattle and can easily be transmitted to humans by mosquitoes' bite. Several herders spend most of their times in bushy environments with animals without any protection measures against mosquitoes despite the presence of contingency plan in the country. As a result, funds need to be availed to enable the implementation of the RVF contingency plan, especially availing mosquito nets for the herders for use at night (ILRI/FAO, 2009).

6.2.3 Risk pathways for RVF in cattle in Ijara District, Kenya

There is need for community awareness on the zoonotic nature of RVF and training on handling animal carcasses in Ijara since animals are locally slaughtered and eaten when sick. On the other hand, RVF vaccination of cattle in Ijara district has never been carried out. Though the sero-survey carried out during this study indicates exposure of RVF virus to some of the cattle, most of the cattle are free of RVF antibodies. It then follows that any serious outbreak can lead to huge losses to the locals in Ijara whose main economic activity is pastoralism. There is therefore a need to carry out RVF vaccination in cattle in this region.

The cattle in Ijara are very mobile traversing between Lamu in the coast to Garissa to the north and Tana River to the west. Ijara is also bordering Somali hence the likelihood of the cattle herds coming in contact with the neighbours given the owners are kinsmen as well as the ongoing trade. There is therefore a huge chance of infected herd being source of infection spread in the whole region since the region is not a closed one. The veterinary inspection of animals at the border is inadequate due to the usage of illegal routes between the Kenya-Somali

border line. There is need to protect the Kenya- Somali border and manage all the illegal routes and all animals screened to avoid possible transmission of RVF.

The close association between cattle and wildlife should be looked into if RVF prevention and control is anything to pursue. Provision of watering points as well as organized grazing pattern with proper land use planning is necessary. Continuous surveillance of RVF in Ijara in the domestic animals, wildlife, human and vectors as well as environmental monitoring of rainfall and flooding should be done together with all the stakeholders to avoid duplication of work while achieving optimum results.

CHAPTER SEVEN

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CHAPTER EIGHT

APPENDICES

Appendix 1: Field serosurvey data sheet

Sample data					**Place:**
Herd ID:		**Sampling date:**		**Way point:**	
S:		**E:**		**Elevation:**	
Animal ID	**Age**	**Sex**	**Clinical signs**	**Vaccination history**	**Any other Information**

Appendix 2: Checklist for (KII) for Information on Risk factors and Risk Pathways for RVF in Ijara

Background Information

Date……Division……Village……Long……Lat……

Institutional affiliation _____Place of interview_____

1.0 Livestock information

1.01 (Pair wise ranking of livestock species)

	Cattle						
Cattle							

1.02 List cattle diseases affecting your area

1.03 Ranking of cattle diseases- randomly

Disease	**Ranking**

1.04 Pair wise ranking of cattle diseases

	RVF					
RVF						

2.0 Risk factors RVF

2.01 List the risk factors

2.02 Ranking of risk factors- randomly

(Risk factors checklist - interviewees will name risk factors then rank)

Risk factors	Dambos	Rainfall	Drought	Flood	Soil type	Bushy Vegetation	Mosquitoes	Wild life
Dambos								
Rainfall								
Drought								
Flood								
Soil types								
Vegetation								
Mosquitoes								
Wildlife								

RVF risk pathway checklist

3.01 Release Assessment (respondent to name and rank)

3.011 List pathways of entry and reduction measures of RFV in Ijara

Release Risk pathway	Risk reduction measure

3.012 Ranking release/ entry risk pathways randomly

Release Pathway	Random Ranking

3.013 Qualitative ranking of entry risk pathways (scoring)

-Qualitative ranking as below	High	Medium	Low	Negligible
Infected mosquitoes				
Infected domestic animals				
Infected Aborted foetuses and fluids				
Infected wild animals within				
Smuggling live virus				

3.02 Exposure Assessment (Respondent to name and rank)

3.021 List risk pathways of exposure and reduction measure of RFV in Ijara

Exposure Risk pathway	**Risk reduction measure**

3.022 Ranking exposure risk pathways

Exposure Risk Pathway	**Random Ranking**

3.023 Qualitative ranking of exposure risk pathways (scoring)

	High	Medium	Low	Negligible
Infected mosquitoes in water points				
Infected mosquitoes in cattle bomas				
Infected mosquitoes in bushy areas				
Infected mosquitoes in contact with cattle				
Infected aborted foetuses in contact with cattle				

3.03Consequence Assessment (Respondent to name and rank)

3.031 List consequences and reduction measures of RFV in Ijara

Consequence	Reduction measure

3.032 Ranking consequence randomly

Consequence	Random ranking

3.033 Qualitative consequence ranking (scoring)

	High	Medium	Low	Negligible
Morbidity				
Abortions				
Mortality				
Loss of replacement stock				
Reduced production				
Quarantine				
Control (vaccination in surrounding areas with no outbreak)				
Ban on trade				

Any other information

_____

_____

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Appendix 3: List of individuals of people interviewed during the Key Informant interviews

Mr Abdi Ibrahim, Sangailu Division, Ijara District - Owner of Herd number 1178

Mr Rashid Bare, Sangailu Division, Ijara District – Area Chief

Ahmed Hassan Bare, Sangaiulu Division, Ijara District – Head Herder

Mrs. Amin Mohamed, Sangaiulu Division, Ijara District, CBAHW

Mr Mohamed Ali, Ijara Division, Ijara District – Owner of Herd number 1179

Mr Mohamed Omar, Ijara Division, Ijara District- Area Chief

Mr Omar Mwachatsi, Ijara District, Deputy DVO

Mr Abdi Malim, Ijara Division, Ijara District, AHA

Ijara District, Public Health Officer

Ijara District, Senior Game Warden, KWS

Mr Adan Hared, Ijara Division, Head Herder

Mr Dennis Njeru Gitonga, Ijara District, District Livestock Production Officer

Mr Khalif Duble Hassan, Masalani Division, Ijara District – Owner of Herd number 1181

Mr Abdi Bashir, Masalani Division, Ijara District – Area Chief

Mr Hamed Mohamed, Masalani Division, Ijara District – Owner of Herd number 1172

Mr Mohamed Yusuf, Fafi Division, Bura District – Owner of Herd number 1174

Mr Mohamed Bile, Fafi Division, Bura District – Area Chief

Mrs.FaizaRamathan, Fafi Division, Bura District Community Health Worker

Mr Ahmed Farah Haruni, Sankuli Division, Garissa District – Owner of Herd number 1175

Mr Abdulla Ahmed, Sankuli Division, Garissa District – Area Chief

Mrs.Abdalla Salim, Ijara Division, Ijara Slaughter house worker

Appendix 5: Photo gallery

8.5.1 Photo of a flooded low lying grassland called dambos

8.5.2 Photo of a section of Bony forest