RIFT VALLEY FEVER VIRUS MOSQUITO VECTORS SURVEILLANCE, VECTORS DISPERSION MODELING, AND ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PERCEPTION OF THE COMMUNITY ON THE DISEASE IN BORANA, ETHIOPIA

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By

Golo Dabasa Jaldessa

Department of Clinical studies

Advisor: Dr. Haileleul Negussie (DVM, MSc, PhD, Associate Professor)

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion
AP61	Aedes Pseudoscutellaris 61
BHK	Baby Hamster Kidney
CDC	Center for Diseases Control
COVID-19	Corona Virus Disease 2019
DIVA	Differentiate Between Infected and Vaccinated Animals
ELISA	Enzyme-Linked Immunosorbent Assay
ENSO	El Nino-Southern Oscillation
EQA	External Quality Assessment
FDA	Food and Drug Administration
HIA	Haemagglutination Inhibition Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NSP	Non Structural Protein
NVI	National Veterinary Institute
PBS	Phosphate-Buffered Saline
PDS	Participatory Diseases Surveillance
qRT-PCR	quantitative Real-Time Polymerase Chain Reaction
RPA	Recombinase Polymerase Amplification
RT-LAMP	Reverse-Transcription Loop-mediated isothermal amplification
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever Virus
SPSS	Statistical Package for Social Science
SSI	Semi-Structured Interviews
TEA	Tri Ethyl Amine
TSI-GSD-200	The Salk Institute–Government Service Division–200
WRBU	Walter Reed Bio Systemics Unit
YRVL	Yabello Regional Veterinary Laboratory

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ABSTRACT

Rift Valley fever (RVF) is a zoonotic, arthropod-borne viral disease important in domesticated ruminants. This disease is characterized by high mortality rates in young animals and abortions in pregnant ruminants. In Ethiopia, clinical RVF has never been detected and reported. However, it occurs almost in all neighboring country. Hence, this study was aimed for entomological surveillance of Rift valley fever virus mosquitoes vectors, assessment of community knowledge, attitude and perception toward the RVF and vector distribution model based risk mapping. A total of 200 mosquitoes were collected from the study areas of Borana Zone. Based on morphological identification, these mosquitoes were belonging to three genera; *Culex* (n = 48; 24%), *Aedes* (n = 147; 73.5%), and *Anopheles* (n = 5, 2.5%). A total of 244 participants were involved in the KAP assessment,among which majority of the participants 231 (94.67%) were replied that they had observed livestock diseases outbreak in their herds with different symptoms, 151(61.89%) responded that they had seen the sign of mass abortion in their herds, 130 (53.28%) replied that there was the history of heavy rainfall that precedes the event of mass abortion, and 168 (68.85%) mentioned that there had been mass young mortalities, and again 203 (83.20%) responded that they had seen huge mosquito population, 139 (56.97%) described that there are transboundary movements of animals. Ensemble model were developed using 11 explanatory variables. The variables contribute different amount where water Vapor pressure (54.4%) and soil type (29.9%) contribute the most while global land coverage and elevation were contributed the least which is about 1%. The model showed border regions of Ethiopia from southeastern to northwestern of the country were suitable for distribution of RVFV vector mosquitoes where Somali region has wide while Afar region has few patchy suitable areas.many parts of central and Northern Ethiopia is not suitable for RVFV vector mosquitoes. The questionary survey results suggest that the disease might once have occurred in the area during the historical enzootic periods while occurrence of potential mosquito vectors and model capture of broad risky areas particularly along the borde shared with endemic countries necessitates further studies of the diseases.

Keywords: *Borana, KAP, Ensemble model,Mosquito,Rift Valley Fever*

1. INTRODUCTION

Rift Valley fever (RVF) is a zoonotic, arthropod-borne viral disease important in domesticated ruminants. This disease is characterized by high mortality rates in young animals and abortions in pregnant ruminants. Rift Valley fever can affect many species of animals including sheep, cattle, goats, buffalo, camels, monkeys as well as gray squirrels and other rodents (Oluwayelu *et al*., 2018).

It is caused by the RVF virus (RVFV), an RNA virus in the genus *Phlebovirus*, family *Bunyaviridae* (Murphy *et al.,* 2012), that is primarily transmitted by mosquitoes of the *Aedes* and *Culex* spp. The virus appears to survive in the dried eggs of *Aedes* mosquitoes and epidemics are associated with the hatching of the eggs during years of heavy rainfall and localized flooding (Gerdes, 2004; Logan *et al.*, 1991). The primary amplifying hosts are sheep and cattle. Once it has been amplified in animals, RVFV can also be transmitted by other vectors, including many mosquito species and possibly other biting insects such as ticks and midges. In addition, animal products such as fresh, chilled and frozen meat, milk and milk products, wool, bones, skin and hides have been speculated to be potential sources of the virus (WHO 1982). The virus can also be transmitted in utero to the fetus (Gerdes, 2004).

Mosquitoes transovarially infected with RVFV may emerge following unusually heavy, sustained rainfall and are associated with RVF outbreaks among susceptible breeds of sheep and cattle. Potential mosquito vectors of the RVFV belong to the genera *Aedes, Anopheles, Culex, Eretmapodites,* and *Mansonia.* 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. Stagnant and permanent water bodies are the habitats of *Eretmapodites* and *Mansonia*, respectively (Tantely *et al.*, 2016). In most African regions, including East Africa, RVF epidemics have been unpredictable but have typically occurred in an 8 to10 years cycle following heavy, sustained rainfall, associated with the ‘El-Ni ˜no’ phenomenon (Linthicum *et al.,* 1999). In the Horn of Africa, all of the past seven major RVF outbreaks have been preceded by heavy widespread rainfall (Anyamba *et al*., 2009). These increased rains in Eastern Africa were caused by a weather pattern known as El Niño. This is associated with increased sea surface temperatures (SST) in the Pacific and Indian Oceans (Linthicum *et al*., 2016). Rains must be both prolonged and higher than average to support sequential emergence of large populations of *Aedes* and *Culex* mosquitoes (FAO RVF Manual).

Human infections with the virus occur following handling of contaminated fetuses and slaughtering of infected animals as well as via virus transmission following mosquito bites (Van Yelden *et al*., 1977, *LaBeaud et al*., 2015, Anyangu *et al.*, 2010, Madani *et al*., 2003, and McIntosh *et al.*, 1980).

RVF was first reported during an epizootic of the disease in sheep in Kenya (Daubney *et al.,* 1931), and since then there have been several reports of outbreaks elsewhere in Africa (Meegan, 1979, Jouan *et al*., 1988, Zeller and Akakpo, 1995 and Woods *et al.*, 2002). The last two epidemics in Kenya occurred in 1998 and in late 2006/early 2007 were responsible for huge economic losses to livestock farmers. Several hundred human lives were lost in the 2006/2007 epidemic alone. More recently, outbreaks have occurred in South African regions during 2008-2011(Pienaar *et al* 2013). During the 2010 outbreaks, a total of 302 human infections were diagnosed with a case fatality rate of 8%. Most Recent outbreak of RVF during September and October 2019 in Sudan, 19 samples from sick patients that were tested, 9 were positive for RVF. Heavy rains on Aug 13 amplified mosquito populations, and the outbreak in humans began on Sep 19, the WHO said. As of Nov 11, officials have reported 293 suspected cases, 11 of them fatal (Rania and Osama, 2019). 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).

The recent research done in the selected areas of mid Rift Valley, Borana and Segen valley showed that there are diversified species of primary vectors (*Aedes* spps) and secondary vectors (*Culex*, *Anopheles*, and *Mansonia*) but no RVFV genome was detected from all mosquito pools (Megarsa, 2019 unpublished research).

In Ethiopia, clinical RVF has never been detected and reported. However, considering the 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 be vulnerable to clinical RVF during the epizootic periods of the diseases in the Horn of Africa region (Ministry of Agriculture, 2008). Livestock mobility is an essential part of the way of life in a pastoral environment. It is motivated by the need to access natural resources and livestock trade channels. Current mobility practices are dictated by the geo-climatic and sociocultural conditions on the African continent. Other factors also justify the mobility of farmers and their herds, namely the lack of water sources in the dry season or periods of drought, flooding, the need to move from areas affected by disease or inter-ethnic conflict, and banditry (Bouslikhane, 2015).

Pastoral mobility widely recognized around the border of the Borana 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 increased risk of disease transmission between wild animals and livestock (FAO, 2017). Kenya is RVF endemic country and Borana zone share a long boundary with the country and also pastoralists share pasture and water, this zone is at a higher risk. Therefore, the aim of this study was to surveillance of mosquitoes of Rift valley fever virus vectors, risk modeling of the vector distribution, and the assessment of community knowledge, attitude, and perception regarding the disease.

2. LITRETURE REVEIW

2.1. Etiology

RVF is caused by RVF virus which belongs to the family *Bunyaviridae* and the genus *Phlebovirus.* These are spherical virions with diameter of 80-120 nanometers and a host cell derived, bi lipid layer envelop through which virus coded glycoprotein spikes project (Kahn, 2005). This single stranded Ribose Nucleic Acid (RNA) virus has a lipid envelope and two surface glycoproteins, G1 and G2. The genome has three segments: L (Large), M (Medium) and S (Small). RVF virus replicates in the mosquitoes and in the vertebrate animals. The liver, spleen and brain are the major sites of viral replication. The Virus is resistant in alkaline environments but inactivated at pH <6.8.The virus can be inactivated by disinfectants such as calcium hypochlorite, sodium hypochlorite and acetic acid; and be maintained for 8 years when stored below 0°C (Davies and Martin, 2003).

2.2. Epidemiology

The epidemiology of RVFV is complex and involves multiple players, including mosquitoes, wild animals, domesticated livestock, and humans.

2.2.1. Mosquitoes

RVFV has been isolated from a wide range of mosquito species spanning several genera. *Aedes* mosquitoes are the primary reservoir and vector, while *Culex*, *Anopheles,* and *Mansonia* are important secondary vectors that contribute to amplification of epizootics and epidemics (Linthicum *et al.*, 2016 and Clements, 2012). Virus has also been isolated from other mosquito genera, as well as ticks, flies, and midges (Fontenille *et al.*, 1998, Davies and Highton, 1980 and Van Yelden *et al.*, 1977). But their role in biological transmission is unknown.

A unique aspect to the biology of RVFV is that the virus is maintained by transovarial transmission within *Aedes* mosquito eggs, meaning that live virus can be passed from parent mosquito to offspring by maintenance within eggs (Linthicum *et al.*, 1985). During inter-epidemic periods, the virus remains infectious within dormant desiccated *Aedes* mosquito eggs in dry floodplains; infected mosquitoes will emerge during flooding. As would be expected, outbreaks are associated with unusually heavy rainfall, especially cyclical El Nino-Southern Oscillation (ENSO) weather patterns (Davies *et al.*, 1985b).

2.2.2. Mammals

Like most arboviruses, RVFV alternates between mosquitoes and vertebrate hosts. Evidence of RVFV infection (as determined by hemagglutinin inhibition or plaque-reducing neutralizing antibody titers) has been found in many wild mammalian species in Africa, including camels, bats, lions, and elephants (Boiro *et al.*, 1987and Davies *et al.*, 1985a). The virus causes mild or inapparent illness in these species. It is not known whether any of the wild animal species are amplifying hosts.

Unlike wild animals, RVFV is highly pathogenic in domesticated ruminants, which are the amplifying hosts, meaning they develop sufficient viremia to infect feeding mosquitoes and potentiate further transmission. The most severe disease occurs in developing fetuses and very young animals immediately after birth; older animals are somewhat more resistant. Disease is most common in the three domestic animals cattle, sheep, and goats. High titers of virus are found in the blood of infected animals for approximately a week after onset of illness. Direct animal-to-animal transmission of RVFV neither occurs among herds nor experimentally in the laboratory. *Culex* and *Mansonia* mosquitoes are thought to be responsible for horizontal transmission between viremic animals and humans (McIntosh, 1972).

