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MASTER OF VETERINARY SCIENCE THESIS

DETECTION OF RIFT VALLEY FEVER VIRUS FROM MOSQUITO VECTORS AND MOSQUITO DISTRIBUTION MODEL BASED RIFT VALLEY FEVER RISK MAPPING IN ETHIOPIA

BY
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DEPARTMENT OF CLINICL STUDIES MVSc PROGRAM IN VETERINARY EPIDEMIOLOGY

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Detection of Rift Valley Fever Virus from Mosquito Vectors and Mosquito Distribution Model Based Rift Valley Fever Risk Mapping in Ethiopia

BY

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DEDICATION

This thesis is dedicated to my late father Bedasa Jaleta, my late sister Zenebu Bedasa and my late uncle Wegari Jaleta with love and eternal appreciation for their unreserved help, wish and loves.

SIGNED DECLARATION SHEET

First, I declare that this thesis is my actual work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at College of Veterinary Medicine and Agriculture, Addis Ababa University and is deposited at the University/ College library to be made available to borrowers under rules of the library. I solemnly declare that this thesis is not submitted to any other institution any ware for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

CDC	Center of Diseases prevention and Control
cDNA	Compliment deoxy ribonucleic acid
DIVA	Differentiation of Infected from Vaccinated Animals
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FAD PReP	Foreign Animal Disease Preparedness and Response Plan
GLW	Gridded Livestock of the World
HWSD	Harmonized World Soil Database
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MODIS	Moderate resolution Imaging Spectroradiometer
NAHDIC	National Animal Health Diagnostic and Investigation Center
NDVI	Normalized Difference Vegetation Index
NVI	National Veterinary Institute
OIE	*Office of International Epizootics* (World Organization for Animal Health)
PANVAC	Pan African Veterinary Vaccine Center
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
RNA	Ribonucleic acid
ROC	receiver operating characteristics
RT-PCR	Reverse Transcriptase - Polymerase Chain Reaction
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
SSA	Sub-Saharan Africa
TSS	true skill statistic
VBDs	Vector-borne diseases
VIF	Variance Inflation Factor
VNT	Virus Neutralization Test

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ABSTRACT

Mosquito-borne arboviral diseases are a big health challenge worldwide. Rift Valley Fever virus (RVFV) is one of the most important mosquito-borne emerging diseases that threaten human and animal health particularly in Africa. So far, the status of RVFV circulating in mosquito vectors is unknown in Ethiopia. Thus, this study was conducted with the aims of investigating RVFV vector mosquitoes, viral detection, and RVF risk mapping based on RVF mosquito vector distribution model in Ethiopia. Entomological survey was conducted between December, 2018 and April, 2019 in selected areas of mid-Rift Valley, Borena and Segen Valley, Ethiopia and the result showed diversified species of primary vectors (*Aedes spp.*) and secondary vectors (*Culex*, *Anopheles*, *and Mansonia*) were collected and identified. A total of 2,322 adult mosquitoes were collected and four genera: *Aedes* (n = 404; 17.40%), *Culex* (n = 466; 20.06%), *Mansonia* (n = 210; 9.04%), and *Anopheles* (n = 493; 21.23%) were identified while the remaining (746; 32.12 %) mosquitoes were unidentified. *Aedes ochraceus* (126; 8.0%), *Cx. quinquefasciatus* (141; 9.01%), the *M. uniformis* (210; 13.32%) and *An. gambiae* (64; 4.06%) were predominant species from the four genera. Among identified mosquitoes 45.55% and 22.78% were collected near lake shore and near pond, respectively, while the remaining were collected from others habitats. A total of 38 mosquito pools, containing 20-25 mosquitoes per pool, were tested by reverse transcriptase-PCR using the virus specific primers. No RVFV genome was detected from all mosquito pools. Ensemble model were developed using 11 predictor variables. The variables contribute different amount where water vapor pressure (23%) and soil type (21%) contribute the most while mean annual maximum (4%) and mean annual minimum temperatures (2%) contribute the least. Model showed border regions of Ethiopia from southeastern to northwestern of the country were suitable for distribution RVF vector mosquitoes. All regions have variable geographical range suitable for RVFV vector mosquitoes where Somali region has wide while Harari region has few patchy suitable areas. Many parts of central Ethiopia is not suitable for the RVFV vector mosquitoes. Occurrence of potential mosquito vectors and model capture of broad risky areas particularly along the border shared with endemic countries necessitates further studies of the diseases most importantly through one health approach.

Key words: *Ensemble modeling, Mosquito survey, Predictor variables, RVF, Viral detection,*

1. INTRODUCTION

Vector-borne diseases (VBDs), particularly those transmitted by mosquitoes, comprise a significant proportion of recent emerging infectious disease events (Kularni, 2016). Arboviral diseases are caused by viruses that are spread by the bite of an infected arthropod, predominantly mosquitoes (Wilder-Smith *et al.*, 2017). Mosquito-borne arboviral diseases are becoming big health challenge of human and animal worldwide (Cooper *et al.*, 2015). Among mosquito borne arboviral diseases, Rift Valley Fever (RVF) is the classic transboundary animal and zoonotic diseases transmitted by mosquito vectors (Pepin *et al.*, 2010; Nanyingi *et al.*, 2015).

RVF is an emerging mosquito-borne zoonotic infectious disease caused by RVF virus (RVFV), which is a member of the *Phlebovirus* genus of the family *Bunyaviridae* (Linthicum *et al.,* 2016). The disease was first identified in 1930 during an outbreak of sudden deaths and abortions among sheep along the shores of Lake Naivasha in the greater Rift Valley of Kenya (Pepin *et al.*, 2010). Further epizootics were subsequently confirmed in Zimbabwe, Zambia, Sudan, and other East African countries. Outside of East Africa the disease recognized in South Africa in 1951 (Davies, 2010), outbreak occurred in 1977 in Egypt, 1979 in Madagascar, 1989 in Mauritania (Pepin *et al.*, 2010). Out of Africa and Madagascar, outbreak of RVF was first recognized in human and livestock in Saudi Arabia and Yemen in 2000 (Miller *et al.*, 2002).

Natural hosts for RVFV include mosquitoes, sheep, cattle, buffalo, camels, goats, other ruminants, and humans. Sheep are the species of domestic animal most susceptible to RVFV infection, and new-born lambs in particular (Mansfield *et al.*, 2015). RVF is adapted to wide range of vectors, predominantly mosquitoes (Arum *et al.*, 2015). It usually transmitted to mammals by mosquitoes (Diptera: *Culicidae*). The virus has been isolated from more than 53 mosquito species in 8 genera in regions where epizootics occurred (Linthicum *et al.,* 2016). The mosquito vectors transmitting RVF can be classified into two major groups, namely primary and secondary vectors (Arum *et al.*, 2015). Floodwater mosquitoes of genus *Aedes* have been considered the primary maintenance host and source of RVFV that initiate disease outbreaks (Himeidan *et al.*, 2014; Arum *et al.*, 2015; Sang *et al.*, 2017). The genera *Culex*, *Anopheles, Eretmopodites,* and *Mansonia* constitute the secondary

vectors which take over flooded grounds for breeding, contribute to the amplification of the virus due to their ubiquitous biting patterns, consequently resulting in outbreaks (FAD PReP, 2013; Arum *et al.*, 2015). The virus also isolated from various other arthropods (FAD PReP, 2013; Lumley *et al.*, 2018).

RVFV has a complex, multispecies epidemiology (Chevalier, 2013; Iacono *et al.*, 2018). RVF is transmission mainly depends on the availability of competent vectors, susceptible hosts, and suitable ecological and environmental conditions that favour mosquito survival and reproduction (Arum *et al.*, 2015). Numerous environmental, agricultural, epidemiological, and anthropogenic factors are implicated in RVF spread (Lancelota *et al.*, 2017). A major risk factor for both humans and animals was exposure to competent mosquito vectors. Movement of livestock during the viraemic phase of infection to areas with high mosquito density and naïve livestock populations incriminated for disease spread and poses a high risk of human infection especially among persons handling livestock (Nanyingi *et al.*, 2015). Once it introduced into permissive ecologies, the virus becomes enzootic/endemic, making the region vulnerable to periodic outbreaks with the potential to spread further into non-endemic environments that have favorable conditions. Although the virus is endemic to sub-Saharan Africa, it already crossed significant natural geographic barriers such as the Indian Ocean, the Sahara Desert and the Red Sea to reach naive ecologies (Baba *et al.*, 2016).

RVF has remarkable socio-economic and public health impacts; once introduced it kills tremendous number of livestock and people. RVF in livestock can devastate agricultural communities; it causes almost 100% mortality rates among young animals and high abortion rates among livestock (Wilson *et al.*, 2014). When outbreaks cover a wide geographic area, hundreds of thousands of livestock are affected, leading to tens of thousands of human infections and hundreds of hospitalizations (Aradaib *et al.*, 2013). The Horn of Africa has been historically affected by RVF. For instance, Kenya has experienced several outbreaks around 23 in between 1912 to 2007 which resulted in number of human cases with a high case fatality and considerable loss of livestock (Mosomtai *et al.*, 2016; Baba *et al.*, 2016). Furthermore, RVFV is also considered as a potential bioterrorism tool that could have direct (morbidity and death) and indirect (restriction in international trade) impact in countries that are free from the virus (Sindato *et al*., 2011).

Considering the zoonotic threat of RVF and the increasing risk of spread to a disease-free areas, there is a need for better understanding of the socio-economic impact of RVF to integrate it within the disease management and policy decision process (Peyre *et al.*, 2015). Taking these impact into account, many of disease free countries has been modeling the risk of introduction into their countries (Turell *et al.,* 2010; Fischer *et al.*, 2013; Rolin *et al.,* 2013).

The RVF has long history in East Africa (Mosomtai *et al.*, 2016). Despite the fact that Ethiopia shares borders with the disease endemic countries, namely, Kenya, northern Somalia, Sudan and South Sudan (Tran *et al.*, 2016), occurrence of RVF hasn't been reported in Ethiopia. Increasing health and economic importance as well as broad geographical expansion (intentional and non-endemic) of RVF need timely prediction of the disease outbreak (Wilson *et al.*, 2014). Early detection and implementation of appropriate measures, which are essential to minimize the consequences of outbreaks, require a deep understanding of transmission, spread and persistence mechanisms (Chevalier, 2013). It also useful particularly for optimal allocation of resources for surveillance and control and identifies geographical areas characterized by a paucity or absence of information (Clements *et al.,* 2007).

Identification of mosquito species is one important step to control mosquito borne-diseases. It allows to acquire biological information such as breeding sites, biting and resting habits that differ among mosquito species (Taira *et al.*, 2012). Although they have similar morphology, mosquitoes have considerable differences in ecological, epidemiological significance, and physiological features, including food preferences (Das *et al.,* 2016). Similarly, mapping the distribution of vectors using the range of geographical determinants is essential for health planning (Kraemer *et al.,* 2015). Vector distribution modeling and risk mapping of RVF using disease different data like occurrence data, vector population dynamics, anomalous climatic conditions, and surrogate environmental variables provide a foreseeable strategy for RVF surveillance (Nanyingi *et al.*, 2015).

This helps to improve efforts to understand the spatial epidemiology of arboviruses and to predict how these could change in the future and could be used for early warning detection and implementation of control measures are essential.

In Ethiopia entomological study, particularly regarding RVFV vector mosquito presence, distribution as well as their role in the disease transmission has not been investigated. Similarly, there was no report of RVF clinical case or positive sero-or-molecular surveillance yet in Ethiopia. Additionally, information related to the suitability of Ethiopia ecosystems for the transmission of the RVFV is scarce. Risk maps based on vector population, environmental and climatic factors were also not developed yet in Ethiopia. Therefore, this study aimed to achieve the following objectives.

- **General Objective**
  - Entomological survey, viral detection and risk mapping of Rift Valley Fever Ethiopia.
- **Specific Objective**
  - Entomological survey of mosquito vectors of Rift valley fever virus.
  - Molecular detection of RVF virus from mosquito vectors
  - Mosquito distribution model based risk mapping of Rift Valley Fever occurrence in Ethiopia.

2. LITERATURE REVIEW

2.1. Rift Valley Fever Virus

RVFV is an spherical enveloped RNA virus (figure 1) characterized by a genome composed of three single-stranded RNA segments of negative or ambisense polarity: the S segment (prototype strain ZH501: 1690 nucleotides (nt); the M segment (3885 nt); and the L segment (6404 nt) RVF virions consist of an envelope and a ribonucleocapsid (RNP) (Pepin *et al.*, 2010). The S-segment encodes the open reading frames (ORFs) of the nucleocapsid(N) protein and non-structural (NSs) protein in an ambisense manner; M segment encodes for glycoproteins G1 and G2 and two others proteins of 78 and 14 K; and the L-segment encodes an RNA-dependent RNA polymerase, which synthesizes both viral mRNA and genomic RNA (Lee, 1996; Sall *et al.*, 1998; Bird *et al.*, 2007; Ikegami, 2013).

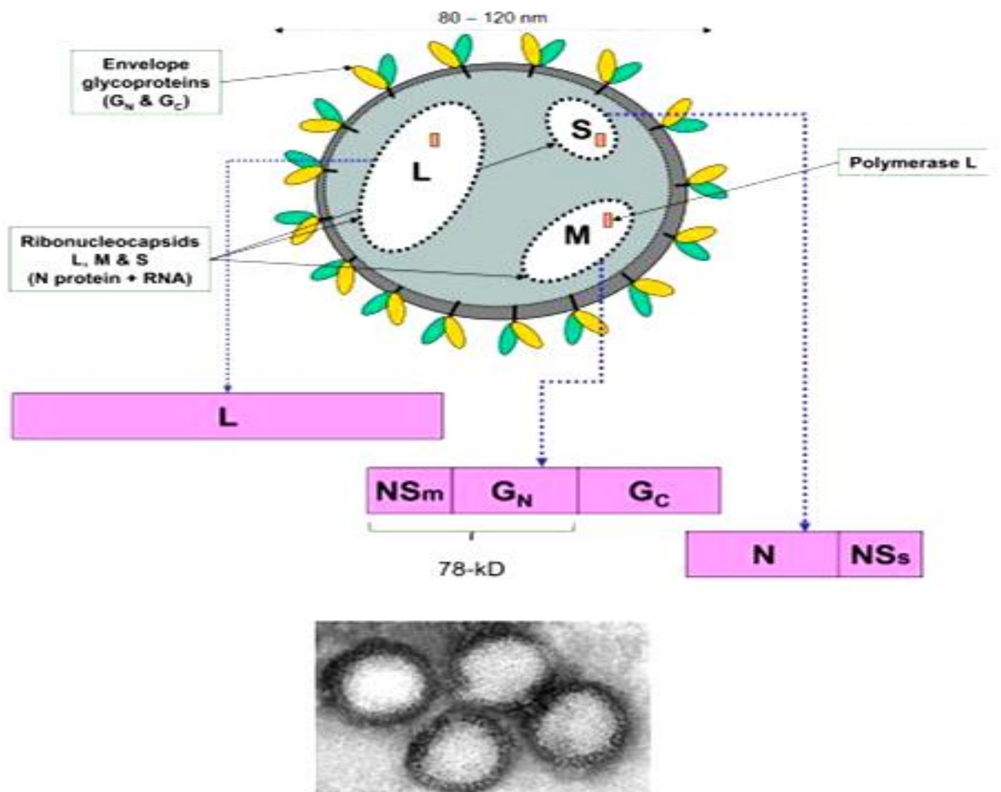


Figure 1: The genetic components of RVF virus RNA segments Source: Epin *et al.*, (2010).

RVFV degrades in the environment; easily destroyed by strong sunlight, however; the virus can persist for a few days in some protein-rich environments such as tissues and can survive in dried blood for up to 3 months. The virus is inactivated by lipid solvents, detergents, and low pH. At neutral or alkaline pH, particularly in the presence of protein material, such as serum, the virus can remain viable for up to 4 months at 4°C, 8 years below 0°C (32°F). It is quickly destroyed by pH changes in decomposing carcasses. Aerosolized RVFV increases in stability as the relative humidity decreases. Under optimal conditions, RVF virus remained viable in aerosols for more than an hour at 25°C (77°F) and 30 percent relative humidity. The Rift Valley fever virus is susceptible to low pH (≤ 6.2) and also solutions of sodium or calcium hypochlorite with residual chlorine content greater than 5000*ppm*. Potassium peroxymonosulfate and sodium chloride is also very effective (FAD PReP, 2013; Spickler, 2015).

RVFV gene flow has been influenced overtime on multiple levels ranging from the macroscopic (i.e., geographic dispersal) to the molecular (i.e., reassortment events). However, RVFV genomics is the relatively low genetic diversity approximately 4% and 1% at the nucleotide and protein coding levels, respectively. This suggests that the virus either has a very low tolerance for mutation within its genome or viruses collectively identified today as RVFV have a relatively recent common ancestor. The significant factor for this identified genetic diversity is that genomic rearrangement via RNA segment reassortment or homologous recombination. However, the impact of these reassortment events on RVFV replication, fitness and, most importantly, host virulence is not fully known (Pepin *et al.*, 2010).