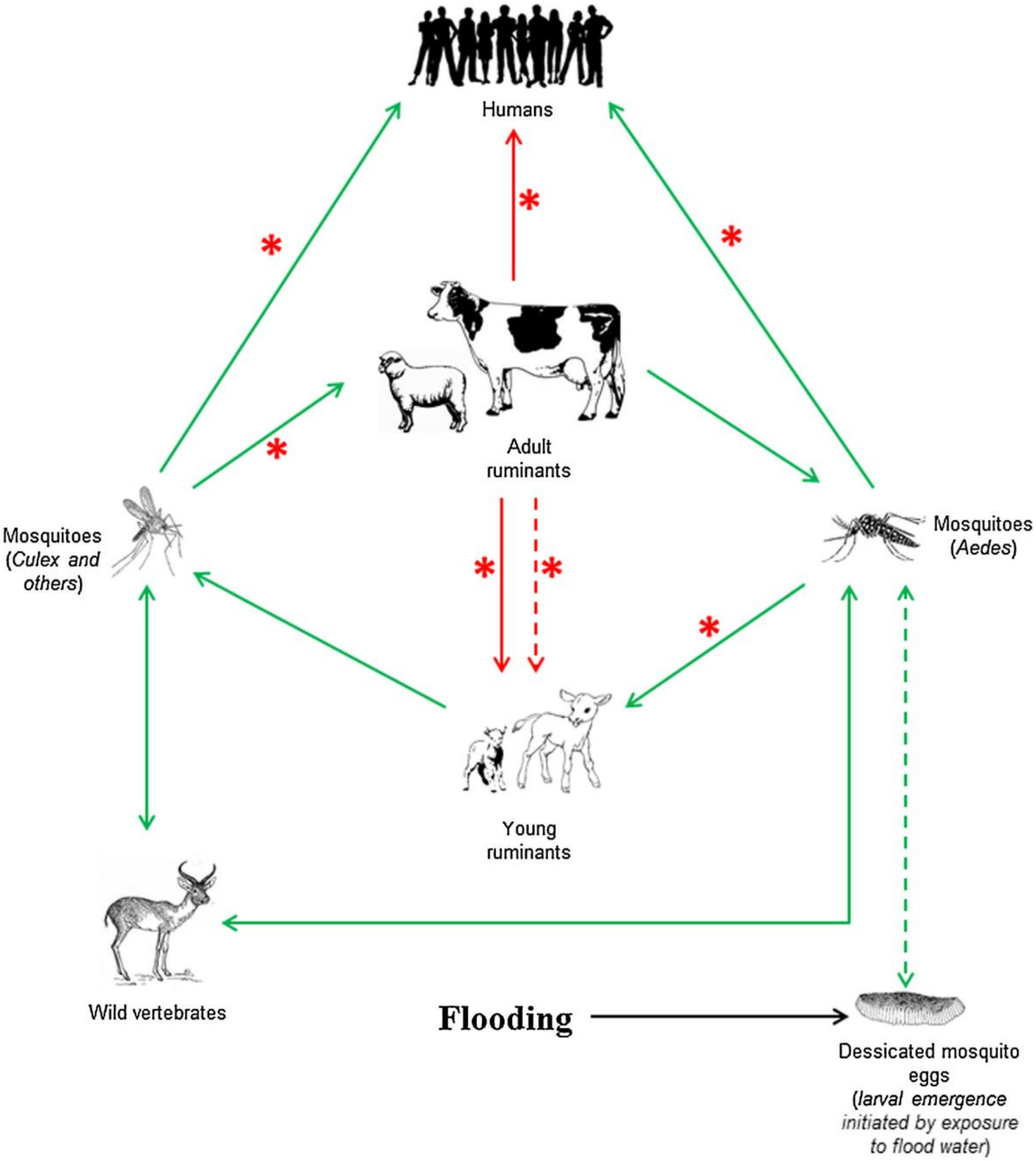
2.2.3. Humans

Humans become infected with RVFV when there is widespread illness and death among domesticated livestock. People can be infected by the bite of a mosquito, but the primary means of transmission of the virus to people is thought to be through mucous membrane exposure or inhalation of viral particles during the handling of infected animals and carcasses. A number of retrospective studies suggest that touching/handling, living close to, and consuming animal products are factors associated with increased likelihood of RVFV infection and possibly more severe outcomes (Van Yelden *et al*., 1977, *LaBeaud et al*., 2015, Anyangu *et al.*, 2010, Madani *et al*., 2003, and McIntosh *et al.*, 1980).

Between 1997–2010, there were 9 RVFV outbreaks, with 1,220 confirmed human deaths and >500,000 estimated human cases (Dar *et al.*, 2013). Most recently in March of 2016, human cases of RVF, associated with an outbreak in goats, occurred in Uganda. One of these patients was a butcher, and the other reported to have interacted with sick animals (Hartman, 2017). The virus has not shown direct human-to-human transmission, and there have been only a few documented cases of vertical transmission (Niklasson *et al.*, 1987,Adam and Karsany, 2008 and Arishi *et al*., 2006).

2.2.4. Transmission

Rift Valley fever virus (RVFV) is transmitted among ruminants by mosquito bites mainly belonging to the *Aedes* and *Culex* genera and by direct contact with body fluids of viremic animals. Moreover, biological or mechanical transmission of RVFV was reproduced experimentally with other hematophagous flies but field relevance of these transmission routes are still unclear (Dohm *et al.*, 2000 and Hoch *et al.*, 1985). Humans are mainly infected by close contact with blood, excreta of infected animals, consumption of raw milk (LaBeaud *et al.*, 2010), and in some rare cases, through mosquito bites (Seufi and Galal, 2010). RVFV circulates between animals within an enzootic cycle during most years, but may become epizootic during wet years in regions such as East Africa. Virus is maintained during dry seasons in desiccation-resistant eggs of several Aedes species which have acquired the virus by vertical transmission (Linthicum *et al.*, 1985). For example, in East Africa, flooding of natural excavations lead to the hatching of large numbers of Aedes (*Aedimorphus* and *Neomelaniconion* subgenera) eggs initiating viral circulation. Movements of viremic animals along trade routes have been suspected to be responsible for the virus spreading (Hoogstraal *et al.*, 1979). Unless vaccines are used on a large scale in Africa, RVF will continue to be a significant problem with the fear of being introduced into western countries.



Source :(https://www.pasteur.fr/en/research/virology/unitsgroups/arboviruses-and-insect vectors/research)

Figure 1: Schematic detailing the transmission cycles of RVFV; vectorial transmission (solid green arrows), direct transmission (solid red arrow) and vertical transmission (dashed red arrows); Red asterixes indicate where vaccine intervention would be appropriate.

2.3. Clinical signs

The sudden onset of large numbers of abortions ('abortion storms') and mortalities among young animals in affected livestock, together with the appearance of the disease in humans, is considered characteristic of an RVF epidemic (Gerdes, 2004). In humans, the clinical recognition of acute haemorrhagic fever cases generally triggers an outbreak investigation in endemic regions. However, clinical diagnosis alone cannot be considered reliable as some animals, for example camels, may have inapparent infections. Moreover, infection of susceptible, adult non-pregnant ruminants is often subclinical and hence outbreaks outside of the lambing or calving seasons can be easily missed. The clinical signs of RVF in animals tend to be nonspecific and differential diagnoses includes brucellosis, Bluetongue, Wesselsbron disease, enterotoxaemia, Bovine ephemeral fever, vibriosis, trichomonosis, Nairobi sheep disease, heart water, ovine enzootic abortion, toxic plant ingestion, bacterial septicaemias, peste des petits ruminants, anthrax and Schmallenberg disease (OIE, 2015).

2.4. Diagnosis

Laboratory diagnosis would ideally rely upon a combination of serological and molecular approaches the usefulness of the chosen assay is dictated by the disease kinetics. The windows in which particular virological markers (e.g. virus, viral RNA, IgG, IgM, hepatic lesions) are likely to be detected. There is likely to be some variability in disease kinetics between humans and animal species, due to variation in susceptibility between species, and even within species (Ikegami and Makino, 2011,Fagbami *et al.*, 1975, Bird and Nichol, 2012). For reporting RVFV in animals, the Office International des Epizooties (OIE); World Organization for Animal Health, require laboratory confirmation by at least two positive results from a combination of different diagnostic approaches preferably for the same specimen i.e. either positive for virus/viral RNA and antibodies or positive for IgM and IgG with demonstration of rising titres between paired sera samples collected 2–4 weeks apart (OIE, 2015).

2.4.1. Histopathology

The collection of diagnostic tissues (e.g. liver) in formaldehyde in the field may be beneficial in remote areas where cold chains do not exist. The histopathological examination of the liver of affected animals (and humans) will reveal hepatic lesions characteristic of RVFV and immunostaining may allow the specific identification of RVF viral antigen in tissue (OIE, 2015, Coetzer, 1982).

2.4.2. Virus isolation

RVFV can be isolated from whole blood or serum collected during the acute (febrile) stage of the disease (Anderson Jr *et al.,* 1989). Post mortem, the virus can also be isolated from the brain, liver, spleen and organs of the aborted fetus. In vivo isolation of the RVF virus can be performed in suckling mice; however, the OIE recommends that this approach should be avoided in favor of in vitro virus isolation, due to animal welfare and biosafety issues (OIE, 2015). 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 12–24 h post infection. Immunostaining or reverse-transcription polymerase chain reaction (RT-PCR) issued to confirm virus isolation.

2.4.3. Serology

Serological diagnosis, usually by virus neutralization 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).Several assays are available for the detection of anti-RVFV anti-bodies in a variety of animal species. Both the Virus Neutralization Assay and Enzyme-linked immunoassays (ELISAs) are highly specific, although there is the potential that they could be hindered by cross-reactivity between RVFV and other phleboviruses (Pepin *et al.*,2010). Alternative techniques such as the Indirect Immunofluorescence, agar gel immunodiffusion (AGID), radioimmunoassays and complement fixation are no longer used (OIE , 2015). The haemagglutination inhibition assay (HIA), once widely applied for serological investigations of suspected animals, is now rarely used due to biosafety and logistical issues.

The Virus Neutralization Assay is the gold standard serological assay, generally used for vaccine potency determination and is the OIE prescribed test for international trade(OIE, 2015). The test is highly specific and, 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. Alternative RVFV neutralization assays are being developed and validated, which eventually may lead to assays which do not involve the hand-ling of highly virulent RVFV in containment (Kortekaas *et al.,* 2011). 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 (Paweska *et al.,* 2005 and Williams *et al.*, 2011). The appearance of antibodies, usually following four to seven days of clinical symptoms clinical symptoms/signs, generally coincides with the gradual disappearance of the virus in the blood. Hence, either serum or blood samples collected for serological analysis may contain live virus and must therefore be inactivated prior to testing outside of bio containment. ELISAs are developed and widely applied by OIE Reference Laboratories

(OIE, 2015 and Williams *et al.*, 2011), and several have been developed using either whole cell lysate derived from infected cells or purified nucleocapsid protein as antigen (Williams *et al.*, 2011,Niklasson *et al*., 1984,McElroy *et al.*, 2009,van Vuren and Paweska, 2010 and Wu *et al.*, 2014). However, the commercially available ELISAs (IgG and IgM) are based upon recombinant RVFV nucleocapsid protein, despite the potential for background issues with this antigen (Faburay *et al.*, 2013).