2.2. Epidemiology

2.2.1. *Susceptible hosts*

RVF virus has wide range of hosts include mosquitoes, sheep, cattle, buffalo, camels, goats, other ruminants, and humans. Sheep are most susceptible to RVFV infection, and new-born lambs, calves, and puppies are highly susceptible (Mansfield *et al.*, 2015). Severe disease can be seen in newborn puppies and kittens, although adult dogs and cats seem to be unaffected (Spickler, 2015). Mice, lambs less than 7 days old, and baby hamsters are the most susceptible experimental animals

(Morrill and McClain, 1996). Serological study have also been shown neutralizing antibodies to RVFV in wildlife in Kenya, including African buffalo, black rhino, lesser kudu, impala, African elephant, kongoni, and waterbuck. This suggests that the possibility of wildlife may be reservoirs for the virus during inter-epidemic periods and play a role in amplifying the virus during epizootics (Spickler, 2015; Muga *et al.*, 2015). A lot of wild rodent species are susceptible to RVF but their epidemiological significance in virus maintenance and transmission is not known. Several avian and reptilian species have been tested for susceptibility and are refractory to RVF virus (Morrill and McClain, 1996).

2.2.2. *RVF mosquito vectors*

RVFV has the potential to infect a wide array of vectors and involved in the diseases transmission, including ticks and a variety of flies (Pepin *et al.*, 2010; Sang *et al.*, 2017). RVFV vectors can be classified into primary ("reservoir/maintenance") and secondary ("epidemic/amplifying") vectors. Reservoir/maintenance vectors include *Aedes* mosquitoes (Diptera: *Culicidae*) associated with freshly flooded temporary or semi-permanent fresh-water bodies and are important vector to maintain the RVF virus and subsequent epidemics of the disease (Fontenille *et al.*, 1998), while epidemic/amplifying consisting of *Culex*, *Anopheles, Eretmopodites,* and *Mansonia* associated with more permanent fresh–water bodies (Pepin *et al.*, 2010; Tran *et al.*, 2016). *Aedes ochraceus* and Aedes *mcintoshi* is the most important maintenance vector of RVFV in East and Southern Africa. Similarly, *Aedes vexans* were found to be responsible for a large outbreak in West Africa and the likely maintenance vector in Saudi Arabia during the emergence of RVFV in 2000 (Pepin *et al.*, 2010). In West Africa its reported that other arthropods like *Culicoides sp.*, and *Amblyomma variegatum* were also involved in the disease transmission during peak of epizootic (Clements *et al.*, 2007). Abundance, longevity, distribution and feeding behavior of all are important facets of what constitutes a good vector, as are inherent capabilities such as threshold susceptibility to infective virus (Pepin *et al.*, 2010; Tran *et al.*, 2016).

2.2.3. *The disease transmission*

Predominant mode by which animal become infected is through a bite from an infected mosquito particularly in early stage (Mansfield *et al.*, 2015). Usually, in East Africa flooded '*dambos'* (low-lying areas of soil) induce hatching of trans-ovarially infected eggs of Aedes mosquitoes that are dormant in the soil, which serve as primary vectors. Hatched infectious mosquitoes transmit the virus to nearby livestock and wildlife vertebrate hosts as depicted in figure 2 below which serve as amplifiers of the virus, infecting more mosquitoes, and thereafter secondary vectors of the virus (*Culex*, *Anopheles, Eretmopodites*, *Mansonia* mosquitoes and other arthropods) amplify the transmission of the virus to non-infected domestic animals and humans (Sindato *et al.,* 2011; Balenghien *et al.*, 2013). Flooding in areas with a high density of livestock and/or wildlife creates a conducive environment for RVF transmission, and under such conditions, the virus is maintained within the ecosystem (Baba *et al.*, 2016).

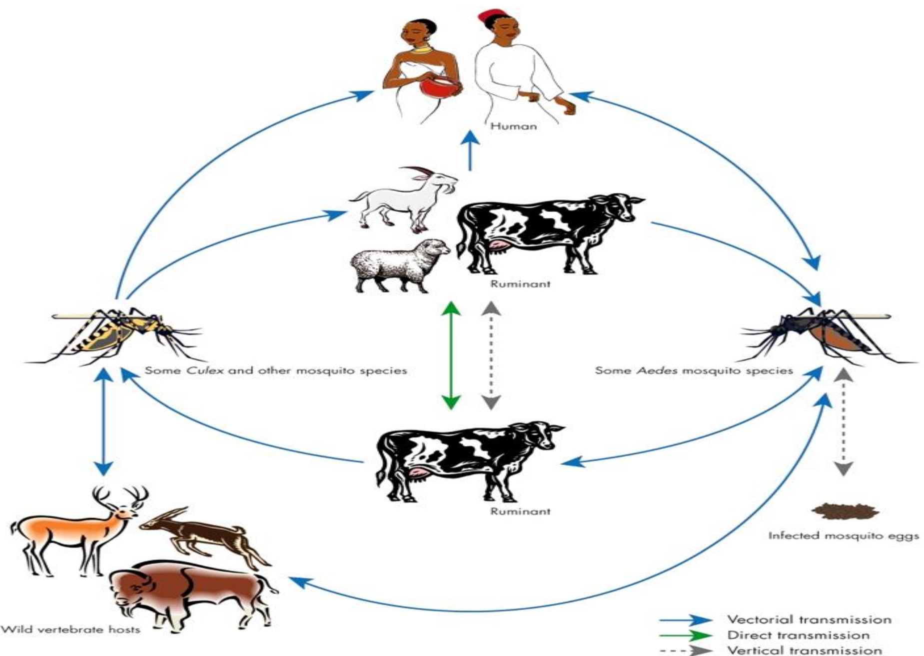


Figure 2: RVF transmission cycle. Source: Balenghien *et al.*, (2013)

In addition, there can also be animal to animal infection through direct contact with infected animal tissues, bodily fluids and fomites mainly associated with abortions. Aborted foetal materials and placental membranes contain large numbers of virus particles which can either contaminate the local environment directly or infect animals in close contact. Animals may also become infected through

the re-use of needles during vaccination; particularly in regions with limited resources. There may also be the potential for lactating animals to infect their young via milk (Pepin *et al.*, 2010; Mansfield *et al.*, 2015).

Human infection can also arise from a mosquito bite. However, humans can become infected through exposure to infected animals or animal tissues, since infected animals, and sheep in particular, can display high titre viraemia (Mansfield *et al.*, 2015). Direct exposure to fluids of infected animals can occur during slaughter or through veterinary and obstetric procedures. Hence, the risk of infection is greatest when slaughtering in the context of traditional sacrificial practices, on which occasions aerosols of infected blood are likely to be generated. This is the major reason that outbreak of RVF is commonly associated with people whose livelihoods revolve around livestock rearing (Muga *et al.*, 2015). Few cases of vertical transmission infants have been demonstrated in human. However, transmission between human does not seem to occur, but the blood and tissues of patients might be sources of exposure for medical workers (Spickler, 2015).

To date, the presence of RVFV in the feces or urine of infected animals has not been demonstrated. Generally, virus shedding in secretions and excretions from infected ruminants is poorly understood, although it is not thought to be important in spreading Rift Valley fever except when these are contaminated by blood (Pepin *et al.*, 2010; Spickler, 2015).

The majority of the factors driving mosquito vector presence and abundance, thus driving the risk of RVF transmission, are related to climate, water and landscape. The *Aedes* genus is mostly associated with temporary water bodies such as flooded area, temporary pond, puddles, and rice fields. *Culex* and *Anopheles* mosquito breeding areas are diverse and could be temporary (rice fields, swamps) or permanent (lakes, ponds) bodies of water. However, stagnant and permanent water bodies are the habitats of Eretmapodites and Mansonia, respectively (Tran *et al.*, 2016).

RVF is driven by a complex interaction of mosquito vector populations and vertebrate hosts in different habitat types under varying environmental conditions (Arum *et al.*, 2015). The RVF virus occur in several ecological climates such as arid in western Africa and the Arabic Peninsula; sub-humid in East Africa; wet forests in central Africa; dam and irrigated agricultural land under hot

climatic conditions in Egypt, Mauritania and Sudan; and humid highlands in Madagascar (Tran *et al.*, 2016). The large epidemics of RVF have occurred in southern and eastern Africa have been associated with above average rainfall, a large amount of vector activity and the presence of susceptible livestock (WHO, 2009). Typically the virus is associated with pastoral regions where habitat conducive to the maintenance of arthropod vectors is present (Morrill and McClain, 1996). In east Africa, RVF has been reported in arid and semiarid areas in the form of sudden and dramatic epidemics at intervals of approximately 10 years associated with widespread flooding and the resultant swarms of mosquitoes (Muga *et al.*, 2015).

Environmental conditions determine the interactions of various elements (vertebrate host, vectors, and virus) transmission cycle of RVF in which rainfall play key role (Ba *et al.*, 2005). Irrigation, rainfall and human population density were the main drivers of RVF cases, independent of seasonal, climatic or spatial variation (Redding *et al.*, 2017). High densities of vectors and the abundance of naïve ruminant populations, including highly susceptible, acted as facilitating factors for the amplification of the virus, with a high impact on humans (WHO, 2009).

2.3. Clinical Sign

Rift Valley fever is usually most severe in young animals. Nonspecific signs of fever, anorexia, weakness and lymphadenopathy are common in lambs. Hemorrhagic or fetid diarrhea, melena, regurgitation, signs of abdominal pain, a serosanguineous or bloodstained mucopurulent nasal discharge and elevated respiratory rate may also be seen. Very young lambs and kids with clinical signs rarely survive longer than a few days, and often die with 24 hours. Older lambs and kids may die acutely or peracutely, recover from the illness, or become infected with few or no clinical signs. Similar signs have been reported in young calves, although some sources have reported that icterus is more likely, and survival rates appear to be higher, Mortality rate is significantly influenced by the age of the animal; newborn lambs are highly susceptible, with a mortality rate of greater than 90% in lambs less than a week old, associated with acute necrotic hepatitis. However, the mortality rate in adult ruminants is generally lower, at 10–30%. The abortion rate can range between 40 and 100% ((Morrill and McClain, 1996; Spickler, 2015; Mansfield *et al.*, 2015).

Abortions, apparently unrelated to the gestation period, are the most characteristic sign in adult sheep, goats and cattle. There are also reports of abortions in wild ruminants including African buffalo, a waterbuck, a springbuck and a blesbuck. Some pregnant animals have few or no clinical signs other than abortion, while others become ill or die. Ocular signs (corneal opacity/ edema and erosions, and anterior uveitis) occurred 8-9 days after inoculation in some minimally affected animals. Hemorrhagic diarrhea has been seen in both naturally and experimentally infected sheep, and may be more severe in some breeds. Infections are often subclinical in adult cattle, but some animals can have a few days of fever, anorexia, weakness, excessive salivation, lacrimation, nasal discharge, bloody or fetid diarrhea, and decreased milk production (Spickler, 2015; Mansfield *et al.*, 2015).

Clinical signs in camels during outbreak include sudden death or an acute syndrome characterized by fever, neurological signs (e.g., ataxia), expiratory wheeze, ventral positional dyspnea, edema at the base of the neck, icterus, blood-tinged nasal discharge, hemorrhages on the oral mucosa and abortions. Camels that developed hemorrhagic signs usually died within a few days. Severe conjunctivitis and blindness occurred in some animals (Spickler, 2015).

Descriptions of illnesses in other species are based mainly on laboratory infections. Some wild or laboratory rodents, including rats, mice and hamsters, have nonspecific signs (e.g., weight loss, fever) of varying severity, with neurological signs and deaths in some cases. RVF virus can cause nonspecific signs of illness (fever, anorexia, depression), neurological signs, or hemorrhagic signs (petechiae, ecchymoses and bleeding from the nose, gums or venipuncture sites) in some species of nonhuman primates, while some other species seem to be unaffected or minimally affected (Spickler, 2015; Mansfield *et al.*, 2015).

Human infections are generally subclinical or self-limited although it can be associated with a severe febrile illness and jaundice. Less than 5% develop the three major complications: hepatitis, encephalitis, retinitis and other ocular lesions, and a haemorrhagic fever (Khan and Smith, 2016). The overall case fatality ratio is estimated to be between 0.5% and 2%. Human cases with jaundice, neurological disease, or haemorrhagic complications are at increased risk of fatality. Generally, most of the sign in human cases are fever, nausea, vomiting,

abdominal pain, diarrhea, jaundice, CNS manifestations, haemorrhagic manifestations, and ocular complications (Pepin *et al.*, 2010).

2.4. Diagnosis

Early detection of suspected cases is pivotal to ensure timely control measures are implemented to reduce the disease burden.

2.4.1. *Clinical sign*

The classical hallmark of RVF epizootics is the sudden onset of large numbers of abortions ('abortion storms') regardless of the stage of pregnancy and mortalities among young animals in affected livestock. This epizootic together with the appearance of the disease in humans is considered characteristic of an RVF epidemic (Mansfield *et al.*, 2015). These massive abortion events have been referred to as ''abortion storms'' and allow for the differentiation of RVF from many of the other common infectious causes of abortion in ruminants such as: Q fever *Coxiella burnettii*, *chlamydiosis*, *salmonellosis*, *listeriosis* or *toxoplasmosis* (Pepin *et al.*, 2010). Signs of disease in adult animals included vomiting, diarrhea, and listlessness. In pregnant ewes, often the only sign of illness was abortion of the fetus, while the ewes themselves displayed few symptoms prior to being found dead (Mcmillen and Hartman, 2018).

2.4.2. *Postmortem lesion*

The most consistent lesion in all species is hepatic necrosis, which tends to be more extensive and severe in younger animals. In aborted fetuses and newborn lambs, the liver may be very large, yellowish-brown to dark reddish-brown, soft and friable, with irregular patches of congestion. Multiple gray to white necrotic foci are usually present, but may not be grossly visible. The liver lesions are often less severe and more localized in calves and adult animals, and may consist of numerous pinpoint reddish to grayish-white necrotic foci. The walls of the gallbladder may be edematous, and can contain visible hemorrhages. Hepatic necrosis is also the most prominent and consistent microscopic lesion, and eosinophilic oval or rod-shaped intranuclear inclusion bodies

may be found in the liver in up to 50% of cases. Additional gross lesions may include jaundice, widespread subcutaneous hemorrhages, petechial and/or ecchymotic hemorrhages on the surface of other internal organs and fluid in the body cavities. The peripheral lymph nodes and spleen are typically enlarged, congested and/or edematous to some degree, and may contain petechiae. A variable degree of inflammation or hemorrhagic enteritis can sometimes be found in the intestines of ruminants and experimentally infected puppies and kittens. Ruminants may have hemorrhages and edema in the abomasal folds, sometimes with blood in lumen of the intestine. Aborted fetuses are often autolyzed (Spickler, 2015).

2.4.3. *Serology*

A vigorous adaptive immune response is developed rapidly following infection, with the production of detectable neutralizing antibodies from the 4th–8th day after infection. These antibodies, which are primarily directed against the viral glycoproteins, *Gn* and *Gc*, are also accompanied by the production of IgM and IgG antibodies raised against the nucleoprotein, N, and the nonstructural protein, NSs (Pepin *et al.*, 2010).

Serological diagnosis, usually by virus neutralization assay (VNA) or enzyme-linked immunosorbent assay (ELISA), is commonly used to confirm RVFV infection in an affected individual (animal or human), during outbreak management and also to determine the prevalence of exposure to RVFV in a susceptible population (surveillance). There are also alternative techniques such as the Indirect Immunofluorescence, agar gel immunodiffusion (AGID), radioimmunoassay and complement fixation, however; are they no longer used. Similarly, haemagglutination inhibition assay (HIA) is rarely used due to biosafety and logistical issues (Mansfield *et al.*, 2015).

The VNA is the gold standard serological assay used for determination of vaccine potency and is the OIE recommended test for international trade (Mansfield *et al.*, 2015). The test is highly specific and sensitive, unlike some ELISA based assays, can be applied to serum from a wide range of host species. However, virus neutralization assays can only be performed in appropriate biosecurity facilities as they involve the manipulation of live virus. The classical VNT is based on the incubation of a standard amount of RVFV with serial dilutions of sera followed by addition of cells.

The induction of cytopathic effect (CPE) after 4–7 days is used as the readout (Mansfield *et al.*, 2015). Alternative novel RVFV neutralization assays are being developed and validated, which eventually may lead to assays which can be performed of outside of classical biosafety containment (Wichgers *et al.*, 2017).

ELISAs can be employed to confirm the presence of either specific IgM antibodies, which appear transiently from 4 days after infection or specific IgG antibodies, which appear from 8 days after infection and may persist for several years. Hence, either serum or blood samples collected for serological analysis may contain live virus and must therefore be inactivated prior to testing outside of biocontainment. ELISAs are developed and widely applied by OIE Reference Laboratories, and several have been developed using either whole cell lysate derived from infected cells or purified nucleocapsid protein as antigen. However, the commercially available ELISAs (IgG and IgM) are based upon recombinant RVFV nucleocapsid protein, despite the potential for high background issues with this antigen. The detection of IgM would suggest a current or recent infection. However, for IgG-based ELISAs cannot distinguish between past and current infection unless paired serum samples are analyzed (acute and convalescent) and a four-fold increase in antibody titre observed (Mansfield *et al.*, 2015).

An indirect ELISA based on the recombinant nucleocapsid protein of RVFV has been developed for the detection of specific antibodies in human and animal sera. This dual target assay is designed to differentiate between infected and vaccinated animals (DIVA). In naturally occurring infections, an antibody response against both N and NSs would be expected whereas in individuals vaccinated with the attenuated vaccines (NSs) only an antibody response to the N protein would be observed. It an important factor when considering the financial burden of movement restrictions on suspect livestock and the regulatory requirements for vaccine licensing (Pepin *et al.*, 2010; Mansfield *et al.*, 2015).

2.4.4. *Virus isolation*

RVFV is readily isolated from serum or whole blood during the febrile stage of the disease as well as from the liver, spleen and brain of fresh carcasses/cadavers or aborted fetuses. Isolation of the

RVF virus is isolated in hamsters, mice and in various cell cultures (Meegan *et al.*, 1989; Pepin *et al.*, 2010). There are various cell cultures that can be successfully employed for in vitro isolation of RVFV including African green monkey kidney cells (Vero), baby hamster kidney (BHK) cells and AP61 mosquito cells. Mammalian cell lines are generally preferred for RVFV isolation due to the consistent cytopathic effect (rounding of the cells), with destruction of the cell monolayer within 12–24h post infection. Immunostaining or reverse-transcription polymerase chain reaction (RT-PCR) can be used to confirm virus isolation (Mansfield *et al.*, 2015).

2.4.5. *PCR assay and gene sequencing*

A range of highly sensitive nucleic acid based molecular tests have been developed for RVFV including nested RT-PCR methods, quantitative real-time PCR, multiplex PCR-based macroarray assay, RT Loop-mediated isothermal amplification (RT-LAMP) and recombinase polymerase amplification (RPA) (Mansfield *et al.*, 2015). Real-time reverse transcriptase-PCR has high sensitivity in detection of the RVFV genome in infected animal sera and in mosquitoes (Garcia *et al.*, 2001). Quantitative real-time PCR is also used to quantify virus load in mosquito samples during RVFV outbreaks (Mwaengo *et al.*, 2012a).

The ability to sequence entire viral genomes relatively quickly should lead to rapid progress in understanding the detailed ecology of RVFV. Ongoing surveillance and RVFV characterization also should help determine the pattern of virus maintenance between epizootic events. As prediction tools become more accurate and available, these data will provide public health authorities an opportunity to anticipate and prepare for RVF outbreaks (Aradaib *et al.*, 2013). The successful high-throughput generation of the complete genome sequence was achieved for 33 diverse RVF virus strains collected from ten Africa countries and Saudi Arabia from 1944 to 2000 (Bird *et al.*, 2007). Phylogenetic analysis of these isolates revealed that seven main viral lineages were categorized as A, B, C, D, E, F, and G among the 33 viruses (Nanyingi *et al.*, 2015). While several distinct virus genetic lineages were determined, which approximately correlate with geographic origin, multiple exceptions indicative of long-distance virus movement have been found. Virus genetic diversity was low (<5%) and primarily involved accumulation of mutations at an average of $2.9*10^{-4}$ substitutions/site/year (Bird *et al.*, 2007).

2.5. Public and Economic Importance

Economic loss associated with RVF disease is due to a combination of livestock deaths and strict trade bans imposed and enforced by the World Organization for Animal Health (OIE) preventing animal export during outbreaks (Glancey *et al.,* 2015). The socio-economic impact of RVF disease on people's livelihoods and on trade can be high due to significant losses in livestock production (meat and milk), closure of livestock markets and bans on livestock movement and slaughtering (Aziz *et al.*, 2018). Economic impacts of RVF arise due to abortion and mortality in livestock, which tends to be higher in young animals and the restrictions on animal movement and embargo on livestock and animal products trade during an epidemic (Clements *et al.,* 2007).

The disease disproportionately affects vulnerable communities with low resilience to economic and environmental challenges, particularly pastoralists (Dar *et al.*, 2013; Muga *et al.*, 2015; Baba *et al.*, 2016). The potential risk of RVF virus spread is high. A single introduction of RVF virus, given the proper environmental conditions, could spread rapidly, resulting in catastrophic economic losses and significant human disease (Bird *et al.*, 2007). For example, the overall economic loss during the 2006–2007 RVF outbreaks in East Africa was estimated to exceed $60 million (Baba *et al.*, 2016). By fearing such potential impact of the RVF disease on human and livestock many disease free countries are modeling RVF outbreak and risk of introduction into their countries; as instance, Netherlands (Fischer *et al.*, 2013), European Union (Rolin *et al.,* 2013), and North America (Turell *et al.,* 2010).

2.6. Prevention and Control Method

There is no specific treatment for RVFV infection in humans and animals and therefore management of clinical cases is only through supportive therapy (Baba *et al.*, 2016). Neither effective antiviral commercial, licensed vaccines are available for human. Phylogenetic data supports the conclusion that the relatively small genetic diversity suggests antivirals effective for one strain should be active against all (Lang *et al.*, 2019).

RVF is regional problem; hence, cooperation between neighboring countries is essential for surveillance, prevention and control. To prevent RVF outbreak, World Animal Health Organization (OIE) recommended: systemic ongoing surveillance in sentinel animal, immediate notifications of clinical cases upon detection; coordinated sharing of information between animal health and public health services, controlling vector (mosquito) population, monitoring variations climatic conditions and vaccination of animals in endemic areas are the list of options recommended (OIE, 2018).

Two types of vaccine are there conventional and novel vaccines. Conventional vaccine include inactivated or attenuated live vaccine while novel vaccine formations based on DNA techniques (OIE, 2018). Vaccination against RVF has been practiced for many years in Africa, as a control measure against the economic losses sustained in highly susceptible breeds of sheep and cattle. Veterinary vaccines used in endemic and epidemic countries include a formalin-inactivated vaccine and an attenuated vaccine strain (Smithburne neurotropic strain) that was developed in 1949 (Wilson *et al.*, 2014). A modified live virus strain, the Smithburn, is inexpensive to produce and despite potential for vaccine associated abortions, has been extensively used for many years in high production livestock systems as a prophylactic measure. Livestock owners have vaccinated routinely, when animals were not pregnant, and have maintained a high level of immunity in their herds or flocks. This has been a cost-effective commercial practice (Davies, 2010). The neutralizing antibodies are the key factor for the initial and persistent protection of infected animals and are, consequently, a good correlate of the protection induced by vaccines against RVFV: if vaccinated animals produce a high level of neutralizing antibodies, they will very likely be protected against experimental challenge or subsequent natural viral infection under field conditions (Wilson *et al.*, 2014).

In non-endemic countries, an active surveillance strategy employing the use of sentinel herds is cost prohibitive. However, robust passive surveillance-based systems that rely on the detection and rapid reporting of significant abortion events to national authorities could provide a cost effective means to detect the emergence of this significant veterinary and human health threat (Pepin *et al.*, 2010).

3. MATERIALS AND METHODS

3.1. Study Area

The current study conducted in different part of Ethiopia where the sites (Figure 3 below) were selected based on the ecological significance for introduction and maintenance (mosquito breeding sites) of RVFV. The areas classified into two as introduction risk (Borena zone) area and near water bodies (Lake Ziway, Lake Hawassa, Lake Abay and Segen River) which are good breeding area for the vector mosquitoes.

Adami Tullu Jido Kombolcha is one of the districts in East Shoa zone of Oromia region in the Great Rift Valley of Ethiopia. Lake Ziway found on the northeast side of the district. The main town of woreda is Batu (Ziway) which found on the western shore of Lake Ziway. Lake Ziway is one of the lakes in the Rift Valley used for multiple purposes like irrigation, fishing, domestic water supply, transportation, recreation, supply of fresh water and horticulture farming (Abera *et al., 2*018). The main rainy season generally extends from June to October. Study area in this district located within the range of geographical area of 7.96 -7.86N, 38.71-38.83E.

Hawassa city is the largest city in the Great Rift Valley of Ethiopia. Main part of the city is on the eastern shore of Lake Hawassa. It is located between 7.012 - 7.098N and 38.45 - 38.52E; 273 km south of Addis Ababa via Bishoftu 130 km East of Sodo, and 75 km North of Dilla. The town serves as the capital of the SNNP region and is a special zone of this region. Hawassa has a mean annual rainfall of about 950 mm and temperature of 20°C. The main rainy season generally extends from June to October (Kebede *et al.*, 2014; Admasu, 2015). Arba Minch city is the separate district in the Gamo Gofa Zone of the SNNP, Ethiopia. It located about 500 km South of Addis Ababa. It is also in the heart western side of the Great Rift Valley, on the shores of two major lakes; Lake Chamo to the South and Lake Abaya to the North. Mosquito collection in this area were carried out around lake Abay which is 5km far north from Lake Chamo (Tirados *et al.*, 2011; Feki *et al.*, 2014). Current study areas from Arba Minch were located between 5.99 - 6.10N, 37.55- 37.61E. Konso is a special Wereda in North Omo Zone which is 90 km south of Arba Minch. The seasonal Segen River

(which originates in Lake Chamo) flows through Konso (1,640 m) on its way to join the Weyto, and then terminating in Lake Chew Bahir. On the western side of Amarro horst the valley through which the river flows is called Segen Valley (BirdLife International, 2019). Mosquitoes were collected from Segen Valley within location of 5.234 - 5.236N and 37.527 - 37.528E.

The entomological survey in the mid Rift valley and Segen valley was conducted during the dry part of the year and the four study areas were include around Ziway town, around Hawassa and Arba Minch city and in Segen valley. The areas were selected based on the assumption that three main lakes and river in the rift valley of Ethiopia *viz.,* Lake Ziway around Ziway town, Lake Hawassa around Hawassa city, Lake Abaya around Arba Minch city and Segen valley around Segen River were considered the best breeding sites for mosquitoes during study periods.

Borena is one of zone in Oromia region of Ethiopia bordered on the South by Kenya, on the West by the Southern Nations, Nationalities and Peoples (SNNP) region, on the North by West Guji and Guji zone, and on the East by the Somali region (Lasage *et al.*, 2010). Yabello is the capital town of the zone and lies 570 km South of Addis Ababa. It has thirteen districts; five of them include Miyo, Dillo, Dire, Moyale and Taltelle are share border with Kenya. The current entomological survey conducted in Borena within three districts: Moyale, Yabello and Elwaye. The region is semi-arid savannah, marked by flood plains vegetated predominantly with grass and bush land. The zone consists about 75% of lowland and it frequently is exposed to droughts (Lasage *et al.*, 2010). People are predominantly involved in small-scale subsistence agriculture production and mainly on livestock husbandry. Peoples rear different animals, predominantly cattle, sheep, goat and camels.

Pastoral mobility widely recognized around the border of Borena zone. It is one of the most successful strategies used by pastoralists to deal with uncertainty and risk prevailing in drylands ecosystem. Mobility is not only a survival issue, but also a resource management strategy to exploit spatial and temporal variation in rangeland resources distribution (Sara, 2010; Lind *et al.*, 2016). In search of feed and water for their livestock, migration is increasing both within and across national borders. Some pastoralists are migrating to protected conservation areas, having negative consequences for environmental gains and increasing risk of disease transmission between wild animals and livestock (FAO, 2017).

There are no perennial rivers and rainfall varies highly, both spatially and temporally. The water sources experience high evaporation rates, frequently drying up early in the dry season. Drought is a common phenomenon in many parts of Borena; particularly lowland parts are severely affected by recurrent droughts. The rainfall pattern is highly erratic often do not occur at the expected time and sometimes above rainfall and sometimes it is far below normal. Bimodal rainy season has experienced in area from October to December and March to May (Lasage *et al.*, 2010). According to CSA, 2017 reports the zone has cattle 1,056,040 cattle, 573, 36 sheep, 868,571 goats, 51,607 camels (CSA, 2017).

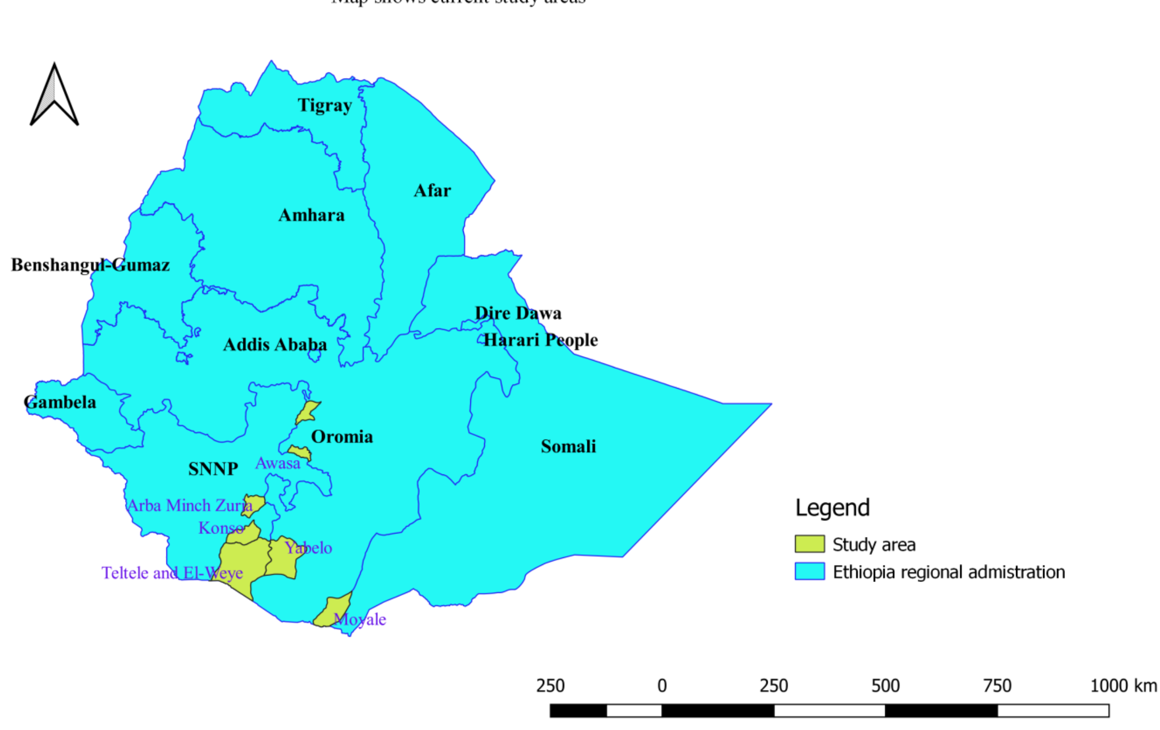


Figure 3: Study area for entomological survey

3.2. Mosquito Collection and Identification

Mosquito trapping with survey of other localities' information (Annex-VII) were conducted in study areas for three rounds. The first term was from 02-15 December, 2018 around Ziway and Hawassa lakes, the second term was from 14-28 February, 2019 around Arba Minch and the third was from 10-24 April, 2019 in Borena zone and Segen Valley. Mosquitoes were collected by using three different types of traps: CDC light traps, UV light traps, and Modified UV light traps. CDC light trap baited with sugar-yeast solution (i.e., used as attractant instead of CO_2). The traps were set in proximity to potential mosquito breeding and feeding sites include indoor and outdoor (near water bodies near animal pen, and on field of where there are human and livestock population). The trap placed at 18:00 PM and collected at 6 -7:00AM in succeeding day. The next morning the traps were collected and using collection cup and put in deep freeze (-20°C) for 15 minutes to kill mosquitoes, then sorting and identification into genus and species level were made by using dichotomous keys of Walter Reed BioSystemics Unit (WRBU) (Potter, 2016) and Edwards, (1941) under the aid of a stereo light microscope and with the assistance of entomology expert. Then, 20- 25 mosquitoes were pooled into eppendorf tube (2ml) according to species, sex, collection date and trap number. The pool then transferred into container containing nitrogen liquid and transported to National Veterinary Institute (NVI), Bishoftu and stored at -80°C until further analysis.

3.3. Mosquito Processing

For viral detection, preserved mosquito pools were processed in the National Veterinary Institute of Ethiopia (NVI) research laboratory. The mosquito pools were ground and homogenized in biosafety level II laboratories at the NVI. The mosquitoes were ground using sterile mortar and pestle by adding 2-3ml of sterile PBS containing antibiotic. The supernatant was then harvested by centrifuged at 12,000 rpm for 10 minutes and stored in a 1.5 ml cryovial at -20°C for further testing.

3.4. Viral RNA Extraction

RNA was extracted using the RNAeasy Mini kit for nucleic acid extraction (QIAGEN, AMBION, Inc., Austin, Texas, USA) in accordance with the manufacturer's instructions (Annex-I). Briefly,

300μl mosquito samples were homogenized with 600μl of RLT buffer by centrifuging for 3 min at maximum speed. From these up to 700μl of the sample transferred to an RNeasy Mini spin column placed in a 2 ml collection tube, and centrifuge for 15s at ≥8000 x g; then flow-through discarded. Alternatively, 700μl RW1 Buffer and 500μl RPE Buffer added to the RNeasy spin column and each centrifuged for 15s at ≥8000 x g, the flow-through were discarded at both step. Another 500μl RPE Buffer were added and centrifuged for 2 min at ≥8000 x g. Finally, RNeasy spin column placed in a new 1.5 ml collection tube, 30–50μl RNase-free water were added directly to the spin column membrane, and centrifuged for 1 min at ≥8000 x g to elute the RNA. Eventually, viral RNA extracted was immediately stored at -20°C until amplification.

3.5. Reverse transcription PCR and Visualization of RT-PCR products

RVFV genome detection in mosquito pools was conducted using reverse transcription PCR (RT-PCR). RT-PCR amplification was performed using specific primers (forward primers S432: 5'-ATGATGACATTAGAAGGGA-3' and reverse NS3m: 5'-GATGCTGGGAAGTGATGAG-3') targeting the region of S-segment of the viral genome to amplify 292bp according to Pan African Veterinary Vaccine centre (PANVAC) standard operative procedure (SOP) (unpublished). RT-PCR was performed by using one-step RT-PCR kit (Invitrogen, USA) and the master mixes were made according to manufacturer's instructions (Annex-II). Briefly, a total volume of 30μl of master mix was prepared, containing 5μl of template RNA, 8µl of nuclease free water, 5μl of 5x one-step conventional gel based-PCR buffer, 5μl of 5x Q solution, 1μl of 10mM each deoxynucleoside triphosphate (dNTP) mix, 1μl one-step enzyme mix, 2.5μl of each forward and reverse primers. RT-PCR was performed using conventional RT- PCR (BIO RAD, CFX^{TM} Real-Time System). The region of interest was amplified with the following conditions: 50°C for 30 min, followed by 94°C for 5 min, then 30 cycles of denaturation at 94°C for 30 sec, primers annealing temperature at 40°C for 30sec, and extension at 72°C for 30 sec. The reaction was then subjected to final extension at 72°C for 5 min. A positive control for RVFV was kindly provided by PANVAC while nuclease free water was used as a negative control.