The detection of IgM would suggest a current or recent infection. However, 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. An indirect enzyme linked immunosorbent assay based on their combinant nucleocapsid protein of RVFV has been developed for the detection of specific antibodies in human and animal sera (McElroy *et al.*, 2009).

This dual target assay is designed to differentiate between infected and vaccinated animals (DIVA), an important factor when considering the financial burden of movement restrictions on suspect livestock and the regulatory requirements for vaccine licensing. 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. A selection of European laboratories recently participated in a ring trial, to directly compare their own ELISAs with those from other laboratories, along with a number of commercially available ELISAs (Kortekaas *et al.*, 2013). It was concluded that, whilst further evaluations are warranted particularly for IgM ELISAs, their ring trial supports the use of commercially available ELISAs for both primary screening and serological diagnosis.

2.4.4. Molecular methods

A more economical and rapid alternative to virus isolation is the detection of viral RNA during the acute (febrile) phase of the disease, when high levels of viraemia occur in both humans and animals. After an initial incubation period of 2–6 days in humans, the febrile phase occurs, and typically lasts 3–4 days(Ikegami and Makino, 2011 and Smithburn *et al.,* 1949). Such rapid responses are necessary to allow case confinement (to avoid nosocomial infections in humans) and provide appropriate management and control decisions. A range of highly sensitive nucleic acid based molecular tests have been developed for RVFV including nested RT-PCR methods (Sall e*t al.*, 2002), quantitative real-time PCR (Bird *et al.,* 2007,Garcia et al., 2001,Drosten *et al.*, 2002 and Mwaengo *et al.,* 2012), multiplex PCR-based macro array assay (Venter *et al.*, 2014),RT Loop-mediated isothermal amplification (RT-LAMP) (Le Roux *et al.,* 2009) and recombinase polymerase amplification (RPA) (Euler *et al.*, 2012).

The RVF RT-LAMP amplifies specific nucleic acid sequences using a set of six primers by strand displacement activity of a DNA polymerase (Le Roux *et al.,* 2009 and Peyrefitte *et al.*, 2008). Amplification leads to the formation of a DNA precipitate which is detectable by the naked eye. There is no requirement for complex equipment and the assays can be cheaply and easily applied in field situations. The RVF RPA method (Euler *et al.,* 2012), an alternative isothermal amplification method utilizing three oligonucleotides and a specific fluorescent probe, has been shown to be as sensitive as LAMP and qRT-PCR, but with the greater potential to be applied to a hand held battery operated device for point of care or field application. The OIE recommends that viral RNA detection is undertaken using locally validated methods (OIE, 2015), such as conventional gel based-PCR or real-time RT-PCR.

In a recent external quality assessment (EQA), thirty expert laboratories from 16 countries tested a blind panel of RVFV positive and negative samples using their local molecular based RVFV test procedures. Twenty-eight of the 30 labs (93%) reported the use of a quantitative real time RT-PCR assay (qRT-PCR) for which consistently high sensitivity and specificity was observed between the laboratories, although less consistency was observed for the nested RT-PCR assays. (Escadafal *et al.*2014) also highlighted the limitations of employing highly specific probe based or nested molecular assays, which may fail to detect the wide range of potentially circulating RVFV strains. The two more recently developed molecular assays (RT-LAMP and RPA) were also represented in the ring trial, albeit by single laboratories, and compared well to the qRT-PCR. Whilst molecular techniques have proven useful during RVF outbreaks (Gerdes, 2004), the short duration of viraemia (3–4 days) (Ikegami and Makino, 2011 and Smithburn *et al.,* 1949), and the dependence on specialized equipment and skills, still pose challenges to their widespread applicability for monitoring the spread of the virus in the field.

However, molecular approaches allow for the rapid identification of the genetic lineages and hence the source and pathogenicity of the viral strains involved in a RVF outbreak (Ameri *et al*., 2009 *and Bird et al*., 2007). In addition, the RVF viral load is predictive of disease outcome, thus the use of quantitative real time assays may enable clinicians to target individuals with high viral loads (poor prognosis) for special intensive clinical management (Njenga *et al.*, 2009).

Sensitive and specific molecular assays may also be used for the early detection of RVFV RNA in mosquito pools thereby contributing to disease surveillance, vector species identification and disease control (Jupp *et al*., 2002, Mwaengo *et al.,* 2012 and Lutomiah *et al*., 2014).

2.5. Prevention and control method

RVF is a complex disease which readily cycles between mosquitoes, wildlife, livestock, and humans. Mosquito host choice is therefore an important factor affecting the dynamics of mosquito-borne pathogen transmission, including RVFV, and has implications on the efficacy of different control measures (Macdonald, 1957).

Prevention and control of RVF in humans therefore rely on preventing the disease in domestic animals in the peri domestic environment (Gubler, 2002). Vaccination of livestock is essential to prevent major outbreaks (Pepin *et al.,* 2010). The best and most efficient way to control the outbreak is to vaccinate all susceptible ruminants before a predicted outbreak. Several killed and live attenuated vaccines are available for livestock and provide immunity for 3 years. The first vaccine against RVFV was developed by Smithburn (Smithburn *et al.*, 1949). However, because this vaccine induces abortions and teratogenesis in ewes, cows and goats (Botros *et al.*, 2006, Kamal, 2009), its use is restricted during outbreaks and is only given to non-pregnant female animals (Barnard, 1979; Swanepoel and Coetzer, 2004). MP-12 vaccine which was derived by mutagenesis of the virulent Egyptian RVFV strain (ZH548) that was isolated during the 1977 outbreak is efficacious in livestock (Morrill and Peters, 2003). The live attenuated MP-12 vaccine (developed by the USAMRIID) and the C-13 vaccines (developed at the Laboratoire des Bunyavirides, Institut Pasteur, Paris, France) are also promising and have been tested in animals (Lopez *et al.*, 1995).

Although there is no RVFV vaccine approved by the Food and Drug Administration (FDA) for human use, several vaccine candidates are undergoing trials. A killed virus vaccine, TSI-GSD-200 (The Salk Institute–Government Service Division–200), is available primarily for laboratory personnel, and has produced good results for over 12 years of follow-up with only 3.5% of the subjects showing side effects (Pittman *et al.*, 1999).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Borana zone, Oromia Regional State, Southern Ethiopia. Yabello is the capital of Borana Zone located 570 KM South of Addis Ababa (Dabasa *et al.,* 2013). The altitude of Borana rangeland lies at 970 m above sea level in the South, bordering Kenya to 1,693 m above sea level in the northeast. It borders with the Republic of Kenya to the South, Somali Regional State to the East, and Southern Nation and Nationalities and People's Regional State to the West, and Guji and West Guji Zones to the North (Figure 2). The climate is semi-arid, mean annual rainfall measuring below 600 mm. There are four locally defined seasons comprising two rainy seasons (long rains *ganna*, and short rains *hagayya)* and two dry seasons (long dry season *bona hagayya* and the short dry season *adoolessa*) (Coppock, 1994; Tache, 2008).

A prominent feature of the Borana ecosystem is the erratic and variable nature of the rainfall, with most of the areas receiving between 238 and 896 mm annually (Angassa and Oba, 2007), and the annual mean daily temperature varies from 19 ^{o}C to 24 ^{o}C with moderate seasonal variation. The Borana pastoral system is dominated by savannah vegetation containing mixtures of perennial and woody bushland. The major sources of water are ponds and deep wells during rainy and dry periods, respectively (Angassa and Oba, 2007).

Livestock is an integral part of the Borana people that serve several purposes such as a source of food, income generation, and social prestige (Angassa and Oba, 2007). Pastoralism is the main source of livelihood. The society that was once predominantly cattle pastoralists now rears a mixture of livestock species, including cattle, sheep, goats, camels, and equines. Pasture, water, and mineral licks make up the three key natural resources on which the Borana pastoral production depends, each resource having specific management rules that regulate access. Pasture is common property and its management is the collective responsibility of local territorial units constituted by mixed clans, whereas wells as the critical dry season water source are owned by clans (Tache, 2008).

After settling pastoralists the government had organized them into pastoral associations within districts. The agro-pastoralist livelihood is more diversified with a mix of crop production, herding activities, poultry farming, and livestock trading, petty trading, and sale of forest products (Anbacha and Kjosavik have an unpublished manuscript of an in-depth study of livelihood diversification of the Borana). However, this does not mean communities in the pastoral production system never participate in non-pastoral activities. For instance, every Borana household has a small plot of land upon which they produce crops (Anbacha and Kjosavik, 2018).

According to Borana zone Pastoral Development Office 2017 reports, the zone has 1,416,180 cattle, 776,870 sheep, 1,262,782 goats, 237,205 camels, 102,767 Donkeys, 4,433 Mules, 1,841 Horses and 306,057 Chickens (Borana Zone Pastoral development Office Report, 2017).

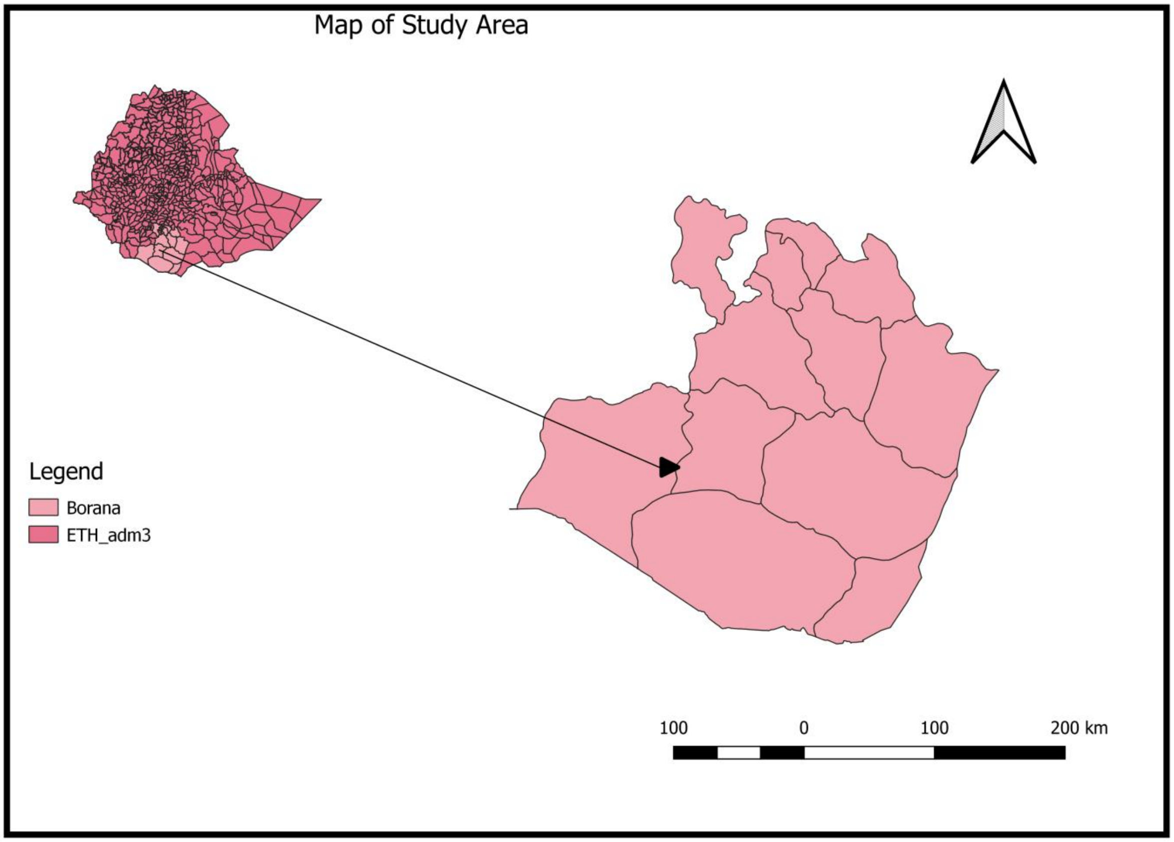


Figure 2: The map of the study area

3.2. Study design

Sampling design was purposive based and the Borana zone woredas that borders Kenya were selected , as Kenya is an endemic country (Linthicum *et al*., 2016). The survey participants may not accurately represent the true population in Borana. However, given that over 240 participants were interviewed in the study area, focusing in part on high risk populations, it is believed that the study is still very useful.

3.3. Collection of mosquitoes

Mosquitoes were collected from purposively selected woredas that border Kenya which included Moyale, Miyo, Dirre, Dillo, and Taltalle districts from October 2019 to March 2020. Using the CO2 baited CDC light traps and CO2 baited mosquito trap BG-Sentinel 2. Initially, the plan was set to collect the mosquitoes in two seasons, during short wet season *Hagaya* and long wet season *Gana* in the study area. The first plan which was during December 2019 – January 2020 was successful but the second round collection which was planned in April 2020 was failed due to COVID-19 pandemic. During mosquito collection, the traps were set in proximity to potential mosquito breeding and feeding sites which include indoor and outdoor (near water bodies like flood plains that create damboo, lakes, and dams and also near the animal pen, and on the field of where there are human and livestock population). The trap was placed at 18:00 PM and collected at 6 -7:00 AM in a succeeding day. All necessary information filled in format (attached at annex-2), 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 level were made by using mosquito taxonomy key (Jupp and Smith, 1986) and dichotomous keys of Walter Reed Bio Systemics Unit (WRBU) using stereomicroscope and Dino-Lite digital microscope in Yabello regional veterinary laboratory (YRVL). Then, 20- 25 mosquitoes were pooled into Eppendorf tube (2ml) according to species, sex, collection date, and trap number. The pool was then transferred into the container containing liquid nitrogen, transported to National veterinary Institute (NVI), Bishoftu for molecular detection of RVFV. Unfortunately due to COVID-19 pandemic the laboratory works that were planned at NVI were not accomplished.

3.4. Participatory epidemiological investigation

3.4.1. Participatory disease surveillance (PDS)

The contacts were made purposively with livestock keepers and key informants based on their knowledge about the local livestock disease situation. Livestock keepers and the interviewer were discussed animal health issues together, and pastoralist knowledge and experience were listened to and also were respected, even if it seems to contradict formal (biomedical) veterinary knowledge (Catley *et al.*, 2002). The method that was used in this study was a questionary survey.

3.4.2. Questionnaire survey

A pre-study field visit to the study area was conducted, to build trust explain the study objectives, mobilize the community leaders, and ask the community to be involved in all parts of the study accordingly we had explained the contents of the questionary and if they believed that they can interact then we mark them as key informants. Therefore during the real data collection, as we did during our pre-study visit, we introduced ourselves that we come from College of Veterinary Medicine and Agriculture of Addis Ababa University and tell them that we are veterinarian working research and would like to ask them questions regarding livestock health, livestock disease and livestock diseases driving factors and then if they agree to answer we thank them and proceed to the interview.

This was lead to a sense of ownership and empowerment. Thus, successful face-to-face interviews with the heads of households at their home were taken place in a friendly environment. The questionnaires included the demographic data that are related to the livestock owners such as sex, age, educational status, disease-related questions such as disease outbreak in their area that characterized by abortion storm and mass young mortality, and that associated with seasonality of unusual rain. And the driving factors of the diseases were also assessed by asking whether they ever encounter a huge mosquito population, where mosquito reared such as laying water.

Questionnaires were prepared in English but, administered in *Afan Oromo* accordingly individual interviewee of the households was interviewed to know their knowledge, attitude, and perception regarding the RVF disease and environmental aspects of RVF. But the questionnaires were not designed in the way that directly asks about specific disease RVF, in steady, it was based on syndromic surveillance which was based on the clinical signs. The questionnaire is attached as an annex.

Convenience sampling was used; all participants who accept to cooperate in the questionary survey were eligible for the study. From Moyale, Bokola and Arballe kebeles are chosen for their accessibility, both kebeles are periurban and community there lead livestock rearing with small business, like labor work in Moyale, animal trade, petty trade owning shop and self-business. From Miyo woreda, there was an outbreak of other diseases in cattle in Chari Liche and Melbana kebeles and together with the investigation team from Yabello regional veterinary laboratory that went there, we carried out the questionary survey regarding this research. From Dirre, Madhacho, and Gololcha kebeles were selected; Communities in Madacho practice animal trade, petty trade, and crop production along with pastoralism. The households are located on the main road from Addis Ababa to Moyale. And Gololcha kebele which is mainly pastoralist is found about 15 Km west of Madhacho, which is situated on the main asphalt. From Elwaye woreda Sarite and Dhedhertu kebeles were interviewed the community of these kebeles are mostly pastoralist but there are scatter plot of land cultivated in Dhedhertu but the community of Sarite are all pastoralist. The community of Bule Korma and Bila Kebeles of Taltalle are Agro pastoralist they practice cultivation of different crops integrating with livestock rearing, this both kebele found on the road from Milami town the capital of Taltalle woreda to Hobok the kebele bordering Kenya. The community at Chirate and Bilala Kebeles of Dillo woreda are pure pastoralist and their livelihood depends on rearing of livestock.

The survey was written in English but was read aloud in a private setting to participants in *Afan Oromo*. The survey questionnaire asked participants about socio-demographic variables as well as epidemiological risk factors and exposures, perception regarding the livestock diseases and signs and factors that were incorporated into the questionary intentionally that are specific to RVF disease, environmental aspects of RVF and mosquitoes.

Out of a total of 244 persons participated in the study, 40 (16.39 %) was selected from Moyale, 40 (16.39 %) from Dirre, 40 (16.39 %) from Elwaye, 40 (16.39 %) from Miyo, 40 (16.39 %) from Taltalle and 44 (18.03%) was selected from Dillo. A total of 139(56.97%) participants were males whereas 105(43.03%) were females. Among the study participants, a higher number of respondents 92 (37.7%) were having age ranges between 20-30 years, 75 respondents (30.74%) were having age ranges between 30-40 years, 45 respondents (18.44%) were having age ranges between 40-50 years, 26 respondents (10.66%) were having age greater than 50 years and relatively few numbers of respondents 6(2.46%) were having age less than 20 years.

The majority of the total respondents 191 (78.28%) were without formal education, 40(16.39%) attended elementary school 8(3.28%) attended High school and only the small proportion of the respondents 5 (2.05 %) educated at different levels in College and University. Regarding the marital status, majority of the total respondents 215(88.11%) were married, 15(6.15%) were single, and 14 (5.74%) were divorced.

The greater proportion of the participants, 167 (71.44 %) of the study participants primary occupation was nomadic/transhumance pastoralism, 42(17.21%) practiced sedentary pastoralism and other agricultural activities, 25(10.25%) sedentary pastoralist, were as a small proportion 10 (4.1 %) were replied that they practiced mixed type of livelihood.

All of the interviewed households 244(100%) included in the study was keeping livestock at least one species, and among the respondents, 89(36.48%) replied that they kept cattle, goats and sheep, 49(20.08 kept cattle, goats, sheep, camel and equids, 29(11.89%) kept cattle, goats, sheep and Equines, 23(9.43%) kept cattle, goats, sheep and camel and small proportion also replied owns pet. The participants also asked the purpose why they kept livestock and majority 182(74.59%) replied that they kept for meat, milk, draught power and for sale whereas 43(17.62%) kept for meat, milk and for sale and 12(4.92%) kept for milk and for sale. (**Table 1**)

Table 1: Socio demographic background of the study participants (N=244)

Factors	**Categories**	**n**	**%**
Site	Elwaye	40	16.39
	Dirre	40	16.39

	Moyale	40	16.39
	Miyo	40	16.39
	Dillo	44	18.03
	Taltalle	40	16.39
Gender	Male	139	56.97
	Female	105	43.03
Age	<20 years	6	2.46
	20-30 years	92	37.7
	30-40 years	75	30.74
	40-50 years	45	18.44
	>50 years	26	10.66
Educational status	Illiterate	191	78.28
	Elementary	40	16.39
	High school	8	3.28
	College and University	5	2.05
Marital status	Single	15	6.15
	Married	215	88.11
	Divorced	14	5.74
Lifestyle respondent	Nomadic pastoralist	110	45.08
	Transhumance pastoralist	57	23.36
	Sedentary pastoralist	25	10.25
	Agrarian	4	1.64
Livelihood	Salary	5	2.05
	Self-business	12	4.92
	Sale of animal products	19	7.79
	Live animal sale	23	9.43
Livestock Owned by species	cattle, goats, sheep,	49	20.08

	camel, and equids		
	cattle, goats, sheep,	89	36.48
	cattle, goats, sheep, and equids	29	11.89
	cattle, goats, sheep, and camel	23	9.43
Purposes of rearing	meat, milk, draught power and for sale	182	74.59
	meat, milk and for sale	43	17.62
	Milk and for sale.	12	4.92

3.4. Method of data analysis

Microsoft excel 2010 database system was used for entry, coding and simple calculation of recorded data. Statistical data analysis was done by using STATA Statical software Version 13, Quantum geographic information system (QGIS Desktop 3.4.5 software) and R software version 3.5.1. was used for mosquito distribution modeling. Descriptive statistical data analysis tests were used and the score of knowledge and attitude of the community regarding livestock diseases and diseases driving factors was generated and Pearson chi-square test p-values below 5% were considered as indicators of statistical significance.

3.5. Mosquito Dispersal Modeling

3.5.1. Potential explanatory variables of RVF vector mosquitoes occurrence

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).

Models for determining areas at risk for RVF outbreaks consider several factors. However, factors related to vector ecology were very 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).

Surplus and extended rainfall have a positive linear relationship with vegetation development in most RVF-endemic regions. 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.

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). NDVI data of six months based on rainy seasons in Borana zone were downloaded. Accordingly, NDVI data of September-December 2019 (short rainy season called *Hagaya)* and NDVI data of January-March 2020 (long rainy season called *Ganna*) were downloaded from website of Famine Early Warning Systems (FEWS)https://earlywarning.usgs.gov/fews/datadownloads/East%20Africa/eMODIS%20NDVI%20C6. 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).

Globe cover information is becoming more and more important for landscape ecology and environmental monitoring (Zhang and Mei, 2016). A strong influence of human land-use change 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 globe 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 *et al.*, 2010), based on the Land Cover Classification System (LCCS) at a high resolution (300m).

Climatic factors have obvious impacts on the distribution of RVF mosquito vectors, data of climatic factors were downloaded from the WorldClim database (http://worldclim.org/). A precipitation creates an ecologically moist 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). WorldClim version 2 (Fick and Hijmans, 2017) has average monthly climate data including minimum, mean, and maximum temperature as well as precipitation for the years 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.

The soil 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/). 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). 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 populations play a major role in the 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 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 population densities were downloaded from the FAO Gridded Livestock of the World (GLW) web (http://www.fao.org/livestock-systems/global-distributions/en/). 2010 data is the latest version of GLW and it includes global distributions of cattle, sheep, goats, horses, pigs, buffaloes, chickens and ducks at a spatial resolution of 5 minutes of arc, approximately 10 km at the equator (Gilbert *et al.*, 2018).

3.5.2. GIS Operation

In the current study, eleven explanatory variables (Elevation, Globecover, HWSD, Livestock density, NDVI, precipitation, Max temp, Min temp, sun radiation, vapor and wind) 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 5 arcminutes 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, 2015; Naimi *et al.*, 2014). 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.

3.5.3. 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.