The RT-PCR products were visualized by electrophoresis in a 1.5% agarose gel in 0.5 x Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer (SERVA, Heidelberg, Germany) stained with GelRed

nucleic acid stain. Each well was loaded with 5μl of the PCR product and 1μl of 6x DNA loading dye (Promega, Madison, USA). Samples were separated along with DNA ladder (Promega, Madison, USA) at 150 V for 30 min. The PCR products were visualized using a gel documentation system and scoring was done based on the size of the PCR products.

3.6. Mosquito Distribution Modeling

3.6.1. Potential predictors of RVF vector mosquitoes occurrence

Models for determining areas at risk for RVF outbreaks consider several factors. However, factors related to vector ecology utmost important. Knowledge of vector ecology is important in understanding the transmission dynamics of vector borne disease (Arum *et al.*, 2015). RVF-relevant ecological factors have the advantage that most of them can be mapped effectively over larger areas and be used as disease trigger mechanisms in early warning systems (Mosomtai *et al.*, 2016). Excess rainfall results in flooding and hatching of dormant *Aedes* virus-infected mosquito eggs in *dambo* habitats and then infect livestock and humans (Balenghien *et al.*, 2013). Poorly drained soils are prone to flooding and provide good habitats for floodwater *Aedes* mosquito species. Surface water hydrology is influenced by rainfall, soil type, vegetation type, humidity, and temperature (WHO, 2009). The significant role of mosquitoes in RVFV transmission has resulted in the generation of climate and environmental-based models to predict the risk of RVF outbreaks using a combination of temporal, spatial and remotely sensed satellite environmental data, including vegetation indices, temperatures, and proxy indicators of rainfall, that directly affect RVFV vector mosquito development and survival in RVF-endemic regions (Britch *et al.*, 2013).

In most RVF-endemic regions, excess and prolonged rainfall has a positive linear relationship with vegetation development. The ‘‘greening’’ of habitat indicated by increases in normalized difference vegetation index (NDVI) in turn has a direct relationship with RVFV mosquito vector development, emergence, and survival. Specifically, 3 months of sustained excess rainfall and resultant persistent increases in NDVI are strongly indicative of impending high levels of RVFV transmission in large part due to extremely favorable vector mosquito habitat (Britch *et al.*, 2013). Thus, these data are used as an indicator of breeding and upsurge patterns of some insect pests and vectors of disease,

including locusts and mosquitoes (Balenghien *et al.*, 2013). The NDVI is a dimensionless index that describes the difference between visible and near-infrared reflectance of vegetation cover and can be used to estimate the density of green on an area of land.

NDVI data is downloaded from website of Famine Early Warning Systems (FEWS) https://earlywarning.usgs.gov/fews/datadownloads/East%20Africa/eMODIS%20NDVI%20C6.
The NDVI are the product of a temporally smoothed 250m spatial resolution NDVI data set which have invalid value of 201 – 255. Calculations of NDVI for a given pixel always result in a number that ranges from minus one (-1) to plus one (+1) valid values. Hence, by using NDVI = (value - 100) /100 invalid value convert into it to valid values (Swets *et al.*, 1999). No green leaves give a value close to zero. A zero means no vegetation and close to +1 (0.8 - 0.9) indicates the highest possible density of green leaves (Weier and Herring, 2000).

Rainfall (precipitations) creates an ecologically humid environment that insures the proliferation of breeding sites and the development of RVF vectors. Rainfall floods the mosquito breeding habitats which contain eggs of the primary vector species and reservoirs (transovarially infected *Aedes spp.*), and these subsequently serve as a habitat for development of secondary vectors (e.g., *Culex spp.*) (Bicout and Sabatier, 2004). Similarly, wind has a significant effect on vector distribution and geographical range or distribution of vectors also tends to be limited by a minimum and maximum temperature/humidity (Baba *et al.*, 2016). To account for the impact of climatic factors on the distribution of RVF mosquito vectors, data were downloaded from the WorldClim database (http://worldclim.org/). WorldClim version 2 (Fick and Hijmans, 2017) has average monthly climate data including minimum, mean, and maximum temperature as well as precipitation for 1970-2000. Solar radiation (kJ m^{-2} day^{-1}), wind speed (m s^{-1}) and water vapor pressure (kPa) are also available in version 2 of the Worldclim database. The database provides these climatic layers at different spatial resolutions, from 30 seconds (~1 km^2) to 10 minutes (~18 km^2); 2.5 arcminute resolution data (~5 km^2) were used in this study.

Land cover information is becoming increasingly important for landscape ecology and environmental monitoring (Zhang and Mei, 2016). Strong influence of anthropogenic land use change on mosquito communities could have potential implications for pathogen transmission to

humans and wildlife (Meyer *et al.,* 2016). Habitats productivity, species dynamics and abundance, mosquitoes feeding rates, and sporogony development are presented in relation to temperature changes, deforestation and land coverage changes (decrease), which leads to temperature changes and subsequently increases survivorship of adults and sporogony development in adult mosquitoes' body (Kweka *et al.,* 2016). To account for the impact of land usage on the distribution of RVF mosquito vector, land cover data were downloaded from the European Space Agency's GlobCover Portal (http://due.esrin.esa.int/page_globcover.php). In this study, GlobCover v 2009, released on 21st December 2010, was used. This dataset is the most recent (2009) available and specifies 22 classes (Bontemps, Defourny and Eric, 2010)(Bontemps, Defourny and Eric, 2010)(Bontemps *et al.,* 2010), based on the Land Cover Classification System (LCCS) at a high resolution (300m).

The clay and loamy soil texture support long periods of water retention (flooding) and render it suitable for breeding primary mosquito vectors and the survival of their RVFV-infected eggs. Unlike sandy soil, clay soil texture supports the retention of water for long periods of time, and thereby contributes to the flooding and wetness of the habitat, making it suitable for the breeding and survival of mosquito vectors (Baba *et al.*, 2016). The data were downloaded from FAO's Harmonized World Soil Database (HWSD) (v1.2) (http://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/harmonized-world-soil-database-v12/en/). Raster data of elevation also downloaded from this website. HWSD is a 30 arc-second raster database with over 15000 different soil mapping units that combines existing regional and national updates of soil information worldwide (Nachtergaele *et al.*, 2012).

Livestock densities great play role in transmission of RVF virus assuming the existence of direct transmission, an increase in domestic ruminant density is expected to increase the number of potentially infectious contacts that a susceptible individual experiences over a given time; therefore, there is a greater risk of amplification. Because infectious ruminants may travel after being sold, ruminant density was also considered as a factor of spread (Tran *et al.*, 2016). The data for livestock densities were downloaded from the FAO Gridded Livestock of the World (GLW) web (http://www.fao.org/livestock-systems/global-distributions/en/). The latest version of GLW has a reference year of 2010 and includes global distributions of cattle, buffaloes, sheep, goats, horses,

pigs, chickens and ducks at a spatial resolution of 5 minutes of arc, approximately 10 km at the equator (Gilbert *et al.*, 2018).

3.6.2. GIS Operation

In the current study, eleven explanatory variables in raster data format have incorporated in modeling, but originally they have different format, extent and resolutions. However, for modeling in R using 'biomod2'all GIS layers have to share the same projection system, extent and resolutions. Hence, by using Quantum geographic information system (QGIS Desktop 3.2.0 software) tools include clip and raster calculation all raster layers were rescaled to have the same extent and resolution. Finally, all layers have clipped to East African countries (-12.0°, 20.0°N: 23.0°, 52.0°E) with resolution of 5arcminutes and the same coordinate system (EPSG: 4326 - WGS 84 - Geographic) and GeoTIFF (*.*tif**) raster format.

Multicollinearity among explanatory variables was checked using variance inflation factor (VIF) analysis (Graham, 2003), with the "*vifstep"* command in the "*usdm"* package of R (Naimi *et al.*, 2014; Naimi, 2015). A stepwise selection routine was implemented to select a set of variables with sufficient low multicollinearity and only variables which had VIF values less than or equal to 10 were considered in the analysis (Craney and Surles, 2002). As an indicator of multi-collinearity, the larger the value of VIF the more collinear the variables. As a rule of thumb, if the VIF of a variable exceeds 10, which will happen if multiple correlation coefficients for a variable R^2 exceed 0.90, that variable is said to be highly collinear. Calculates variance inflation factor (VIF) for a set of variables and exclude the highly correlated variables from the set through a stepwise procedure. Final ensemble model out were also plotted and mapped with using QGIS software.

3.6.3. Modeling approach

The current model developed using sampling point (geo-reference data) and different climatic and environmental data. Distribution of RVF vector mosquitoes in Ethiopia were estimated based on ensemble species distribution modeling using the '*biomod2*' package in R software. BIOMOD is a computer platform for ensemble forecasting of species distributions, enabling the treatment of a

range of methodological uncertainties in models and the examination of species-environment relationships. BIOMOD includes the ability to model species distributions with several techniques, test models with a wide range of approaches, project species distributions into different environmental conditions and dispersal functions (Thuiller *et al.*, 2009). It provides a suite of methods and tools relevant to the problem of modeling distributions, such as the ability to quickly build individual models and to combine them in different ways. BIOMOD was first developed in the S-Plus language environment in 2003 and was later ported to the R statistical language environment as a package under the name '*biomod2*'(Hao *et al.*, 2019). *'biomod2'* develop models by using ten different algorithms: general linear models (GLM), general boosted models (GBM, also referred to as boosted regression trees), general additive models (GAM), classification tree analysis (CTA), artificial neural networks (ANN), surface range envelope (SRE), flexible discriminant analysis (FDA), multiple adaptive regression splines (MARS), random forests (RF), and maximum entropy (MAXENT). All of these techniques need absence and presence records to determine the suitability range for the species under question. However absence records were not available, so pseudo-absence background data were generated by Surface Range Envelope (SRE) model. It coerces pseudo-absences to be selected outside of the broadly defined environmental conditions suitable for the species.

All of the models were generated using a calibration (train) subset of 80% of the input data set and an evaluation (test) subset of 20%. The true skill statistic (TSS) and the area under the receiver operating characteristics (ROC) curve were used to assess the models' performance. This two metrics are indicators of discrimination capacity, which quantifies how well the model can distinguish presences from absences (or presences from background samples, when absences are unavailable) (Hao *et al.*, 2019). Three evaluation runs were performed during the modeling, resulting in a total of 30 models (10 modeling methods x 3 folds), from which the average values of TSS and ROC were taken. TSS measures the model performance based on sensitivity and specificity and includes commission errors. The TSS value ranged from −1 to +1, where +1 indicates perfect agreement between predictions and observations and values of 0 or less indicate agreement no better than random classification (Allouche *et al.,* 2006). Only modeling algorithms with an average TSS score above 0.8 were included in the subsequent ensemble modeling procedure. This ensures that the individual models used to build the ensemble models were the best suited and most accurately

predicted occurrences. The model techniques passing the TSS test were used in the final modeling steps using the full data set. The resulting models are referred to as "full models" as they use all the presence and absence points and not just the 80% as the evaluation models. TSS was only calculated for the evaluation models, as the full models do not contain a data-split and, therefore, cannot be evaluated by TSS. Instead, the full models were evaluated by their response plots and environmental variables were excluded from the ensemble if they did not generate an effect response.

The models produced raster cells with values ranging between 0 and 1000. The values indicate how close the climate and ecological conditions within in each cell are to the optimal conditions for the species in question; with higher values indicating higher suitability. As a rule of thumb, sites with suitability higher than 500 predict presence, while sites with suitability lower than 500 indicate absence. The estimated suitability value was divided by 1000 to convert the suitability value into a probability of occurrence. During model development the '*build.clamping.mask*' was set to 'TRUE' to identify locations where predictions could be uncertain. Predictions could be uncertain if values of the variables extend outside the range used for calibrating the models. Models committee averaging, which gives both a prediction and a measure of uncertainty, was also developed during the ensemble modeling.

3.7. Ethical Consideration

Prior to start of the study, official ethical clearance for the project have requested and taken from Animal Research Ethics Committee of College of Veterinary Medicine and Agriculture, Addis Ababa University (Annex-II). In addition, prior to begin study, recommendation from Ministry of agriculture to conduct research on RVF have requested and permitted (Annex-VI). From around study area permission from the districts and respective village authorities have also requested for the study and they agreed before beginning of study.

4. RESULTS

4.1. Mosquito Survey

A total of 2,322 adult mosquitoes were collected from all study areas and many of them were identified. The identified mosquitoes include genus *Aedes* (n = 404; 17.40%), *Culex* (n = 466; 20.06%), *Mansonia* (n = 210; 9.04%), and *Anopheles* (n = 493; 21.23%) while other mosquitoes (n = 746; 32.12 %) were unidentified. The prominent species collected were *Mansonia uniformis* from around Ziway Lake, *Aedes ochraceus* from Moyale Borena, *Aedes geniculatus* and *Culex antennatus* from Hawassa and *Culex univitattus* from Arba Minch as shown in Table 1 below. Abundance of identified mosquito per site was 21.76% (n = 343) in Borena (Moyale), 14.78% (n = 233) in East Shoa (around Ziway), 23.73% (n = 374) in Sidama (around Hawassa), 23.3% (n = 383) in Gamo gofa (around Arba Minch), and 5.45% (n = 86) in Konso (Segen Valley).

Table 1. Mosquito species abundance per site of collections

Mosquito species	Moyale	Yabello	El-weye	Ziway	Arba Minch	Hawassa	Segen Valley	Number and %
Ae. mcintoshi	5	-	-	-	-	-	-	5(0.31%)
Ae. ochraceus	92	6	4	-	9	-	15	126(8.0%)
Ae. vexans	13	4	9	2	-	4	-	32(2.03%)
Ae. geniculatus	5	6	13	-	14	18	4	62(3.93%)
Other *Aedes spp.*	11	9	13	18	99	17	12	179(11.36%)
Cx. antennatus	14	-	14	-	-	92	-	120(7.61%)
Cx. univitattus	17	-	-	-	82	6	-	105(6.66%)
Cx.quinquefasciatus	115	13	5	-	-	-	8	142(9.01%)
Cx. pipiens	9	4	11	-	-	34	6	64(4.06%)
Cx. theileri	17	-	-	18	-	-	-	35(2.22%)
M. uniformis	-	-	-	58	90	62	-	210(13.32%)

An. Arabiensis	19	10	-	9	16	-	-	54(3.42%)
An. gambiae	8	2	12	7	-	6	29	64(4.06%)
Other *Anophelus spp*	15	9	12	121	71	135	12	375(23.79%)
Total	343	63	94	233	383	374	86	1576 (100%)

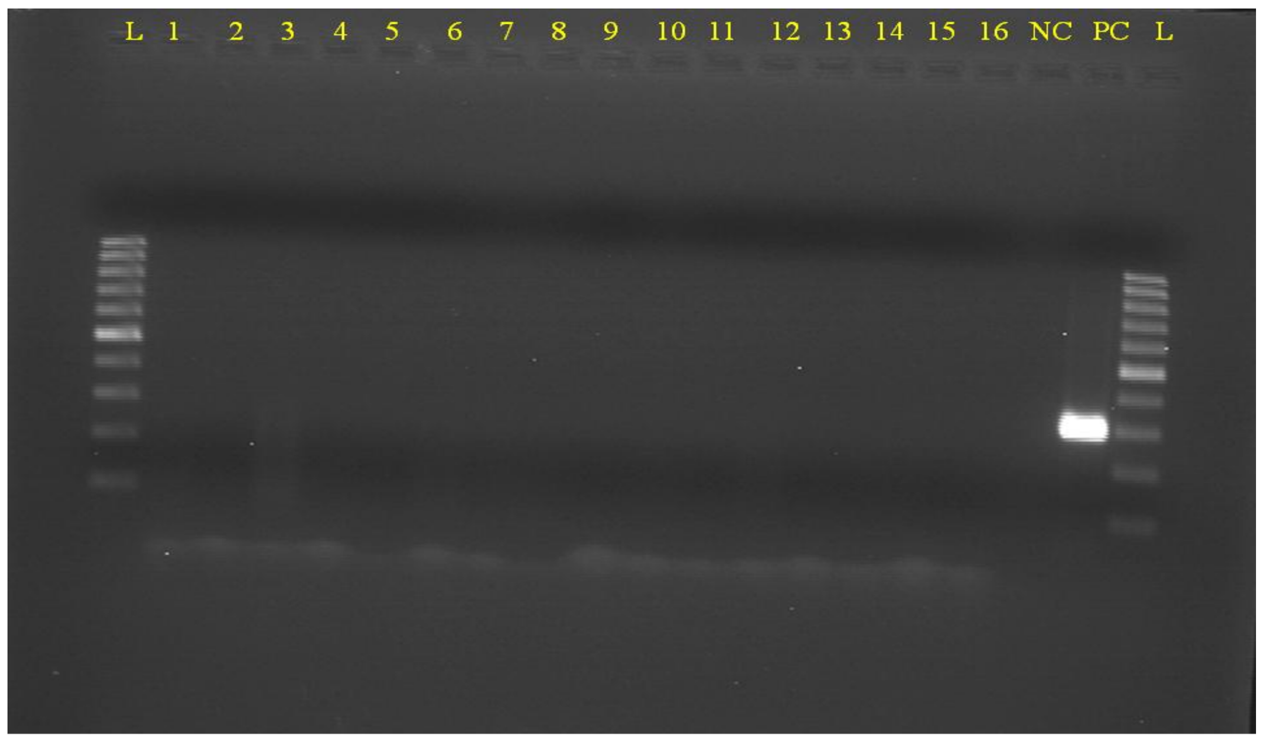
Highest number of mosquitoes were collected and identified from around water bodies include near lake shore (n = 718; 45.55%), near pond (n = 359; 22.78%), near river (n = 88; 5.58%) as depicted in Table 2 below. More *Aedes spp* and *Culex spp* were collected from around water bodies.

Table 2. Mosquito distributions in different habitats

Mosquito species	Indoor	Lake shore	Animal pen	Near pond	Outdoor on field	Segen river	Number and %
Ae. mcintoshi	-	5		-	-	-	5 (0.31%)
Ae. ochraceus	-	8	6	93	4	15	126 (8.0%)
Ae. vexans	4	2	2	15	8	1	32 (2.03%)
Ae. bromalie	-		-	6	-	2	8 (0.5%)
Ae. cumminsii	-	4	-	-	-	-	4 (0.25%)
Ae. geniculatus	-	28	-	16	14	4	62 (3.93%)
Ae. furcifer	6	11	2	-	-	-	19 (1.2%)
Cx. antennatus	70	12	10	14	14	-	120 (7.61%)
Cx. univitattus	3	81	3	16	2	-	105 (6.66%)
Cx.quinquefasciatus	13	-	-	116	5	8	142 (9.01%)
Cx. pipiens	34	-	-	11	11	8	64 (4.06%)
Cx. theileri	15	-	6	14	-	-	35 (2.22%)
M. uniformis	56	111	17	2	24	-	210 (13.32%)
An. arabiensis	8	8	-	29	9	-	54 (3.47%)
An. gambiae	5	-	-	9	3	29	46 (2.91%)
Other Aedes spp	16	90	21	17	18	9	171 (10.85%)
Other *Anophelus spp*	1	358	-	1	1	12	373(23.67%)
Total	231	718	67	359	113	88	1576
%	14.65%	45.55%	4.25%	22.78%	7.17%	5.58%	100.00%

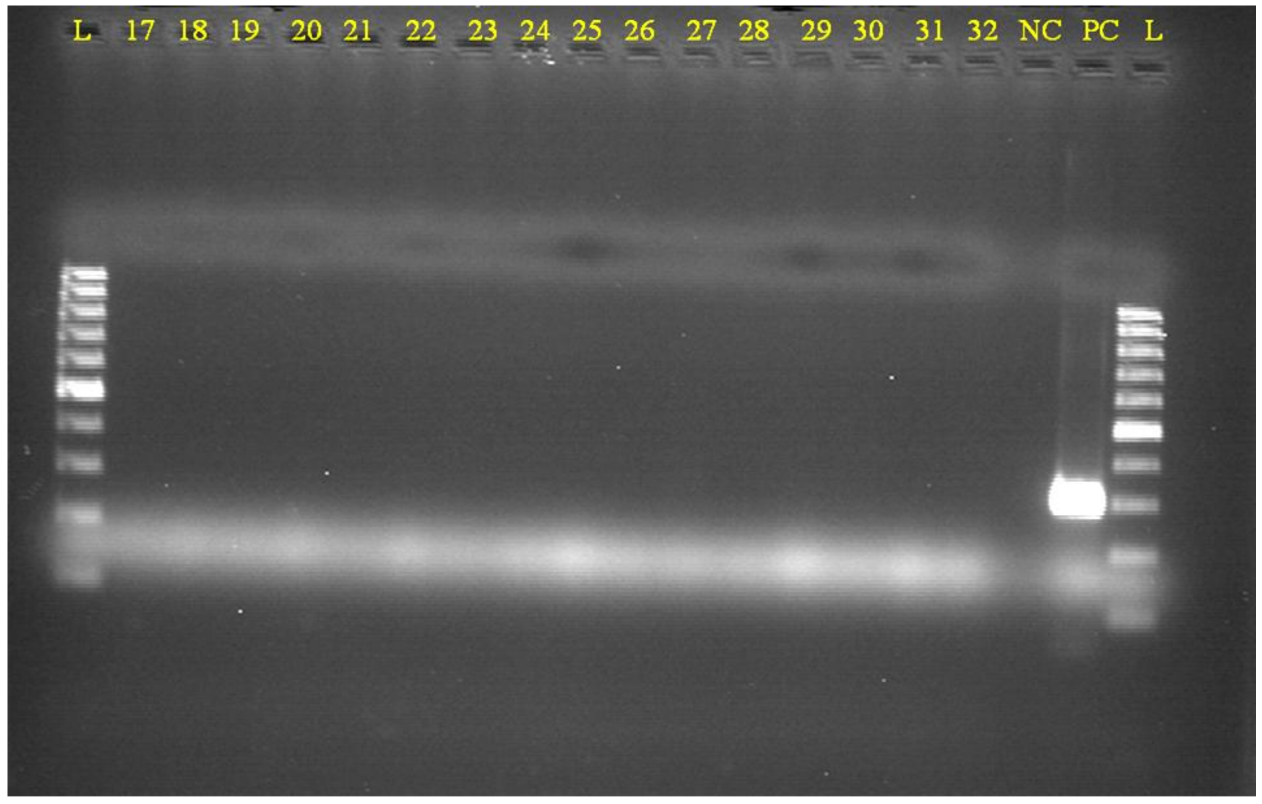
4.2. Viral Detection

For RVFV genome detection, a total of 38 pools of mosquitoes containing approximately 20-25 mosquitoes/pool were examined using RT-PCR, of which none of the mosquito pools were found positive for RVFV genome as illustrated in Figure 4 and 5.



'L'= Molecular ladder, 'NC'= Negative control, 'PC'= Positive control, "1,2,3,4……" = Sample number

Figure 4: RT-PCR result of RVFV genome detection for the mosquito's pool number 1 to 16



‘L’ = Molecular ladder, ‘NC’ = Negative control, ‘PC’ = Positive control, ’17, 18, 19, 20,………32’ assigned number of samples

Figure 5: RT-PCR result of RVFV genome detection for the mosquito’s pool number 17 to 32.

4.3. RVF Mosquito Distribution Model

4.3.1. Model performance and importance of environmental variables

Thirty individual models were developed initially Individual model performance evaluation showed among the 30 models, 26 had ROC > 0.90 ($ROC_{average}$ = 0.94), considered as good accuracy based on the classification of Swets, (1998). On average the most accurate technique was GBM, while the least accurate was ANN. Of these, 24 had TSS > 0.8 ($TSS_{average}$ = 0.83) or excellent accuracy based on the classification of Albouy *et al.* (2010). High-accuracy models (TSS > 0.8) were

combined to form ensemble forecasting of RVF mosquito vectors. The model developed performed very well (TSS = 0.974 and ROC = 0.999).

Table 3. Average performance of individual models.

Performance evaluators	MAXENT Phillips	RF	GAM	GLM	GBM	CTA	ANN	SRE	FDA	MARS
KAPPA	0.72	0.78	0.72	0.50	0.84	0.56	0.50	0.77	0.54	0.75
ROC	0.96	0.98	0.95	0.93	0.99	0.92	0.84	0.92	0.95	0.94
TSS	0.80	0.88	0.90	0.84	0.89	0.84	0.58	0.83	0.85	0.88

GLM = general linear models, GBM = general boosted models, GAM = general additive models CTA = classification tree analysis, ANN = artificial neural networks, SRE = surface range envelope, FDA = flexible discriminant analysis, MARS = multiple adaptive regression splines, RF = random forests, MAXENT= maximum entropy.

The distribution of RVF mosquito vectors is mainly influenced by water vapor pressure (vapr4), soil type (Soil type). Both the individual and the ensemble models identified two temperature variables, namely mean annual minimum and mean annual maximum temperature, as least significant determinants of the distribution of RVF mosquito vectors. The contributions of individual variables are provided in Table 4 below.

Table 4. Overall contribution of the variables in initial models

	MAXENT Phillips	RF	GAM	GLM	GBM	CTA	ANN	SRE	FDA	MARS	Overall contribution
Elevation	0.19	0.01	0.64	0.04	0.13	0.30	0.38	0.20	0.08	0.00	19.71/%
Landcover	0.17	0.01	0.42	0.21	0.02	0.15	0.53	0.22	0.07	0.26	20.61%
Soil type	0.39	0.09	0.52	0.15	0.35	0.53	0.79	0.50	0.15	0.55	40.29%
Livestock	0.11	0.05	0.32	0.04	0.26	0.45	0.36	0.45	0.18	0.32	25.36%
NDVI	0.17	0.04	0.36	0.30	0.21	0.39	0.00	0.35	0.00	0.00	18.24%
Prec	0.25	0.02	0.69	0.16	0.00	0.00	0.19	0.21	0.00	0.37	19.02%

Tmax	0.00	0.01	0.40	0.09	0.07	0.27	0.01	0.29	0.00	0.00	11.32%
Tmin	0.00	0.05	0.52	0.20	0.00	0.00	0.01	0.04	0.25	0.00	10.75%
Srad	0.09	0.04	0.42	0.62	0.04	0.00	0.34	0.19	0.36	0.13	22.40%
Vapr	0.47	0.06	0.80	0.57	0.06	0.00	0.00	0.24	0.67	0.53	34.02%
Wind	0.26	0.05	0.66	0.44	0.01	0.00	0.00	0.03	0.04	0.59	20.79%

Key: Tmax = Mean annual maximum temperature (°C), Tmin = Mean annual minimum temperature (°C) , Srad = Solar radiation (kJ m^{-2} day^{-1}), Prec = Mean annual precipitation (mm/year), Livestock = Livestock population (livestock population/5 arc minute), Vapr = water vapor pressure (kPa), Wind = wind speed (m s^{-1}), Land cover = Land cover type, Elevation(masl), Soil type = Harmonized World Soil Database, NDVI=Normalized difference vegetation index

An ensemble model was developed by incorporating weighted runs from the 24 models which met the inclusion criteria all models (TSS ≥ 0.8). As depicted in table 5 below, to develop ensemble model, water vapor pressure has the highest overall contribution (23%), followed by soil type (21%), while mean annual minimum temperature were contribute the least 2%.

Table 5. Ensemble variable Importance

Variables	EMmean	EMcv	EMca
Elevation	0.08	0.03	0.04
Landcover	0.06	0.02	0.02
Soil type	0.21	0.07	0.09
Livestock	0.13	0.04	0.06
NDVI	0.06	0.02	0.03
Prec	0.08	0.03	0.04
Tmax	0.04	0.01	0.02
Tmin	0.02	0.01	0.01
Srad	0.09	0.03	0.04
Vapr	0.23	0.08	0.10
Wind	0.13	0.04	0.06

4.3.2. Ensemble models

Ethiopia has many suitable areas that support growth and distribution of RVF vector mosquitoes. Most border areas of the country starting from Somali region, southern border of Oromia and SNNP as well as western border of Gambella and Benishangul Gumuz suitable area captured by ensemble model as in figure 6 below. Many of these are found in lowland of humid to dry sub-humid ecological zones. Model showed most central part of the country is not suitable for RVF vector mosquitoes.

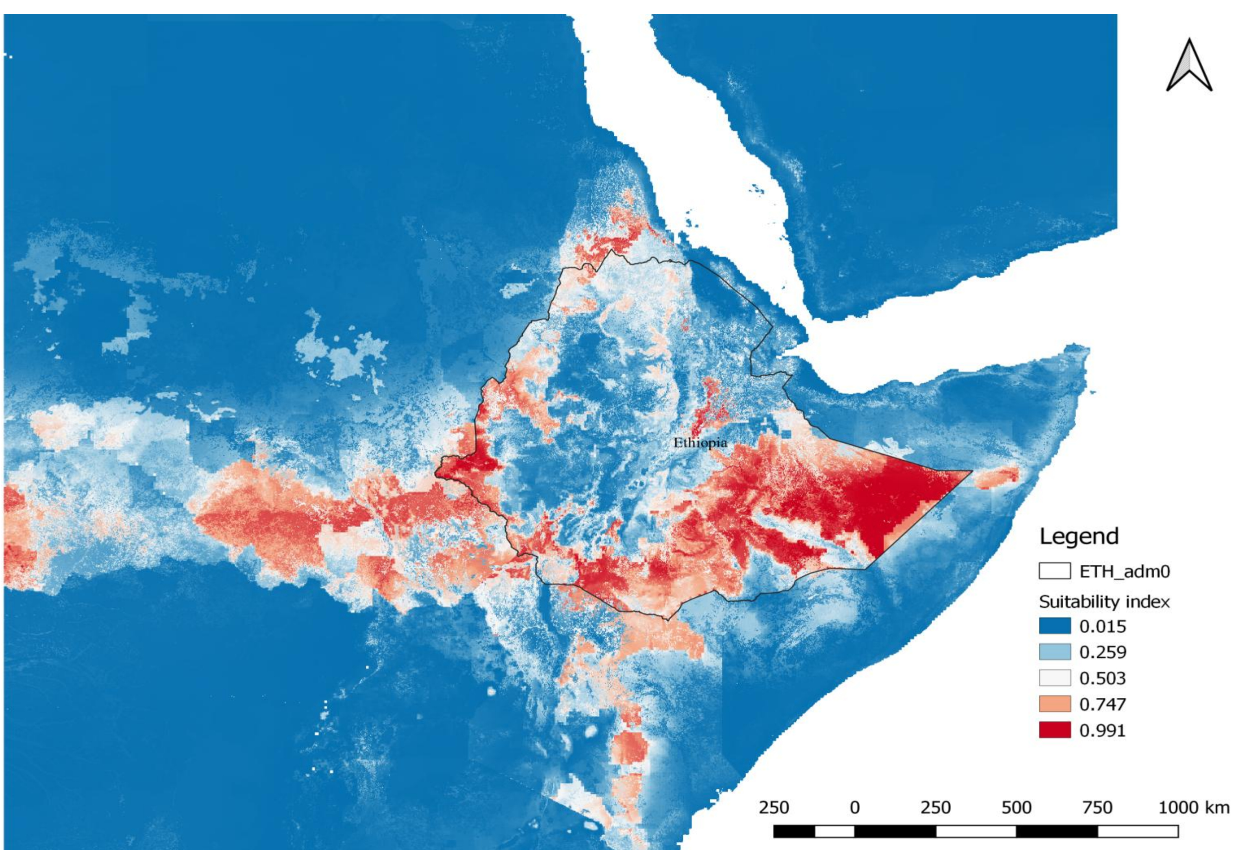


Figure 6: Predicted potential distribution of RVF vector mosquitoes. The scale indicates less suitable environment (blue color) and most suitable environment (red color).

A suitability map of committee average (Figure 7 below) also looks similar with maps of ensemble mean above. Models committee averaging gives both a prediction and a measure of uncertainty.

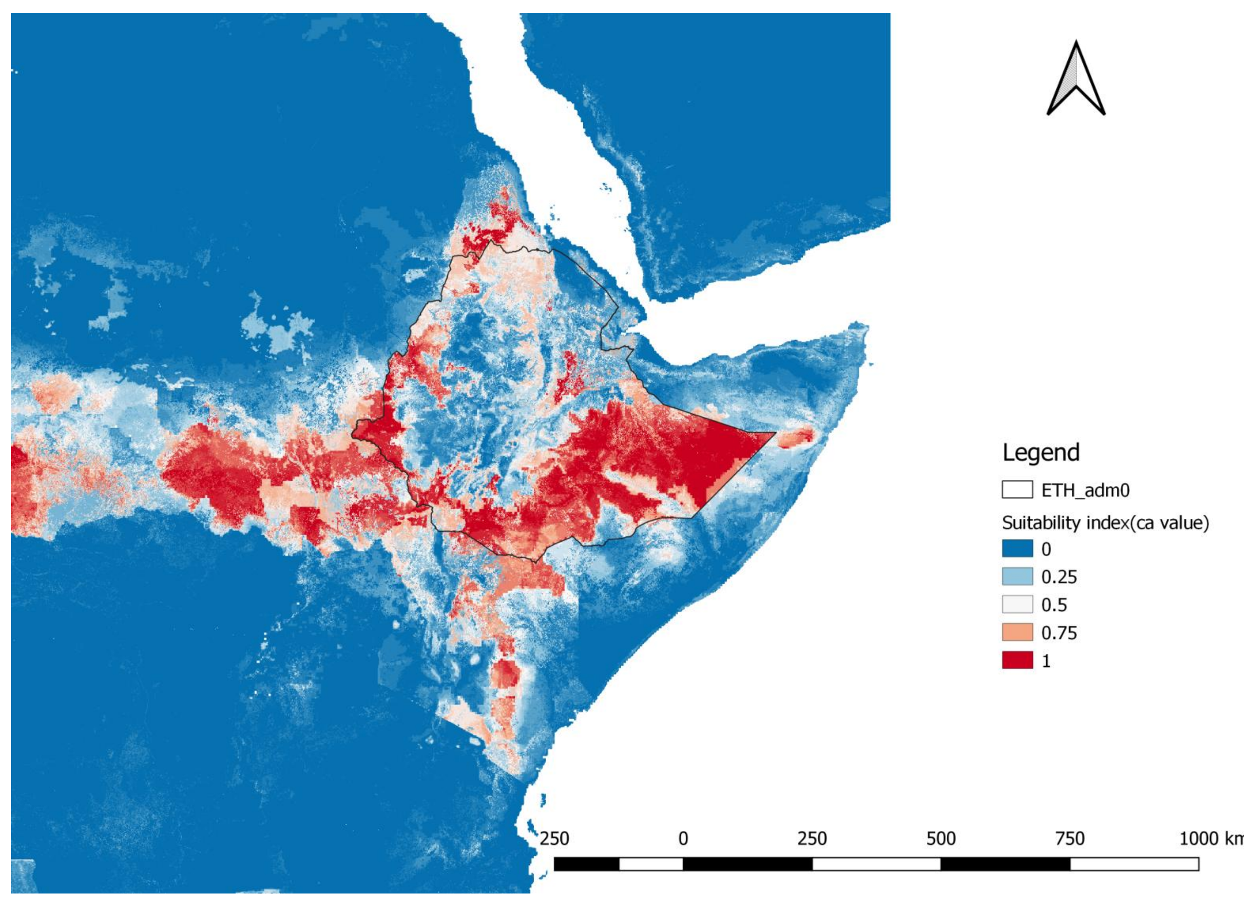


Figure 7: The estimated committee averaging across the selected predictions. The scale indicates unsuitable environment with certain prediction (blue colors), less suitable with uncertain prediction (light colors), and most suitable environment with certain prediction (red colors).