3.6. Ethical Consideration

Prior to the start of the study, official ethical clearance for the project was requested and taken from the Animal Research Ethics Committee of College of Veterinary Medicine and Agriculture, Addis Ababa University. Approval of this study was also obtained from the Ministry of agriculture. (annex 2).

4. RESULT

4.1. Mosquito genera

In this study, a total of 200 mosquitoes were collected from study areas. Based on morphological identification. These mosquitoes were belonging to three genera; *Culex* (n = 48; 24%), *Aedes* (n = 147; 73.5%) and *Anopheles* (n = 5, 2.5%) as shown in **Table 2.**

Table 2: Summary of mosquito genera collected

	Miyo	**Dirre**	**Moyale**	**Dillo**	**Total**
Culex	8	-	27	13	48(24%)
Aedes	5	45	70	27	147(73.5%)
Anopheles	1	-	1	3	5(2.5%)

4.2. Knowledge, Attitude, and practice of the community with regards to the disease and Driving Factors

4.2.1. Community knowledge about livestock diseases

The majority of the participants 231(94.67%) were replied that they had observed livestock diseases outbreak in their herds/locality. Whereas 13(5.33%) replied that they had never observed livestock diseases outbreak in their herds/locality. Those that replied outbreaks occurred in their herds/locality were told different types of symptoms that they had observed such as sudden death, abortion, coughing, salivation, depression, lacrimation, diarrhea, and colic.

Among participants, 151(61.89%) responded that they had seen the sign of mass abortion in their herds whereas 93(38.11%) answered that they had never seen the sign of mass abortion in their herds.

Among 151 participants that replied they had seen the sign of mass abortion in their herd, 52(21.31%) replied that they had seen it in sheep and goats, 46(18.85%) in goats, 14(5.74%) in sheep, 14(5.74%) in cattle, sheep, and goats whereas small proportion 2(0.82%) also reported the case in camel.

Among 151 participants that replied, they had seen the sign of mass abortion in their herd.The season in which such event happened had been asked and 44(18.03%) replied that it happens during all seasons (Winter, Autumn, Summer and Spring), 29(11.89%) during Summer, 20(8.20%) during Spring, 17(6.97%) during Summer and Spring, 17(6.97%) during Autumn and Summer, 12(4.92%) during Winter, 6(2.46%) during Autumn and Spring, 4(1.64%) during Autumn, 2(0.82%) during Autumn, Summer, and Spring, whereas 1(0.41%) replied that it happens during winter and autumn.

Among the participants, 130(53.28%) responded that there was a history of heavy rainfall that precedes the event of mass abortion, whereas 114(46.72%) replied that there was no history of heavy rainfall that precedes the event of mass abortion. Among the 244 participants, 168(68.85%) answered that there were mass young mortalities, whereas 76(31.15%) responded that there were no mass young mortalities.

Among the 168 participants that replied mass young mortalities, the species in which mass young mortalities happened was asked and 124(50.82%) replied it happened in kid and lamb, 23(9.43%) in kid, 7(2.87%) in lamb, 4(1.64%) in kid and calves, 4(1.64%) in kid and camel calves and 1(0.41%) in calves, 1(0.41%) in calves, kid and lamb, 1(0.41%) in kid, lamb and camel calves, 1(0.41%) in lamb and camel calves whereas 1(0.41%) replied it happened in camel calves.(**Table 3**)

Table 3: Community knowledge regarding livestock diseases (N=244)

Factors	Categories	n	%	p-value
Diseases outbreak in your herds/locality	Yes*	231	94.67	0.03
Have you seen the sign of mass abortion in your herd	Yes*	151	61.89	0.00

In which species of animals you have seen mass abortion	Sheep and Goats	52	21.31	
	Goats	46	18.85	
	Sheep	14	5.74	
	Cattle, sheep and Goats	14	5.74	
	Camel	2	0.82	
In which season mass abortion happened	All season	44	18.03	
	Summer	29	11.89	
	Spring	20	8.20	
	Summer and spring	17	6.97	
	Autumn and summer	17	6.97	
	Winter	12	4.92	
	Autumn and spring	6	2.46	
	Autumn	4	1.64	
	Autumn, summer and spring	2	0.82	
	Winter and autumn	1	0.41	
was there history of heavy rainfall before the event	Yes*	130	53.28	0.03
Have seen mass of young animal mortalities in your herd	Yes*	168	68.85	0.00
In which species mass of young animal mortalities seen	Kid and lamb	124	50.82	
	Kid	23	9.43	
	Lamb	7	2.87	
	Kid and calves	4	1.64	

	Kid and camel calves	4	1.64
	Calves	1	0.41
	Calves, kid and lamb	1	0.41
	Kid, lamb and camel calves	1	0.41

Among the 168 participants that replied there was mass young mortalities, the clinical sign they had seen was asked and 65(26.64%) replied diarrhea, 23(9.43%) depression and diarrhea, 8(3.28%) depression, diarrhea and nasal discharge, 8(3.28%) coughing, depression, diarrhea and nasal discharge, 6(2.46%) sudden death, coughing and diarrhea, 6(2.46%) sudden death and diarrhea, 5(2.05%) coughing, 4(1.64%) coughing, depression and diarrhea, 4(1.64%) coughing, diarrhea and nasal discharge, 3(1.23%) sudden death, coughing, depression, lethargy, diarrhea, vomiting and nasal discharge, 3(1.23%) sudden death, depression, lethargy and diarrhea, 3(1.23%) depression, lethargy and diarrhea, 2(0.82%) sudden death, coughing, depression, lethargy, diarrhea and nasal discharge, 2(0.82%) sudden death, depression and vomiting, 2(0.82%) sudden death, depression and nasal discharge, 2(0.82%) sudden death, depression and other sign, 2(0.82%) sudden death and other signs, 2(0.82%) coughing, depression and nasal discharge, 2(0.82%) coughing and nasal discharge, 2(0.82%) depression and vomiting, 2(0.82%) nasal discharge (**Table 4**)

Table 4: Community knowledge about the clinical sign of the diseases (N=244)

Factors	**Categories**	**n**	**%**
What clinical signs have seen during young death storm	Diarrhea	65	26.64
	depression and diarrhea	23	9.43
	depression, diarrhea and nasal discharge	8	3.28
	coughing, depression, diarrhea	8	3.28

	and nasal discharge		
	sudden death, coughing and diarrhea	6	2.46
	sudden death and diarrhea	6	2.46
	Coughing	5	2.05
	coughing, depression and diarrhea	4	1.64
	coughing, diarrhea and nasal discharge	4	1.64
	sudden death, coughing, depression, lethargy, diarrhea, vomiting and nasal discharge,	3	1.23
	sudden death, depression, lethargy and diarrhea	3	1.23
	depression, lethargy and diarrhea	3	1.23
	sudden death, coughing, depression, lethargy, diarrhea and nasal discharge,	2	0.82
	sudden death, depression and vomiting,	2	0.82
	sudden death, depression and nasal discharge	2	0.82
	sudden death, depression and other sign	2	0.82
	sudden death and other signs	2	0.82
	coughing, depression and nasal discharge	2	0.82
	coughing and nasal discharge	2	0.82
	depression and vomiting	2	0.82
	nasal discharge	2	0.82

4.2.2. Community knowledge about the driving factors

Among 244 participants of the survey, 203(83.20%) responded that they had seen huge mosquito population (swarms) during summer and spring, while 41(16.80%) that they had never seen swarm of mosquito during summer and spring. Among those that replied there is huge mosquito population, 181(74.18%) replied that there were laying water in their surroundings, whereas 63(25.82%) replied that there was no laying water in their surroundings.

Out of a total of 244 persons participated in the study, majority 215(88.11%) replied that there are movements of animals from one site/locality to another within the country, while 29(11.89%) answered that there are no movements of animals from one site/locality to another within the country, 139(56.97%) responded that there are transboundary movements of animals, while 105(43.03%) responded, no transboundary movements of animals, all 139 persons that replied there is transboundary movements of animals, 139(100%) responded that the country to which animals move is Kenya.

Among 244 participants of the study, 157(64.34%) responded that there is introduction of neighbor country livestock into their grazing area/herds, 145(59.43%) answered that their livestock transit into the neighbor country for grazing and search of feed, while 99(40.57%) replied that their livestock do not transit into neighbor country for grazing and search of feed. Out of total of 244 persons participated in the study, 150(61.48%) responded that there is an occasion when their herds/flocks mix with herds/flocks from neighbor country, while 94(38.52%) responded that there is no an occasion when their herds/flocks mix with herds/flocks from neighbor country.

Out of total of 244 persons participated in the study, majority 220(90.16%) responded that there is common grazing land in their surroundings, while 24(9.84%) responded that there is no common grazing land in their surroundings. Among 244 participants of the study, 145(59.43%) replied that there was history of heavy rainfalls for 1-2 weeks which was led to flooding in their surroundings, while 99(40.57%) replied that there is no history of heavy rainfalls for 1-2 weeks which was led to flooding in their surroundings, 132(54.10%) responded that they had observed 1-2 weeks laying water associated with flooding from heavy rainfall in their localities, while 112(45.90%) responded that they hadn't observed 1-2 weeks laying water associated with flooding from heavy rainfall in their localities.(**Table 5**)

Table 5: Community knowledge about diseases driving factors (N=244)

Factor	Categories	n	%	P-value
Have you ever seen huge mosquito population during summer and spring?	Yes*	203	83.20	0.001
Were there any laying waters in your surrounding?	Yes*	181	74.18	0.000
Are there movements of animals from one site to another within the country?	Yes*	215	88.11	0.117
Is there any transboundary movement of your animals?	Yes*	139	56.97	0.000
to which country	Kenya	139	100	
Is there any introduction of neighbor countries' livestock into your grazing area/herds/flocks?	Yes*	157	64.34	0.000
Are your animals' transits into neighbor countries for grazing and/or search of feed?	Yes*	145	59.43	0.000
Is there any occasion where your herds/flocks mix with herds/flocks from neighbor country/ies?	Yes*	150	61.48	0.000
Is there common grazing land in your surrounding	Yes*	220	90.16	0.000
Is there history of heavy rainfalls for 1-2 weeks which led to flooding in your surrounding?	Yes*	145	59.43	0.000
Have you ever observed 1-2weeks laying water	Yes*	132	54.10	0.000

associated with flooding from heavy rainfall in your localities?	

4.3. RVF Mosquito Dispersal 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 RF, while the least accurate was ANN. Of these, 23 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 6. Average performance of individual models.

	MAXENT.Phillips	RF	GAM	GLM	GBM	CTA	ANN	SRE	FDA	MARS
KAPPA	0.69	0.89	0.71	0.62	0.87	0.61	0.56	0.83	0.66	0.79
ROC	0.96	0.98	0.90	0.95	0.98	0.91	0.90	0.88	0.93	0.97
TSS	0.87	0.90	0.79	0.87	0.89	0.84	0.74	0.75	0.81	0.89

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 (vapr), 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 7 below.