The model showed variation in the uncertainty index among different parts as the '*clamping mask*' value depicted in Figure 8 shows. The '*build.clamping.mask*' identifies locations where predictions are uncertain because the values of the variables are outside the range used for calibrating/training the models. The values *'clamping mask*' value corresponds to the number of variables that are out of their calibrating range. The values greater than to 0.5 corresponds to uncertainty in models predictions.

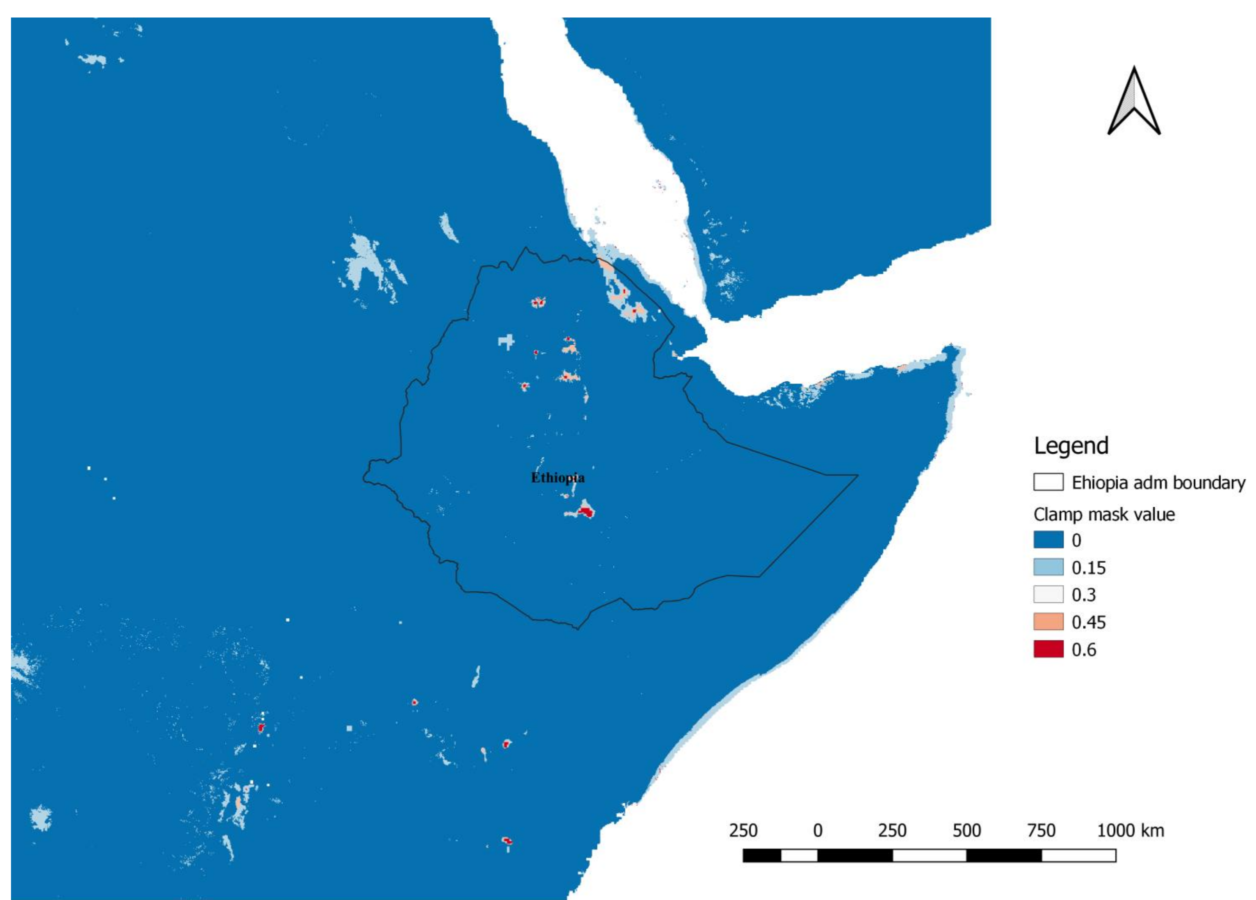


Figure 8: The Estimated ‘clamping mask’ value. Warmer (red) indicate areas where models predictions are uncertain.

5. DISCUSSIONS

5.1.Mosquito Survey

The current entomological surveillance was conducted to define the occurrence and distribution of competent RVFV vectors in Ethiopia as an important step in assessing and mapping high risk area that could be prone to RVF outbreaks. The mosquito survey in the study sites revealed differences in species composition and abundance that *Culex* and *Aedes* were the most prominent mosquito genus in the study area.

Aedes mosquitoes were incriminated as principal vectors for RVFV and believed to play a significant role in maintaining the endemicity of the disease in the environment through transovarial transmission (Alhaj *et al.*, 2017). In spite of their low abundance in current collections, the two known RVF vectors in Kenya such as *Ae. mcintoshi* and *Ae. ochraceus* were identified. In Kenya, many reports of entomological survey indicated these two species are the predominant mosquito collections and frequently incriminated in initiation and spread of RVF during epidemics (Arum *et al.*, 2015; Ochieng *et al.*, 2016; Sang *et al.*, 2017). Mosquitoes of *Culex* genus have also been considered as potential vectors as a result of their bio-ecology in terms of abundance, biting activity, feeding habits and longevity (Brustolin *et al.*, 2017). *Cx. antennatus, Cx. theileri, Cx. quinquefasciatus, Cx. univittatus* and *M. uniformis* are competent vector of RVF in Madagaskar and the virus had been detected from these mosquito species (Luciano *et al.,* 2015). Currently, five *Culex spp.* include *Cx. antennatus*, *Cx. univitattus*, *Cx. quinquefasciatus*, *Cx. pipiens* and *Cx. theileri* were identified and in case the disease outbreak initiated in the localities the diseases can be spread the disease widely these mosquitoes. Two *Anopheles* species, *Anopheles gambiae Complex* and *Anopheles arabiansis* were also identified in the current mosquito survey and in many RVF endemic countries these are also involved in the disease spread.

Mosquitoes usually search water bodies to breed (Che *et al.,* 2013). Most of the current mosquitoes were collected from around water bodies (lakes, ponds and lakes) which thought as mosquitoes' breeding sites. The type of biotope (temporary ponds, river or lake) around the trap points have

significant effects on the abundance of RVFV mosquito vectors (Biteye *et al.,* 2018). The abundance and diversity of these potential vectors were significantly higher in Borena, suggesting that the risk of RVF occurrence is higher in this area than other study sites. The high abundance and diversity of mosquitoes in Borena suggests the potential suitability of collection environments in this zone during the study period for the mosquito species collected.

In the current study, diversity of RVF vector mosquitoes and their potential to be infective to RVFV provides important entomological features for the identification of potential high risk areas for RVF occurrence, which can provide guidance in the design of appropriate prevention and control measures. Given the presence of potential vectors and favorable environmental conditions in some areas, the possibility of an RVF outbreak event in Ethiopia cannot be excluded. This pattern is likely to create variable risk areas of the disease with regards to infection of susceptible livestock.

5.2.Viral Detection

Rift Valley fever virus has been isolated from more than 40 species of mosquitoes belong to eight genera (Che *et al.,* 2013). However, current result of molecular detection for RVFV showed the viral genome wasn't detected from all mosquito pools. Absence of the viral genome in mosquitoes may not suggest that the sites are not risk for the disease. The presence of competent mosquito vectors indicates that an autochthonous outbreak of RVF may occur if the virus is introduced (Brustolin *et al.*, 2017).

Rift Valley fever virus is transmitted between animals and humans by mosquitoes, particularly those belonging to the *Aedes*, *Culex*, *Anopheles* and *Mansonia* genera (Che *et al.,* 2013). In contrary to the current absence of viral genome from mosquitoes, RVFV was detected from similar mosquito species in Madagascar (Ratovonjato *et al.*, 2011), Sudan, and Egypt (Seufi and Galal, 2010) experimental infection in Europe (Brustolin *et al.*, 2017) include *An. Gambiae, An. arabiensis,* and *Cx. pipiens*. Similarly, using qRT-PCR, RVFV have detected from three mosquito species *viz., Aedes mcintoshi, Aedes ochraceus* and *Mansonia uniformis* during the 2006 to 2007 East African outbreaks (Mwaengo *et al.*, 2012b). However, similar to the current results, investigation for RVFV in mosquitoes by RT-PCR were reported as negative from Jazan region, Saudi Arabia (Alhaj *et al.*,

2017), Okavango Delta, Botswana (Pachka *et al.*, 2016) and Ngorongoro district in northern Tanzania (Mhina *et al.*, 2015). This might be due to similar reason of the earlier conclusion that probability of detecting RVFV genome in mosquitoes in the inter-epidemic period is extremely low (Pachka *et al.*, 2016).

In general, the current absence of the virus from the mosquito may not perfectly confirm the absence of virus from the area. There may be the likely circulation of virus below the threshold of detection in the area that may increases in case of favorable conditions created (Pepin *et al.*, 2010). In spite of presence of vectors in the current study areas, clinical outbreaks have not ever reported. Similarly, there is no report of epizootic/epidemic from the neighbor endemic countries during the study periods. The event of high rainfall that resulted extensive flooding were also not happened and observed in region during the time. Therefore, this study supports the previous conclusion that typical RVF epidemics in East Africa have been occurred following ‘El-Nino’ associated heavy and sustained rainfall in the region (Anyamba *et al.,* 2001; Mansfield *et al.*, 2015).

5.3. RVF Vector Mosquitoes Distribution Model

Greater than factors associated RVFV transmission with vertebrates (species, movement, density, susceptibility, and vaccination), mosquito vectors are major components of RVF risk which referred to as entomological risk (Luciano *et al.,* 2015). This means circulation of RVF virus associated with mosquito vectors which in turn need supporting ecological environment (Bicout and Sabatier, 2004). Hence, model of mosquito vector distribution suggesting, not surprisingly that vector suitable habitat is linked to habitat suitability for RVF (Bicout and Sabatier, 2004). Having knowledge of distribution patterns, temporal abundance, and habitat preferences of the disease vectors will allow to accurately predict the location and timing of potential outbreak events of the diseases (Palaniyandi, 2017). The current RVFV vector mosquitoes distribution model provide valuable information on the spatial suitability habitat for RVF mosquito vectors occurrences, thus greatly assist informed risk-based surveillance, prevention and control activities.

The model performed well (TSS = 0.974 and ROC = 0.999), indicating a clear ability to distinguish between suitable and unsuitable habitat. TSS and ROC were selected as robust measures of model

performance (Allouche *et al.,* 2006). The current model maps showed that Ethiopia has wide suitable area found around the border shared with RVF endemic countries. The model depicted all regions have small to wide range of suitable area where Somali region has wide and Harari region has few patchy suitable area. Many parts of the border areas found include most part of Somali region, Hararghe, most part of Bale and Guji zone, South Omo, Benchi Maji, most part of Gambela and Benishangul Gumuz region and western border of Qellam Wollega were found suitable area for RVFV vector mosquito's distribution. Many of these are found in lowland of humid to dry sub-humid ecological zones. Amhara and Tigray region also have some moderately suitable areas, particularly on the northern border of the regions. Similarly, in Afar region patchy of suitable area were revealed surrounding Awash Valley. Wide range of central Ethiopia is found not suitable for the RVF mosquito vectors. The model prediction also extended even to the area where occurrence data have not yet reported.

Many factors influence presence, distributions and abundance of RVFV vector mosquitoes. Although climatic variables influence mosquito abundance, but it seems to be very complex to assess the real impact, for instance, Khan *et al*. (2018) found that no strong significant relationship between relative humidity, temperature and rainfall with *Culex*, *Aedes* and *Anopheles* in Jeddah, Saudi Arabia. Similarly, the current distribution model indicates, precipitation and extreme temperature has minor importance for mosquito occurrences. This go in hand with the report of Mellor and Leake, (2000) that extreme temperatures has detrimental effect to vector populations. In current model solar radiation relatively suitable (9%) for RVF vector mosquitoes in contrary to Sang *et al*. (2018) report that solar radiation was not significant on mosquito vector survival.

RVF virus circulation has been reported in several eco-climatic areas (Tran *et al.*, 2016). Circulation of RVFV has been commonly reported in several sub-humid parts of East African countries. Ethiopia has wide sub-humid eco-zone and suitability map based on satellite data shows that suitable factors are exist for amplification, spread and occurrence of RVFV in Ethiopia (Tran *et al.*, 2016). High relative humidity favors most metabolic processes in vectors towards their prolonged survival, whereas low humidity tends to decrease their daily survival rate due to dehydration (Mellor and Leake, 2000). The current model also illustrated suitable areas that extended over many humid dry to sub humid area of the country those have value range from 1.52 ±0.02 kPa to 1.85±0.02 kPa

and its higher range which not in agreement with report of Khan *et al.*, (2018). Water vapor pressure which means relative humidity was found the leading factor contributing 23% in model development. This means that relative humidity determine distribution of the RVFV vector mosquitoes than the other factors.

The presence of agricultural irrigation in the local area strongly linked to an increased risk of RVF which understandable as irrigation is known to directly benefit mosquitoes by increasing the habitat availability for larvae (Redding *et al.*, 2017). However, land cover contributes minimum (6%) in current model development. This tells that area to have no extensive irrigation or dry environment unsuitable for agriculture.

It is obvious that impermeable soils with high proportions of clay and loamy texture do not easily allow water to filter through resulting in periodic water stagnation and flooding during periods of prolonged rainfall (Sindato *et al.*, 2016). The areas with *solonertz*, *luvisols* and *vertisols* soil types positively associated with RVF (Munyua *et al.*, 2016). Such flooding then leads to the hatching of RVFV infected *Aedes* mosquito eggs which are considered to be the reservoirs and primary transmitters of the RVFV. Colonization of the flooded areas by secondary vectors including *Culex*, *Anopheles* and *Mansonia* mosquitoes contribute to further virus transmission and spread between animals and humans (Sindato *et al.*, 2016). The current model prediction is concord with the Maxent models of Ochieng (Ochieng *et al.*, 2016) for three mosquito species *Culex univitattus*, *Mansonia africana*, and *Mansonia uniformis* that soil type highly determine the distribution of these mosquito species.

The risk of RVF was positively associated with low altitude areas. These are suitable site for breeding mosquito vectors which maintain the virus (Munyua *et al.*, 2016). Lowland zone is the most suitable habitat for RVFV vectors (Ochieng *et al.*, 2016) which is confirmed by current model that captured the suitable area for RVFV vector mosquitoes mostly in lowland area. However, changes in altitude are closely associated with varying ecological conditions (demonstrated by changes in host and vector diversity, climate and physical features such as moisture content) that might be responsible for the variation in the risk levels observed. Ecosystems in low altitudes experience irregular climate patterns that increase turnover rates of livestock and wildlife

populations, compromising the maintenance of appreciable levels of herd immunity (Munyua *et al.*, 2016).

Livestock densities play great role in transmission of RVFV assuming the existence of direct transmission, an increase in domestic ruminant density is expected to increase the number of potentially infectious contacts that a susceptible individual experiences over a given time; therefore, there is a greater risk of amplification. In addition, infectious ruminants may travel after being sold, (Tran *et al.*, 2016), spread of RVF from endemic areas can occur with the movement of livestock and the introduction of viraemic animals in conducive areas, then the virus is imported and subsequently spread between naive ruminants and local mosquitoes that are competent vectors for RVFV such as certain *Culex* and *Anopheles* species (WHO, 2009). Many of 2006 to 2007 RVF epizootics associated with livestock in pastoral and agro-pastoral farming systems (Baba *et al.*, 2016). Currently livestock density contributes 13% in model buildings which mean that livestock population relatively moderate impacts on RVF mosquitoes vector distribution. The geographical placement of the country associated with large commercial ruminant trade and pastoralist movements (Diop, 2015; Lind *et al.*, 2016) makes Ethiopia at risk of RVF occurrence.