Table 7. Overall contribution of the variables in initial models

Variables	**MAXENT**	**RF**	**GAM**	**GLM**	**GBM**	**CTA**	**ANN**	**SRE**	**FDA**	**MARS**
Elevation	0.05	0.01	0.54	0.17	0.02	0.05	0.40	0.17	0.01	0.10
Globcover	0.23	0.01	0.48	0.12	0.09	0.30	0.17	0.37	0.14	0.33
HWSD	0.22	0.10	0.67	0.40	0.45	0.48	0.68	0.36	0.32	0.62
Livestock	0.15	0.02	0.00	0.01	0.19	0.15	0.18	0.24	0.00	0.00
NDVI	0.31	0.04	0.37	0.26	0.08	0.00	0.00	0.26	0.04	0.00
prec	0.06	0.01	0.59	0.29	0.00	0.00	0.29	0.31	0.00	0.00
tmax	0.00	0.02	0.41	0.11	0.00	0.02	0.05	0.19	0.00	0.25
tmin	0.01	0.04	0.47	0.14	0.09	0.03	0.09	0.24	0.10	0.23
srad	0.13	0.06	0.40	0.26	0.08	0.03	0.33	0.31	0.00	0.12
vapr	0.55	0.23	0.78	0.69	0.70	0.64	0.01	0.45	0.59	0.68
wind	0.08	0.06	0.69	0.34	0.00	0.02	0.00	0.12	0.11	0.00

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 represented in table 8 below, to develop ensemble model, the highest overall contribution were made by water Vapor pressure (54.4%), followed by Harmonized World Soil Database(HWSD) (29.9%), while Elevation were contribute the least 1%.

Table 8. Ensemble variable Importance

Variables	EMmean	EMcv	EMca
Elevation	0.013	0.332	0.013
Globcover	0.12	0.32	0.132
HWSD	0.299	0.664	0.29
Livestock	0.027	0.161	0.026
NDVI	0.049	0.198	0.044
prec	0.021	0.549	0.016
tmax	0.018	0.275	0.011
tmin	0.026	0.307	0.02
srad	0.048	0.294	0.041

vapr	0.544	0.677	0.551
wind	0.044	0.222	0.038

4.3.2. *Ensemble models*

Ethiopia has many suitable areas that support growth and distribution of RVF vector mosquitoes. Most lowlands border areas of the country starting from Somali region, southern border of Oromia that is Borana, Bale and Guji zones and and SNNP as well as western border of Gambella and Benishangul Gumuz suitable area captured by ensemble model as in figure 3 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.

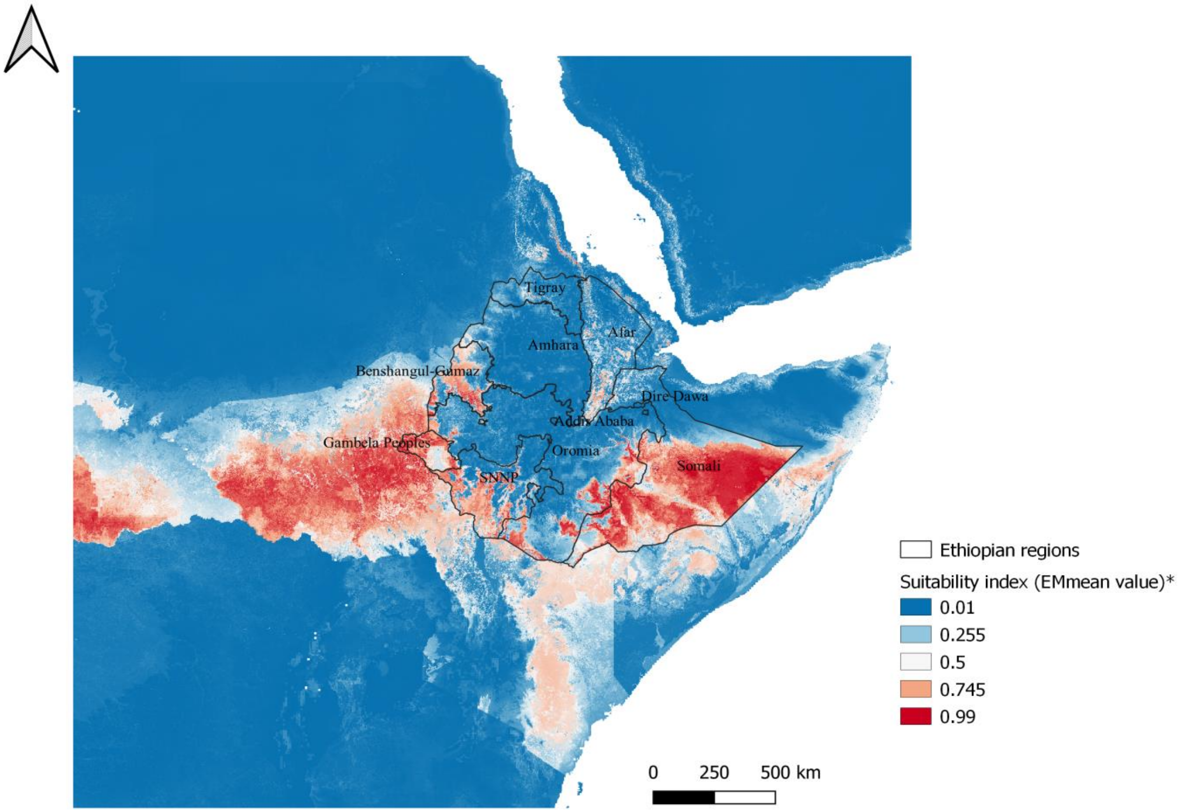


Figure 3: Predicted potential distribution of RVF vector mosquitoes. The scale indicates less suitable environment (blue color) and most suitable environment (red color).

A suitability map of committee average (Figure 4 below) also looks similar with maps of ensemble mean above. Models committee averaging gives both a prediction and a measure of uncertainty.

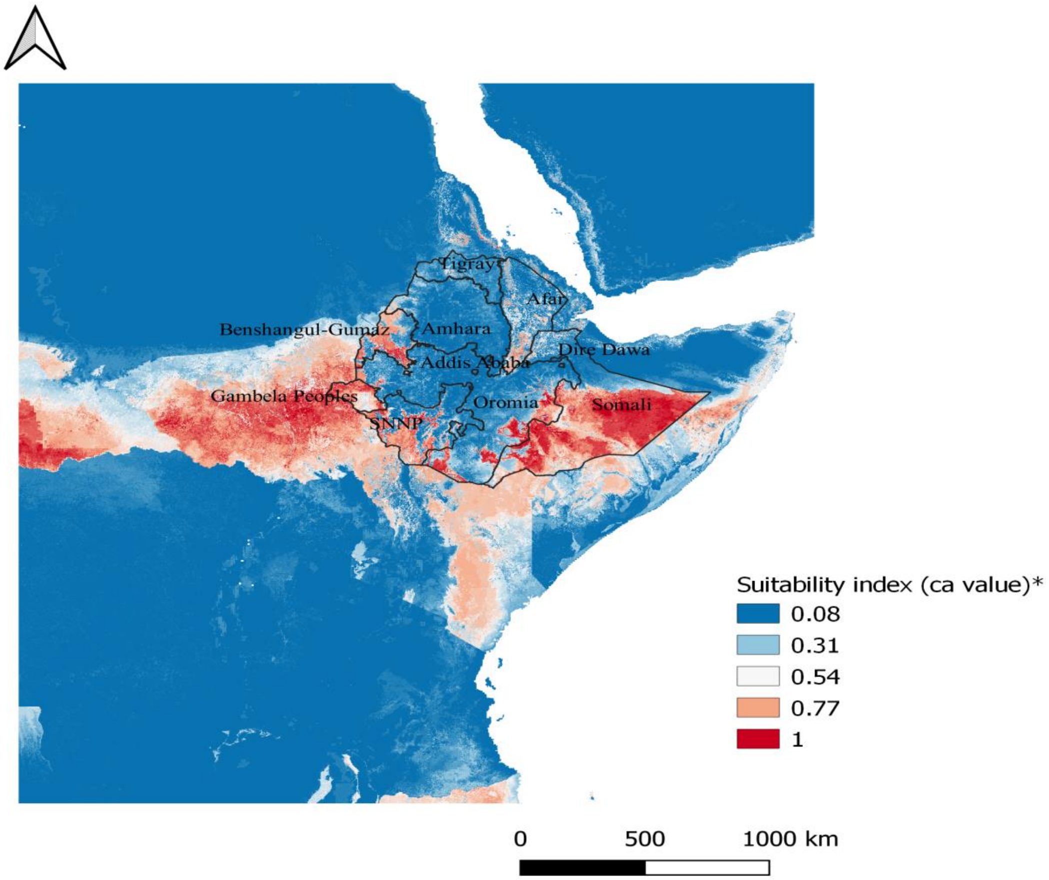


Figure 4: The estimated committee averaging across the selected predictions. The scale indicates unsuitable environment with certain prediction (blue colors), less suitable with uncertain prediction (light colors), and most suitable environment with certain prediction (red colors).

The model showed variation in the uncertainty index among different parts as the 'clamping mask' value portrayed in Figure 5 below. 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.

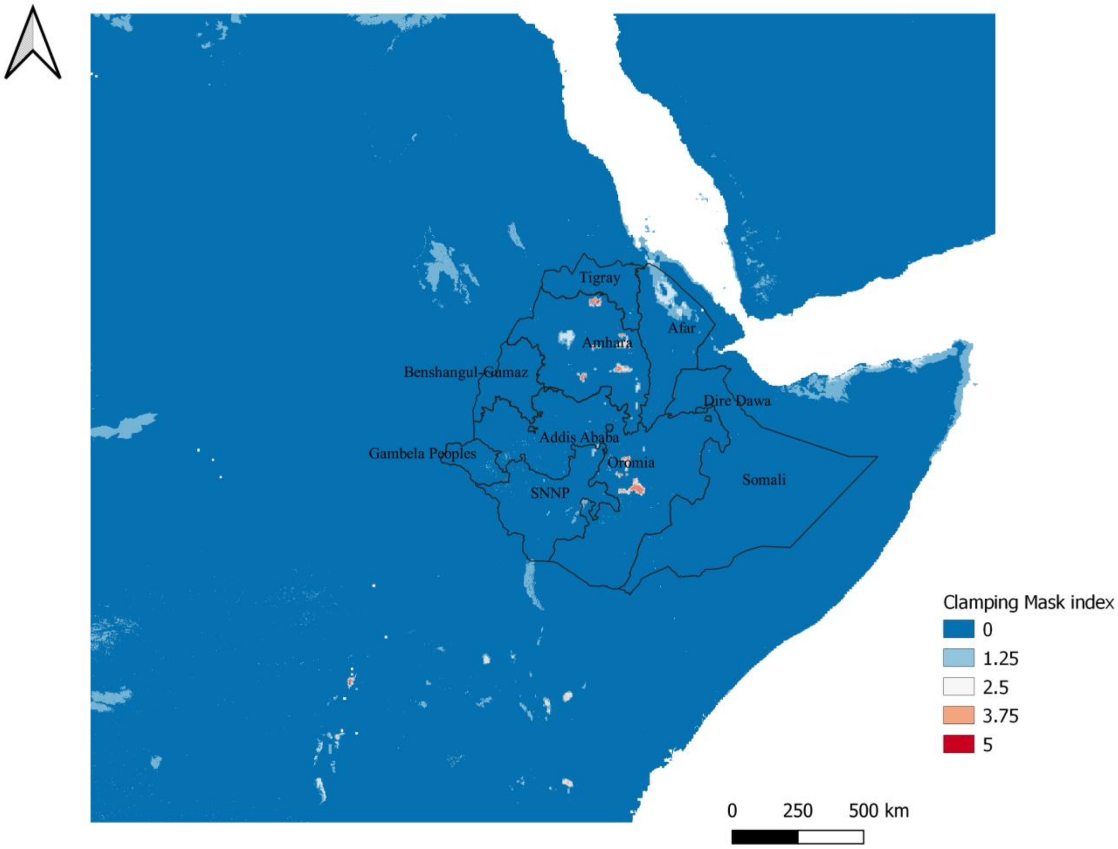


Figure 5: The Estimated 'clamping mask' value. Warmer (red) indicate areas where models predictions are uncertain.

5. DISCUSSION

The current vector surveillance was conducted to define the occurrence and distribution of competent RVFV vectors in Borana, also as to detect virus in the vector, and develop a mapping of high-risk area with modeling. In the initial plan, we proposed to integrate the anticipated molecular detection of Rift valley fever virus from the competent mosquito vector with this participatory based questionary but unfortunately due to the COVID-19 pandemic that occurred all over the world, laboratory part of this study could not be accomplished and we enforced to depend on questionnaires and mosquitoes ensemble dispersion models.

In spite of low collections, the mosquito survey indicates that *Aedes* and *Culex* were the most prominent mosquito genera in the study area. *Aedes* mosquitoes were incriminated as principal vector for RVFV and believed to play a significant role in maintaining the endemicity of the disease in the environment through transovarial transmission (Oluwayelu et al., 2018). 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 (Tantely *et al.*,2015). In the current study there were few mosquito number and distributions. This might be due to inappropriate season of sample collection and technical problem on the field associated with mosquito traps and trap power supply.

The current qualitative study is structured questionnaire, where most of the questions response were yes/no and there were no focus discussion group. In this study majority of the respondents 191 (78.28%) have no formal education, 40(16.39%) attended elementary school 8(3.28%) attended high school and only the small proportion of the respondents 5 (2.05 %) have education at different levels in College and University. An association between higher educational attainment and better health status has been repeatedly reported in the literature. Kawachi *et al.*(2010) have demonstrated that there is evidence to suggest that schooling is causally related to improvements in health outcomes.

5.1. KAB Assessment

This study was based on the knowledge of the participants about general livestock diseases, but also the specific clinical signs that related to Rift valley Fever and its driving factors were also integrated into the questionary to get insight or indicator of the risk of occurrence of the disease. During the 2007 RVF outbreak in Sudan, Hassan *et al*. noted that an RVF outbreak was only recognized after human cases were identified, indicating that improved awareness of the disease in animals should be emphasized (Hassan *et al*., 2011). When at-risk communities have a heightened awareness of RVF symptoms in animals they may be able to improve surveillance and identify an outbreak early. Jost et al. conducted focus groups with pastoralists in Kenya and Tanzania during the 2006-2007 RVF outbreak and found that pastoralists not only recognized changes in weather patterns and mosquito swarms but also recognized RVF signs among animals and humans (Jost *et al.*, 2010).

Accordingly in the current study, the majority of the participants 231(94.67%) were responded that they had observed livestock diseases outbreak in their herds/locality. Among those that replied they had observed the disease outbreak in their herd, even if we didn’t ask the participants by directly naming RVF, 151(61.89%) specifically replied that they had seen the sign of mass abortion in their herds and 168(68.85%) responded that there was mass young mortalities. Storm abortions in adult animals and high mortality of young animals have previously been reported to be alarming signs of RVF outbreak (Glyn Davies and Martin, 2003). The most common clinical signs recognized in this study were coughing and diarrhea; however, this is a rare symptom of RVF.

Among 244 participants of this study,151(61.8%) participants replied they had seen the sign of mass abortion in their herd, 52(21.31%) in Sheep and goats, 46(18.85%) in Goats, 14(5.74%) in Sheep, 14(5.74%) in cattle, Sheep and Goats, whereas small proportion 2(0.82%) also reported the case in camel. This is in agreement with the study by Budasha *et al*. the overall prevalence of abortion was 17.4% and was highest in sheep (7.8%). Intra-species abortion rate was lowest in cattle (9.7%), highest in sheep (24%) and intermediate (18.8%) in goats (Budasha *et al*., 2018).

In this study 130(53.28%) participants answered that there was the history of heavy rainfall that precedes the event of mass abortion, whereas 114(46.72%) replied that there was no history of heavy rainfall that precedes the event of mass abortion. It has been observed in many studies that RVF outbreaks and endemicity are closely linked to heavy rainfall (Alert, 2009).

It was also mentioned in many studies that high numbers of mosquitoes emerged because of high rainfall (Alert, 2009). A study condcuted in Kenya by Owange *et al*. (2014) pastoralists felt that mosquitoes were a very important risk factor for RVF transmission in cattle Likewise, even if our study did not ask directly whether mosquito was the vector of RVF, among 244 participants of the survey, 203(83.20%) responded that they had seen huge mosquito population (swarms) during summer and spring, this implies that huge mosquito population can pose an important risk for RVF introduction.

139(56.97%) participants of the the current study responded that there is transboundary movements of animals. The expansion of the disease since the early 20th century in Africa is most likely due to the movement of infected livestock (Taylor *et al.*, 2016). Introduced breeds of highly susceptible cattle, sheep and goats that have then provided suitable conditions for the rapid amplification of the RVFV in hosts and vectors, aimed at communities with low resilience. Moreover, the limited flight capacity of the main arthropod vectors makes livestock movements a very important factor of expansion (Chevalier *et al.*, 2004).

151(61.8%) participants responded that they had seen the sign of mass abortion in their herd, 168(68.85%) answered that there was mass young mortalities, 130(53.28%) answered that there was the history of heavy rainfall that precedes the event of mass abortion, the storm of abortion, mortality of young animals, history of heavy rainfall that precedes the event of mass abortion generate suspicious that the disease might have once occurred in the study area during the historical epizootic in neighboring countries, and history of transboundary movement of livestock synergized with high numbers of mosquitoes and as revealed by risk modeling, indicated that there is a time bomb threat and the potential suitability of RVF in the current study area.

5.2. Mosquito vector of RVF Dispersion Model

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 mosquito dispersion model provided valuable information on the geographic suitability of habitat for RVF mosquito vector 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 RVFV vector mosquitoes dispersal model of this study also provides valuable information on the spatial suitable habitat for RVF mosquito vector occurrences, The current model maps showed that Ethiopia has wide suitable area found on the periphery of the country that borders with RVF endemic countries, these areas are mostly lowlands. Lowland area is the most suitable habitat for RVFV vectors (Ochieng *et al*., 2016). The model showed all regions have small to wide range of suitable area. Somali region, Oromia region lowland areas of Borana, Bale, and Guji, South western parts of SNNPR, major part of Gambella region, major parts of Benshangul-Gumuz region and western part of Oromia region has wide-ranging suitable area for RVFV vector mosquito distribution. Many of these areas found along the periphery of the country that shares boundary with diseases endemic countries like Kenya, South Sudan and Somalia. Southern Afar that close to the central Ethiopia and western part of Amhara region have also moderate suitable areas for RVFV vector mosquito. While, wide ranging areas of central Ethiopia and Tigrai region were less suitable according to the current study model.

By this study among 11 explanatory variables, ensemble model shows that, the highest overall contribution were made by water Vapor pressure (54.4%), followed by Harmonized World Soil Database(HWSD) (29.9%), while Elevation were contributed the least 1%.

The RVF virus circulation has been reported in several eco-climatic areas (Tran *et al*., 2016) where several report is from sub-humid parts of East African countries. In Ethiopia based on satellite data there are wide sub-humid eco-zone and suitable factors for amplification, spread and occurrence of RVFV (Tran *et al*., 2016). High relative humidity is one factor 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). In the current model development water vapor pressure which means relative humidity was found the leading factor contributing 54.4 % in model development. This means that relative humidity determine distribution of the RVFV vector mosquitoes than the other factors.

It is clear 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 which is 29.9%, in agreement 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.

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). However the model of this study indicates that Elevation was contributed the least 1%.

Even if ensemble model of this study shows that livestock population only contributes about 2.7%, Livestock populations play major 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). This result go in hands with the report by Tylor *et al*, that states The expansion of the disease since the early 20th century in Africa is most likely due to the movement of infected livestock (Taylor *et al.*, 2016). 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). Also 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 (5%) 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.

This study was conducted to define the occurrence and distribution of competent RVFV vectors in Borana, to detect virus in the vector, develop a mapping of high-risk area with modeling and assessemt of Peoples knowledge regarding the disease. it provided good information Nevertheless, there are some limitations. The laboratory part could not accomplished due to COVID-19 and also In the current mosquito survey, very low volume and diversity of mosquitos were collected. Neverthless, results of this study suggest that the disease might have once occurred in the study area during the historical enzootic periods in neighboring country or the occurrence of RVF is inevitable in almost all regions of the country which also includes current study area. Thus, we believe that the results of this study are very useful and greatly assist in early warning, risk-based surveillance, prevention and control of the disease.

6. CONCLUSION AND RECOMMENDATIONS

In Ethiopia, clinical RVF has never been detected and reported. However, our study based on questionary survey and ensemble model highlights that Rift valley fever is historically either once upon the time has occurred in the study areas of Borana. This generalization was come from the study participants response on the clinical signs that particularly indicate the disease and also mosquito dispersal modeling also confirms potential suitability of RVF occurrence in current study area. Considering the geographical nearness of the study area Borana zone to RVF endemic country Kenya, synergized with the nature of livestock movements across the international border lead to the conclusion that Ethiopia in general and Borana zone in particular will always be exposed to clinical RVF during the epizootic periods of the diseases in the Horn of Africa. Based on this conclusion the following points are recommended.

- The risk assessment of the introduction of RVF into Ethiopia should be carried out, as per the guidance of RVF Contingency and preparedness plan for Ethiopia.

- The epidemiological and entomological investigation, gathered by active surveillance targeted to define the range of primary and secondary RVF vector species and likely density should be carried out.

- All proficient mosquito traps with variety of sampling technique to capture enough pool of mosquitoes should be carried out.

- Molecular detection of RVFVin the mosquito vectors and serological study of the virus from the host is recommended should be performed.

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8. ANNEX

Annex 1: Questionary

Addis Ababa University

College of Veterinary Medicine and Agriculture

RVF disease KAP assessment in Borena, Ethiopia

I	**Herdsman background**
1	Locality where herdsman live? Village or kebele_____________/District___________________/Zone_______________
2	Sex : Male Female
3	Age of the herdsman? ☐<20 ☐B/n 20 &30 ☐B/n 30&40 ☐B/n 40&50 ☐ >50
4	Educational status of the respondent? ☐ Illiterate ☐ Elementary ☐High school ☐College and University
5	Marital status of the respondent? ☐ Single ☐ Married ☐ Divorced
6	Lifestyle respondent: ☐ nomadic pastoralist ☐ transhumance pastoralist ☐ sedentary pastoralist ☐ agrarian
7	Main source of income for your livelihood? ☐ Salary ☐ self-business ☐ Sale of animal products ☐ Live animal sale ☐ Other Agricultural production, specify………….. ……..
8.	Are you rearing livestock? ☐ Yes ☐ No
9.	If 'yes' the above question, which species you keep? ☐ Cattle ☐ Sheep ☐ Goat ☐ Camels ☐ Equine
10	The purpose of rearing animals? ☐ Meat ☐ Milk ☐Draught power ☐ Sale
11.	How many the following animals you kept? ☐ Cattle: ☐<20 ☐ 21-40 ☐41-80 ☐ 80-120 ☐ 121-200 ☐ >200 ☐ ☐ Goat: ☐ <20 ☐ 21-40 41-80 ☐ 80-120 ☐120-200 ☐ >200 ☐ ☐ ☐ ☐ ☐ ☐ ☐ Sheep: ☐ <20 21-40 41-80 80-120 120-200 >200 ☐ Camels: <20 ☐ 21-40 ☐ 41-80 ☐ 80-120 ☐ >120
II	**Disease related diseases**
1.	Have you ever observed diseases outbreak in your herds/locality? ☐ Yes ☐ No
2.	If 'yes' question no.1 above, what signs and/or symptoms you observed during the event? ☐ sudden death ☐ abortion ☐ coughing ☐ salivation ☐ ☐