The risk of El Niño-driven RVF outbreaks is high in East Africa, for instance, extensive flooding due to heavy rains in 1997–1998 in East Africa resulted expanded epidemics of RVF disease activity in Sudan, Somalia, Tanzania, and Kenya (Bicout and Sabatier, 2004). Similarly, retrospective analysis of remote sensing data confirmed that suitable conditions for the explosive multiplication of mosquito vectors had existed over extensive areas of eastern Africa including South eastern and southern Ethiopia in association with the 1997/1998 epidemic (Roeder *et al.*, 1999). On 20 Dec, 2015 by using NDVI data United State government agencies on ‘Emerging Health Risk Notification’ reported that Ethiopia had potential RVF epizootic risk areas based prediction of heavy rainfall associated with El Niño (USG agencies Working Group, 2015). However, the current model contribution of the annual NDVI (6%) which derived from ‘vegetation greenness’ from March, 2018 to February, 2019 indicate that there were no anomaly in vegetation index that led to heavy and extended rainfall in the country. Nevertheless, the current suitability map overlap with Anyamba (Anyamba *et al.,* 2001) map of arid areas of East Africa where outbreaks have occurred during the satellite recording period (1981-1998). The map shows that an anomalous positive departure in

vegetation greenness (NDVI anomaly) is an indicator of above-normal precipitation leading to flooding. The later prediction of a RVF outbreak by Anyambaa *et al.* (2009) had showed NDVI anomalies in December 2006 in southern Ethiopia which coincide with the current modeling. This anomaly associated with above-normal rainfall creates ideal eco-climatic conditions for the emergence and survival of large populations of RVF vector mosquitoes from *dambo* habitats.

It is also clear that no significant physical barriers has prevented transmission and spread of RVFV as ongoing increases in the global movement of humans, livestock and mosquitoes (Pepin *et al.*, 2010). RVF risk map serves as an important tool for developing and deploying prevention and control measures against the disease (Munyua *et al.*, 2016).

This study was conducted with great endeavor targeted to RVF mosquitoes' vector survey, RVFV detection and risk mapping of RVF in Ethiopia and provided good information which is the first of its kind. Nevertheless, there are some limitations. In the current mosquito survey, the traps were inefficient to representatively sample different species. Hence, this observation inadvertently fails to clearly show that how much about abundance patterns of these mosquitoes *spp*, which have been incriminated as primary or secondary vectors of the disease. Similarly, this study have likely affected by sampling technique, choice of sampling sites, duration of our study, season of sampling and sample size. Similarly, due to the small sample size of presence data points used to construct the spatial distribution model and in this study, it would be not realistically describe that fully the distribution of RVF mosquito vector occurrence in Ethiopia.

6. CONCLUSION AND RECOMMENDATIONS

The current mosquito survey study, diversified adult mosquitoes were collected from Ziway, Hawassa, Arba Minch, Borena and Segen Valley. RVFV primary vector genus *Aedes* and secondary vectors genus *Culex*, *Mansonia*, and *Anopheles* were collected and identified from study areas. Many mosquitoes were collected near lake, ponds and river indicated that mosquito survival may not far from water bodies. Diversity of these competent RVFV vector mosquitoes put area at risk of diseases outbreak if the virus is introduced. The current RVF mosquito vectors distribution model well indicated that Ethiopia is at the risk of RVF outbreaks from the border side of RVF endemic countries. Parts of the countries which are dry humid and semi-arid mostly south and south eastern ecological zones of the country such as Somali region, Borena, Hararghe, and parts of Bale are suitable for the disease vectors. In spite of the fact that clinical cases of RVF has not yet observed and reported in Ethiopia, by considering presence of vector mosquitoes and geographical proximity of the country to RVF endemic countries like Kenya, Sudan and Somalia; the nature of livestock movements across the international border and the ease with which infected mosquitoes can be moved longer distances by the help of wind lead to the conclusion that Ethiopia will always at risk of RVF outbreak during the periods epizootic of the diseases in the Horn of Africa region. These findings provide information that help health authorities to set up efficient entomological and RVF diseases surveillance and vector control programmes.

Based on the conclusion of this work finding the following points are recommended:

1. Further studies targeting the periods of high mosquito activity with range of sampling techniques, sampling season and sampling sites would improve understanding on the abundance and diversity of the RVF potential mosquito vectors in the study area.

2. The likely circulation of the RVFV under threshold of detection necessitates searching of the diseases from vertebrates hosts. Hence, study targeting livestock and wild life should be conducted for further delineation of the diseases status particularly along the border shared with endemic countries.

3. Geographical proximity of Ethiopia to RVF endemic countries and the model capture closest to endemic area indicates that ongoing surveillance need to be conducted in sentinel animals to monitor the infection status in susceptible animals.

4. Climatic conditions risky area using remotely sensed data targeting anomaly of heavy rainfall which could result extensive flooding should be monitored regularly to provide earning warning information.

5. The complex state of RVF disease epidemiology resulted from its infectious and zoonosis status, multi-factors driven and wide host ranges of the diseases needs shifting of study of the disease through one health approach.

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8. ANNEXES

Annex I. Viral RNA extraction protocol (RNeasy®Mini Kit)

Quick-StartProtocol

RNeasy® Mini Kit, Part 1

The RNeasy Mini Kit (cat. nos. 74104 and 74106) can be stored at room temperature (15–25°C) for at least 9 months.

For more information, additional and more detailed protocols, and safety information, please refer to the *RNeasy Mini Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- If purifying RNA from cell lines rich in RNases, or tissue, add either 10 µl β-mercaptoethanol (β-ME), or 20 µl 2 M dithiothreitol (DTT)*, to 1 ml Buffer RLT. Buffer RLT with β-ME or DTT can be stored at room temperature for up to 1 month.
- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
- Remove RNA*later*®-stabilized tissue from the reagent using forceps.
- For RNeasy Protect Mini Kit (cat. nos. 74124 and 74126), please start with the *Quick-StartProtocol RNAlater RNA Stabilization Reagent, RNAlater TissueProtect Tubes, and RNeasy Protect Kits*.

* This option not included for cells in handbook; handbook to be updated.

350µl

1. **Cells**: Harvest a maximum of 1×10^7 cells, as a cell pellet or by direct lysis in the vessel. Add the appropriate volume of Buffer RLT (see Table 1).
   Tissues: Do not use more than 30 mg tissue. Disrupt the tissue and homogenize the lysate in the appropriate volume of Buffer RLT (see Table 1). Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting, and use it in step 2.
2. Add 1 volume of 70% ethanol to the lysate, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.
3. Transfer up to 700 µl of the sample, including any precipitate, to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.

January 2011

QIAGEN®

Sample & Assay Technologies

Quick-StartProtocol

RNeasy® mini kit

Optional: For DNase digestion, follow steps 1–4 of "On-column DNase digestion" in *Quick-StartProtocol RNeasy Mini Kit, Part 2.*

4. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.

5. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.

6. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g.

   Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to dry the membrane.

7. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.

8. If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µl of RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.

Table 1. Volumes of Buffer RLT for sample disruption and homogenization

Sample	Amount	Dish	Buffer RLT	Disruption and homogenization
Animal cells	<5 x 10^6	<6 cm	350 µl	Add Buffer RLT, vortex (≤1 x 10^5 cells); or use QIAshredder, TissueRuptor®, or needle and syringe
	≤1 x 10^7	6–10 cm	600 µl	
Animal tissues	<20 mg	–	350 µl*	TissueLyser LT; TissueLyser II; TissueRuptor, or mortar and pestle followed by QIAshredder or needle and syringe
	≤30 mg	–	600 µl	

* Use 600 µl Buffer RLT for tissues stabilized in RNA*later*, or for difficult-to-lyse tissues.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

"RNA*later*®" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

Trademarks: QIAGEN®, RNeasy®, TissueRuptor® (QIAGEN Group);

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Sample & Assay Technologies

Annex II. Master mix preparation, PCR thermocycle and Agarose gel preparation

TIONAL VETERINARY INSTITUTE	Document No. **NVI -QMS - QF - 41**		
Title: - Master mix preparation and PCR work sheet	Effective Date 20/05/2019	Issue No. 1	Page No. 1of 1

Date: - 23/05/2019

Reference No MB 105/19

Conventional RT-PCR for RVFV Isolation and Identity test procedure

One step RT-PCR Master Mix preparation

N	Type of reagent	For one reaction	Total	Remark
1	RNase free water	8µl		
2	Primer-RVF S432 forw 5pm/ µl	2.5 µl		
3	Primer-RVF NS3M Reve5pm/µl	2.5 µl		
4	5X RT-PCR buffer	5 µl		
5	5X Q solution	5 µl		
6	10 mM dNTP mix	1 µl		
7	RT-PCR enzyme mix	1 µl		
8	Add Template (DNA)	5 µl		
	Total volume	30 µl		

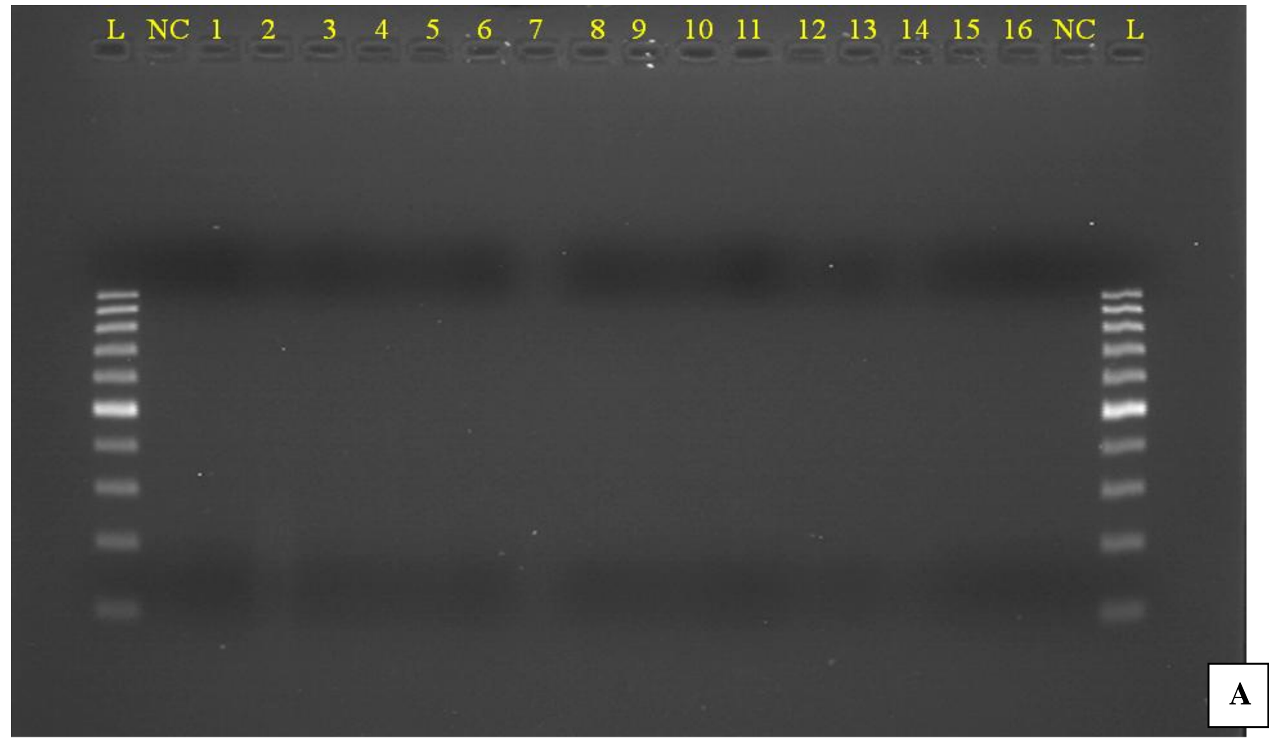
Run RT- PCR

	Temperature	Time	Cycle	Remark
cDNA synthesis	50 °C	30 mints	1 cycle	
Initial Denaturation	94 °C	15 mints	1-Cycle	
Denaturation	94°C	30 sec	30 Cycles	
Annealing	40°C	30 sec		
Extension	72°C	30 sec		
Final Elongation	72°C	5 mints	1-Cycle	
Put at	4°C	Until machine off		

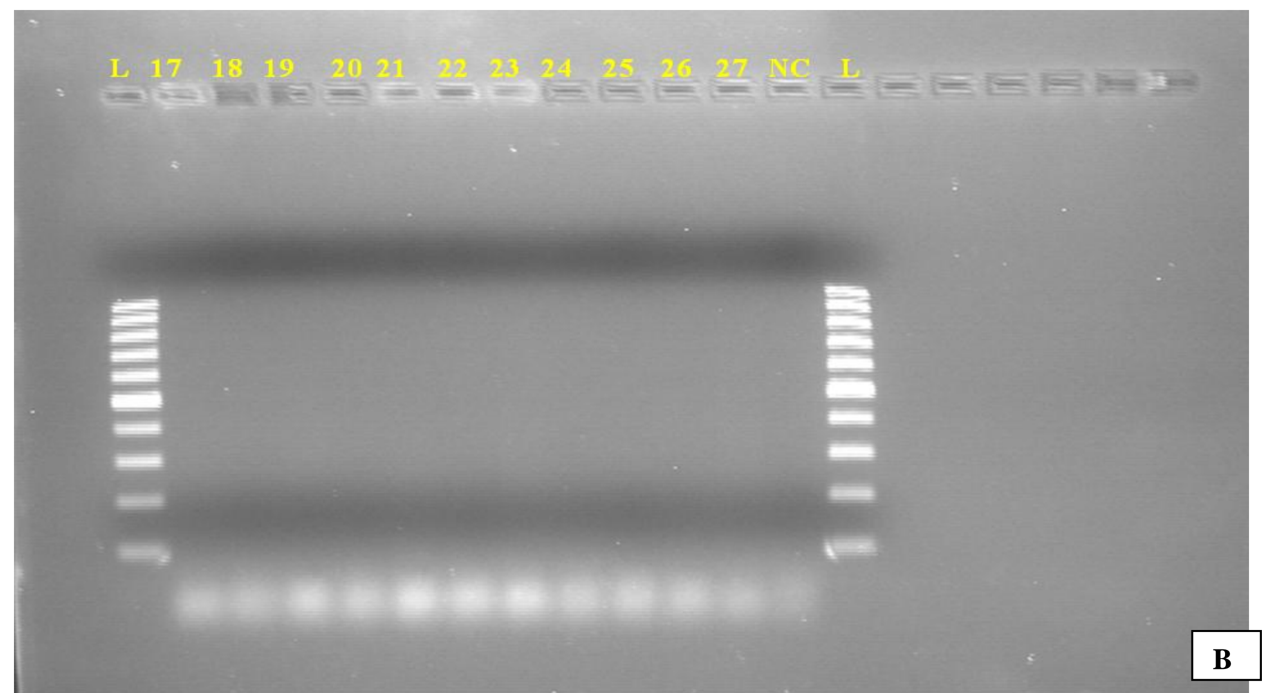
Agrose gel preparation

- Prepare 1.5% Ag arose gel
- Add 4 µ gel red with loading dye
- Load PCR product 10µl in each well and 10µl molecular marker (Ladder) started 100bp plus.
- Run electrophoresis for 1 hour at 120V
- Read the result by using UV light
- It is around 298 bp for positive RVFV result.

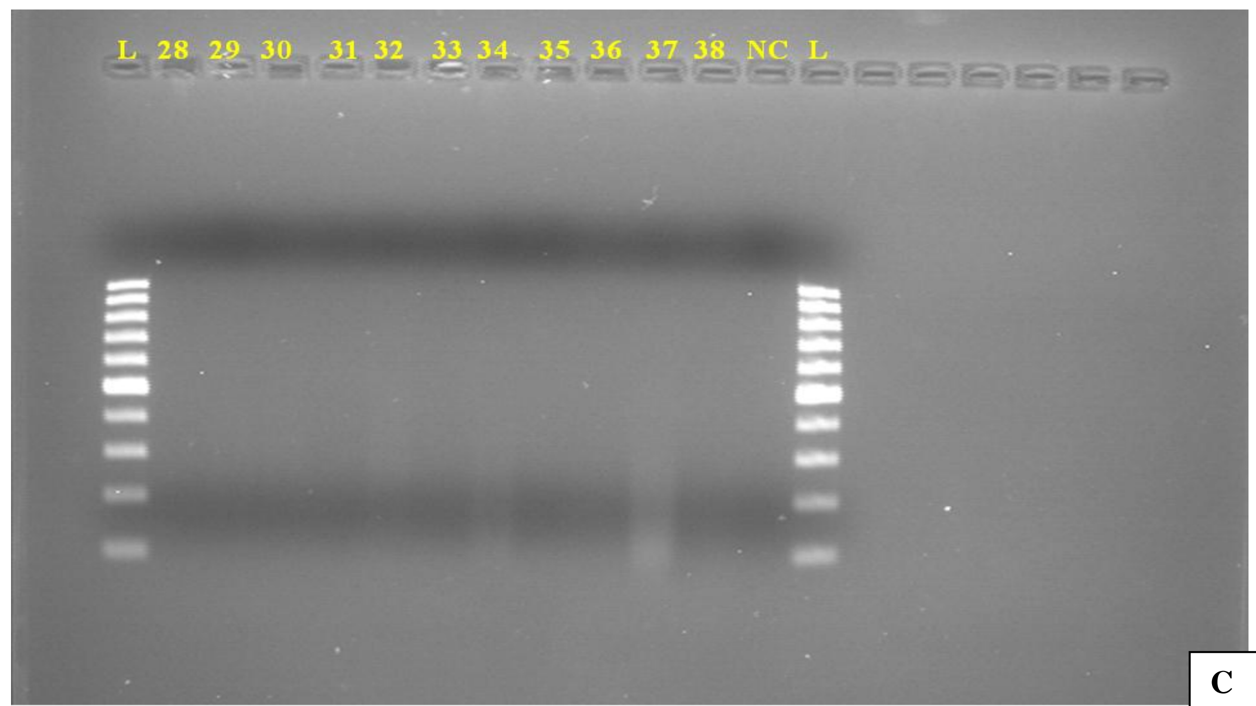
Annex III. The first PCR result without positive control



L = Molecular ladder, NC = Negative control, "1, 2, 3, 4,……" = Numbers representing samples

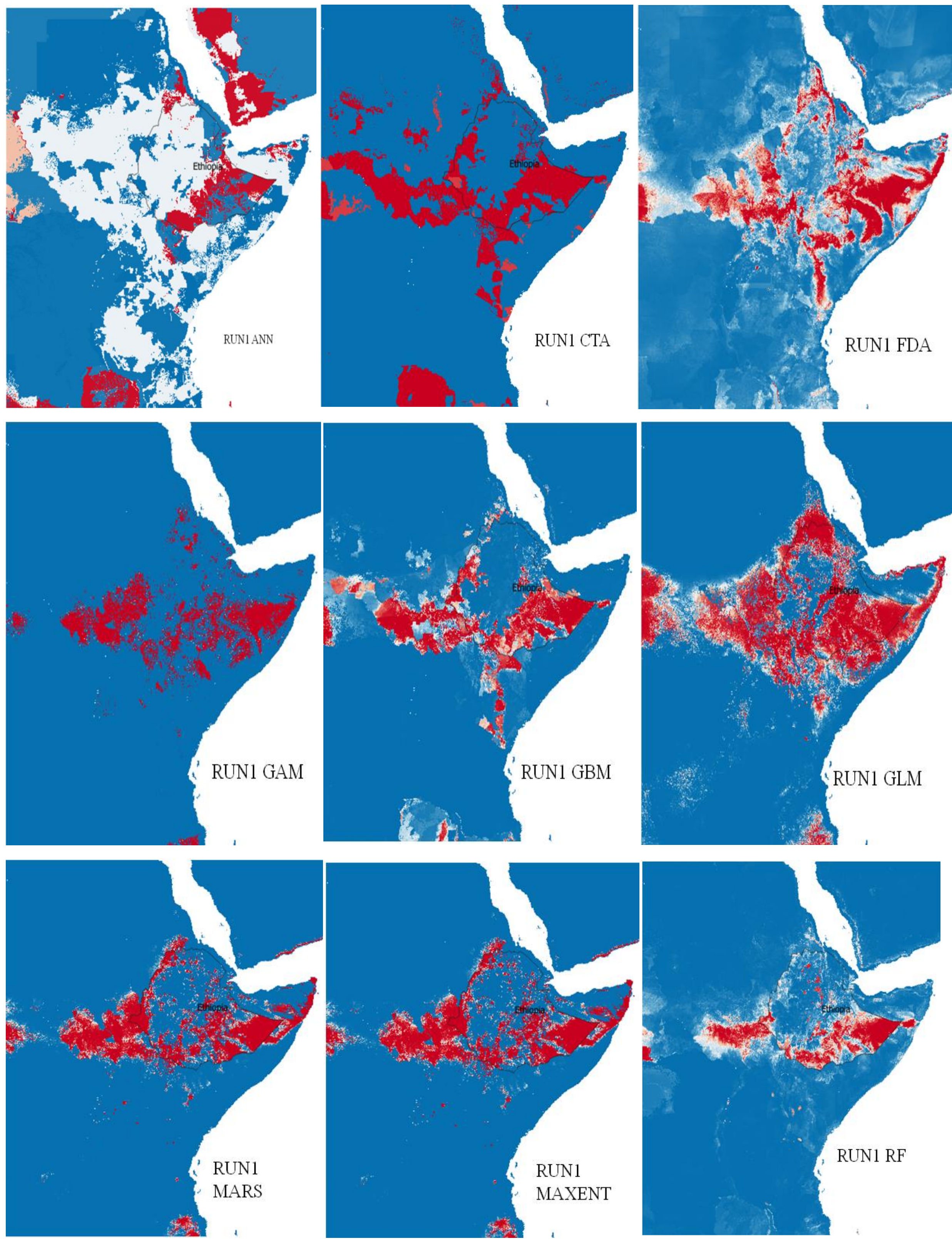


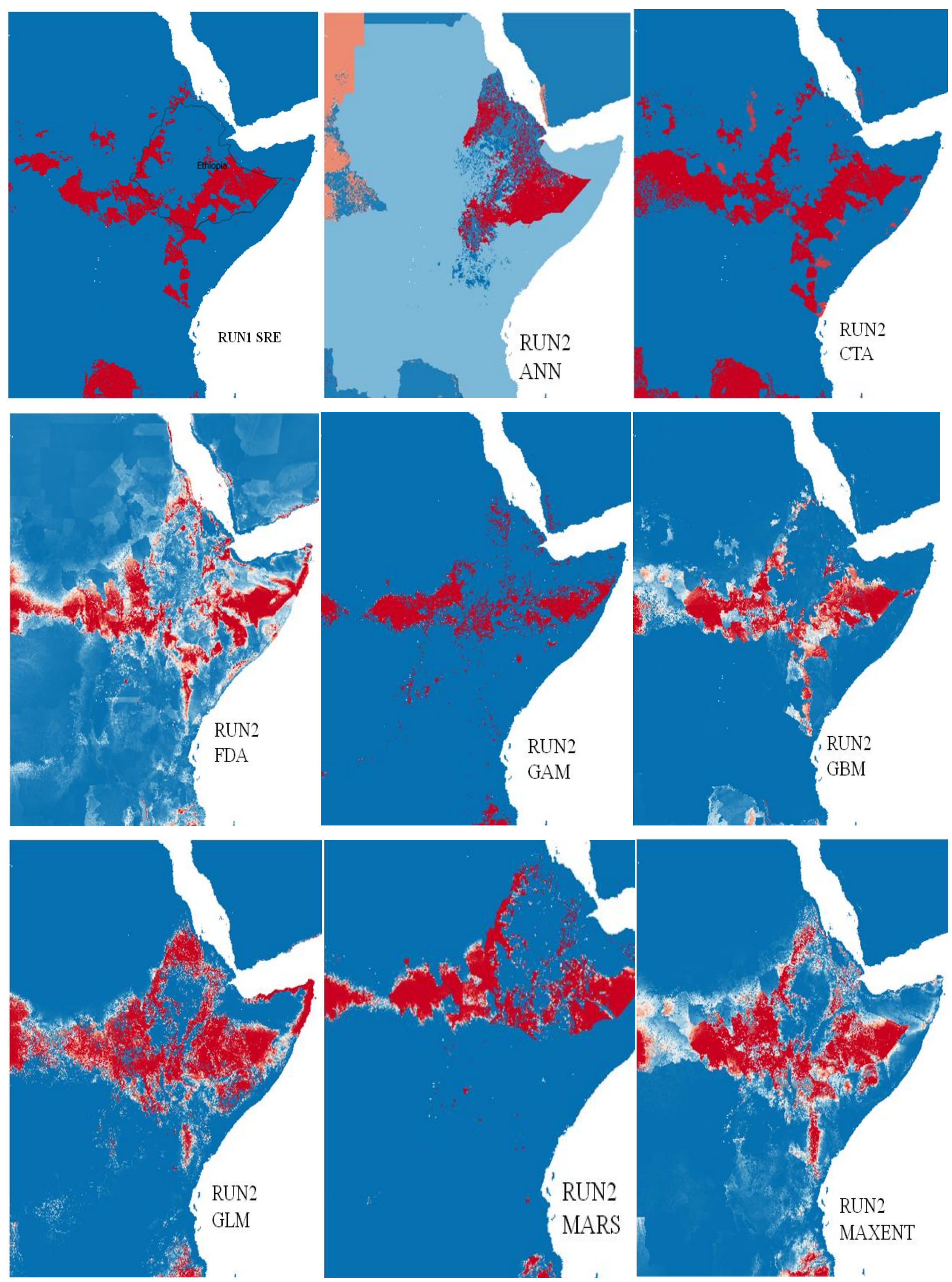
L = Molecular ladder, NC = Negative control, "17, 18, 19, 20,……" = Numbers representing samples

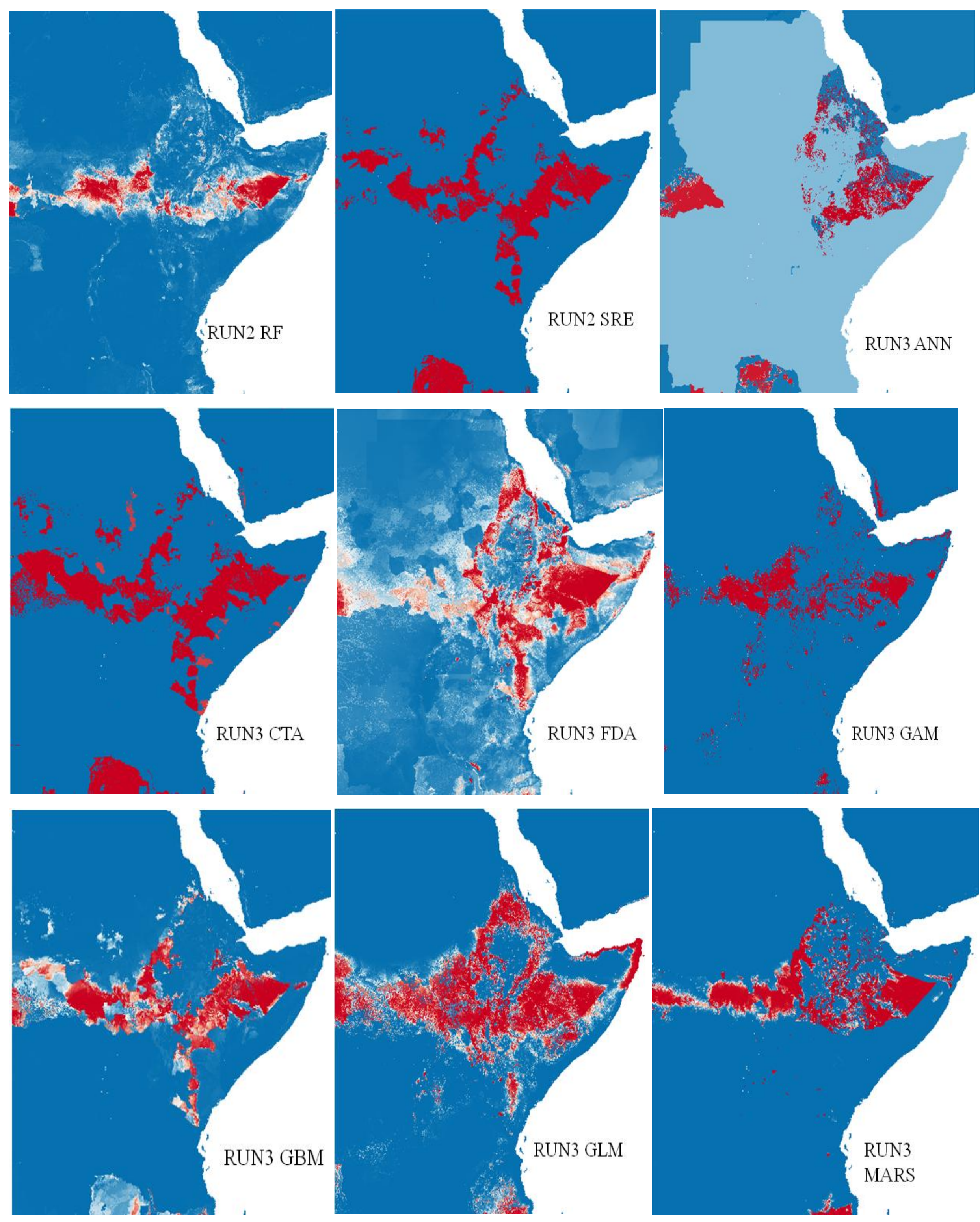


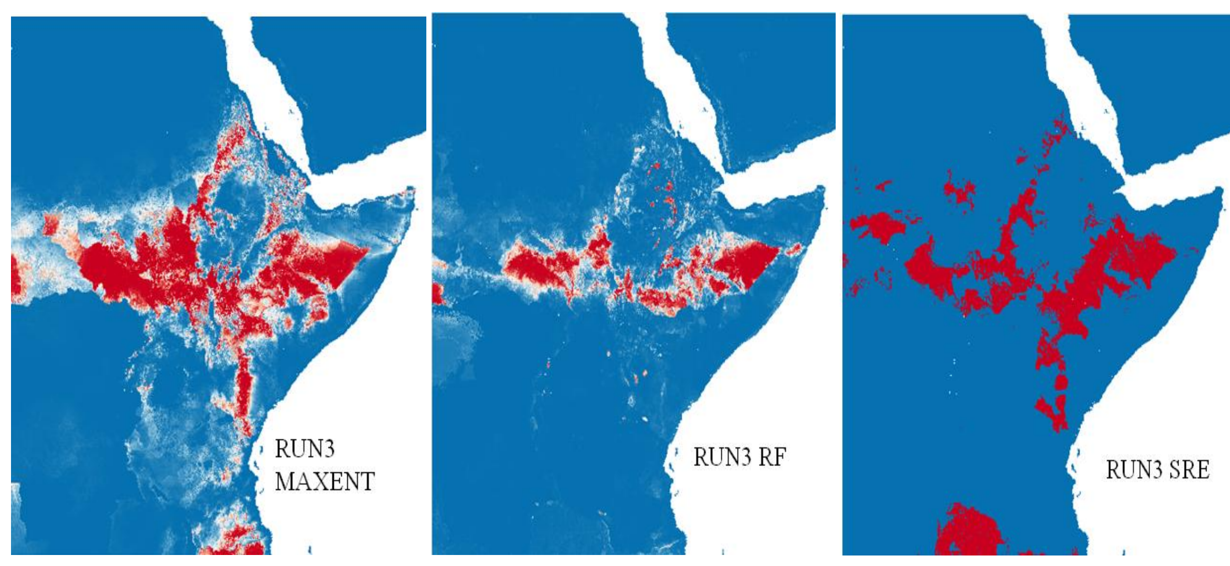
L = Molecular ladder, NC = Negative control, "28, 29, 30, 31,……" = Numbers representing samples

Annex IV. Initial individual model output




Ethiopia
RUN1 SRE
RUN2
ANN
RUN2
CTA
RUN2
FDA
RUN2
GAM
RUN2
GBM
RUN2
GLM
RUN2
MARS
RUN2
MAXENT


RUN2 RF
RUN2 SRE
RUN3 ANN
RUN3 CTA
RUN3 FDA
RUN3 GAM
RUN3 GBM
RUN3 GLM
RUN3
MARS


RUN3
MAXENT
RUN3 RF
RUN3 SRE

Annex V. Ethical Clearance Certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/06/10/2018

Name of Applicant: Samson Leta (DVM, MVSc)

Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *integrating geo-statistical, biological and socio-cultural approaches in the investigation of vector-born diseases of veterinary and public health importance: towards development of innovative disease management system*

Date of application:	**18/05/2018**
Nature of the project:	**non-invasive**
Target animal species:	**invertebrate vectors of diseases**
Number of animals involved:	**essentially none**
Study area:	**Different sites, Ethiopia**

Minutes No. and date of review: **VM/ERC/06/10/018, 31/07/2018**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary

The Federal Democratic Republic of Ethiopia
Addis Ababa University College of Veterinary Medicine and Agriculture

Dr Getachew Terefe
Chairman

Signature

መልሱን በሚጽፉልን ጊዜ ... ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ Fax 251-11-4339933
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ፖ.ሣ.ቁ P.o.x. Box 34
ቢሾፍቱ/ደብረዘይት፤ ኢትዮጵያ Bishoftu/Debre Zeit, Ethiopia

Annex VI. Ministry of Agriculture recommendation on Rift Valley Fever (RVF) research

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የግብርና ሚኒስቴር

Federal Democratic Republic of Ethiopia
MINISTRY OF AGRICULTURE

ቁጥር No. 13/54/10/197
ቀን Date 25/03/2019

To: College of Veterinary Medicine and Agriculture of Addis Ababa University
Bishoftu:

Subject: Recommendation on Rift valley Fever (RVF) research

It is to be recalled that you have requested our Ministry through a letter with ref number D/461/2018 dated 17/12/2018 for permission to undertake research on RVF virus isolation from mosquitoes. Following receipt of the request, the issues has been under discussion with experts of our ministry and document was developed that helps for decision. As it's well known that there are frequent outbreaks of RVF in the neighboring countries. However, clinical cases has never been reported from Ethiopia. This indicates that there is high risk introduction of the virus to our country. Despite undertaking regular risk based surveillance, extensive research has to be conducted to reduce risk of introduction.

Therefore, this is to let you know that we have authorized your college to conduct the research ensuring the following three recommendations are fulfilled:

1. The research team should at least embrace one representative from the federal government
2. The finding of the research should be confidential and responsible authority must be consulted before anything is disclosed of published.
3. Since similar studies have been conducted regarding RVF, it should be more comprehensive including longitudinal study using sentinel herd and identification of risk factors.

Kind regards

G/Egziabher G/Yohannes(PHD)
State Minister

CC:

- **Epidemiology Directorate**
- **Disease Prevention and Control Directorate**
  MoA

Annex VII. Entomological survey form

rap No	type trap	Region	zone	District	Village/Kebelle	Habitat	Date of collection	Latitude	Longitude	Altitude	Mosquito species	Nu of mos