	depression lacrimation ☐ diarrhea ☐ colic ☐ others, please specify……………………
3.	Have you seen the sign of mass abortion in your herd? ☐ Yes ☐ No
4.	If ‘yes’ question no. 3 above, in which species of animals? ☐ Cattle ☐ Sheep ☐ Goat ☐ Camels ☐ Equine ☐ Dog and cat
5.	If ‘yes’, question no. 3, in which season such event happened? ☐ Winter ☐ Autumn ☐ Summer ☐ Spring
6.	If in ‘summer or spring’ seasons, was there history of heavy rainfall before the event? ☐ Yes ☐ No
7.	Have seen mass of young animal mortalities in your herd? ☐ Yes ☐ No
8.	If ‘yes’ question no. 7 above, in which species? Calves (cattle) ☐ kid ☐ lamb ☐ Calves(camel) ☐ foal ☐ puppy
9.	What clinical sign you have seen during young death storm? ☐ Sudden death ☐ Coughing ☐ Depression ☐ Lethargy ☐ Diarrhea ☐ Vomiting ☐ Nasal discharge
III	**Driving factors**
8.	Have ever seen huge mosquito population (swarms) during summer and/spring? ☐ Yes ☐ No
9.	Do you know that Mosquito can transmit diseases from Animal to Animal? ☐ Yes ☐ No
10.	If yes question no. 8 above, were there any laying water in your surrounding? ☐ Yes ☐ No
11.	Are there movements of animals from one site/locality to another within the country? ☐ Yes ☐ No
12.	Are your animals’ transits into neighbor countries for grazing and/or search of feed? ☐ Yes ☐ No
13.	Is there any occasion where your herds/flocks mix with herds/flocks from neighbor country/ies? ☐ Yes ☐ No
14.	Is there common grazing land in your surrounding? ☐ Yes ☐ No
15.	Is there history of heavy rainfalls for 1-2 weeks which led to flooding in your surrounding? ☐ Yes ☐ No
16.	Have you ever observed 1-2weeks laying water associated with flooding from heavy rainfall in your localit☐ Ye☐ No

Annex 2: Entomological survey form

Addis Ababa University
College of Veterinary Medicine and Agriculture
Entomological survey form

Region________________ District__________________Kebele/Village/Lake/Specific name of collection localities________________

Phone number____________ Signiture__________Name of collector______________

Purpose of collection____

____

____

____

____

Collection code (trap no.)	**Type of trap**	**Location(geo-referenced)**			**Type of habitat**	**Meteorological data**				**Date and time of trap**	
		Long	**Lat**	**Alt**		**T(°C)**	**H(%)**	**Wind speed**	**Prec (mm^3)**	**Trap fixing**	**Trap collection**

Annex 3: 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

Dr Getachew Terefe
Chairman

Signature

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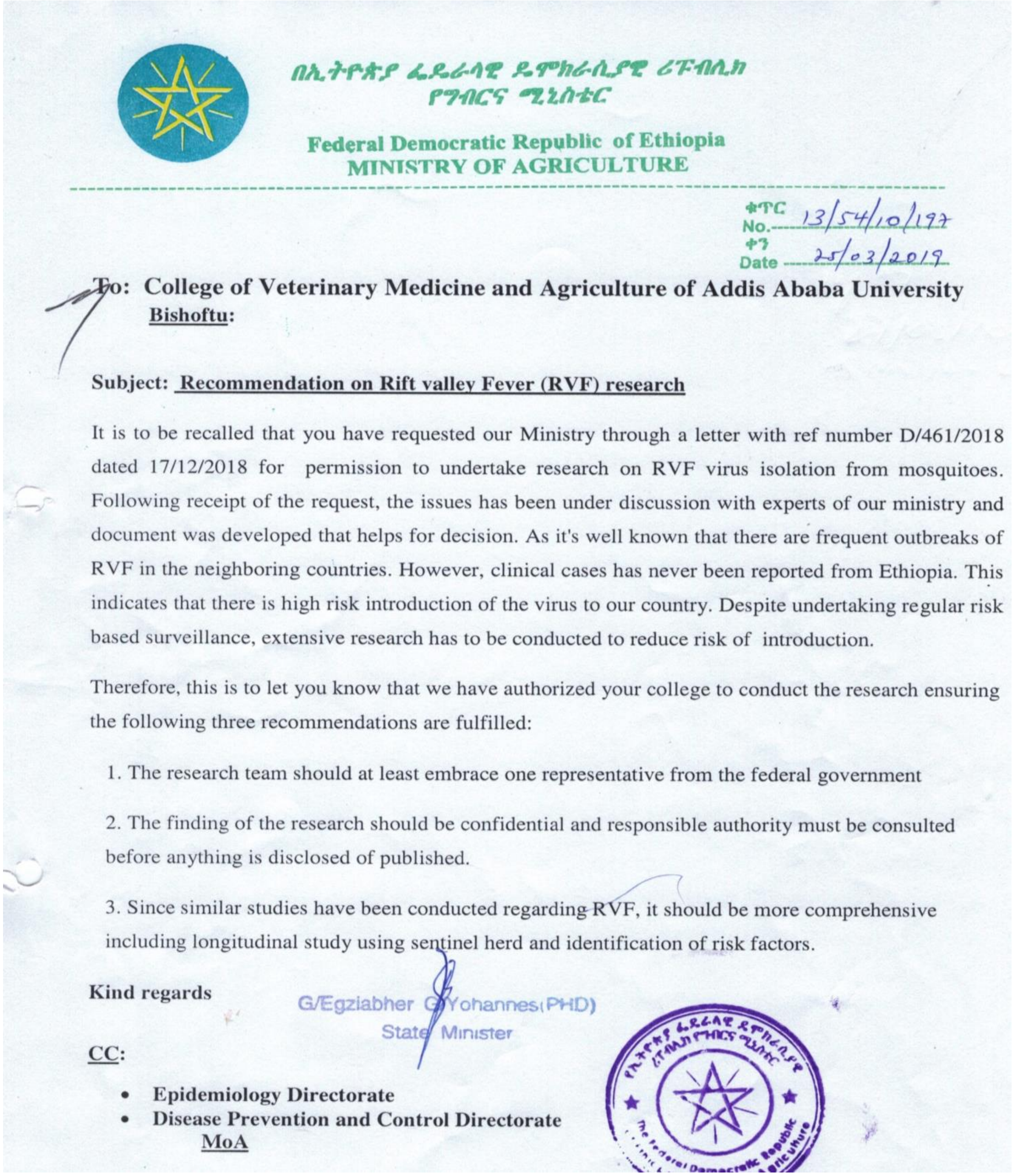
Please quote Our Ref. No. When replying

ፋክስ} Fax 251-11-4339933
ስልክ } Tel. +251 114338450
ፖ.ሣ.ቁ P.o.x. Box}34
ቢሾፍቱ/ደብረዘይት፤ ኢትዮጵያ Bishoftu/Debre Zeit, Ethiopia

Annex 4: *Ministry of Agriculture recommendation on Rift Valley Fever (RVF) research*



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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

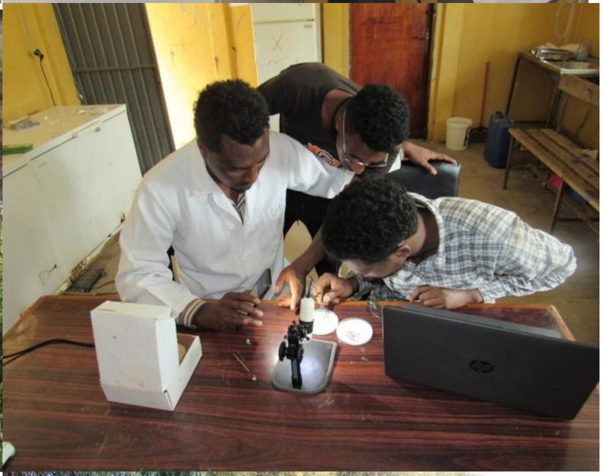
CC:

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

MoA

Annex 5: Photo Gallery



Annex 6: R Script

```
setwd("~/")
library(biomod2)
Riskmap <- read.csv("C:/Users/user/Desktop/Model data/Riskmap.csv")
myRespName <- 'Occurrences'
myResp <- as.numeric(Riskmap[,myRespName])
myRespXY <- Riskmap[,c("Long","Lat")]
##rasterstack
myEpl<-stack("C:/Users/user/Desktop/Model data/Elevation.tif",
        "C:/Users/user/Desktop/Model data/Globcover.tif",
        "C:/Users/user/Desktop/Model data/HWSD.tif",
        "C:/Users/user/Desktop/Model data/Livestock.tif",
        "C:/Users/user/Desktop/Model data/NDVI.tif",
        "C:/Users/user/Desktop/Model data/prec.tif",
        "C:/Users/user/Desktop/Model data/tmax.tif",
        "C:/Users/user/Desktop/Model data/tmin.tif",
        "C:/Users/user/Desktop/Model data/srad.tif",
        "C:/Users/user/Desktop/Model data/vapr.tif",
        "C:/Users/user/Desktop/Model data/wind.tif")
#### checking  for multicollinearity problems using the variance inflation factor

## checking multicollinearity problems using the variance inflation factor
##library(usdm)
##vif(myEpl)  # calculates vif for the variables in ExplVar
##vifcor(myEpl, th=0.9) # identify collinear variables that should be excluded
#vifstep(myEpl, th=10) # identify collinear variables that should be excluded

####################BIOMOD_FormatingData#############################

2.biommodformating
myBiomodData <- BIOMOD_FormatingData(resp.var = myResp,
                       expl.var = myEpl,
                       resp.xy = myRespXY,
                       resp.name = myRespName,
                       PA.nb.absences = 1000,
                       PA.nb.rep = 1,
                       PA.strategy = 'sre',
                       PA.sre.quant = 0.025)
##myBiomodData
# 3. Defining Mododelling options as default options
myBiomodOption<-BIOMOD_ModelingOptions()

## 4. Computing the models
```

```
myBiomodelOut<-BIOMOD_Modeling(
  myBiomodData,
  models = c('MAXENT.Phillips','RF', 'GAM','GLM', 'GBM',  'CTA', 'ANN', 'SRE', 'FDA'
, 'MARS'),
  models.options = myBiomodOption,
  NbRunEval=3,
  DataSplit=80,
  VarImport=3,
  models.eval.meth = c('KAPPA','ROC', 'TSS'),
  SaveObj = TRUE,
  rescal.all.models = TRUE,
  do.full.models = FALSE,
  modeling.id = paste(myRespName,"FirstModeling",sep=""))
# models scores
get_evaluations(myBiomodelOut)
#variable contribution
get_variables_importance(myBiomodelOut)
##4.Ensemble modeling

myBiomodEM <- BIOMOD_EnsembleModeling(modeling.output = myBiomodelOut,
                                      chosen.models = 'all',
                                      em.by='all',
                                      eval.metric = c('TSS'),
                                      eval.metric.quality.threshold = c(0.8),
                                      prob.mean = T,
                                      prob.cv = T,
                                      prob.ci = T,
                                      prob.ci.alpha = 0.05,
                                      prob.median = F,
                                      committee.averaging = T,
                                      prob.mean.weight = F,
                                      prob.mean.weight.decay = 'proportional',
                                      VarImport = 3)
# models scores
get_evaluations(myBiomodEM)
get_variables_importance(myBiomodEM)
##5. Projecting the models over studied area
myBiomomodProj <- BIOMOD_Projection(modeling.output = myBiomodelOut,
                                    new.env = myEpl,
                                    proj.name = 'current',
                                    selected.models = 'all',
                                    binary.meth = 'ROC',
                                    filtered.meth = NULL,
                                    compress = 'xz',
```

```
                            build.clamping.mask = T,
                            do.stack=F,
                            output.format = '.img',
                            on_0_1000=T)
########################################################################
######
Ensemble projections of species over space
myBiomomodEF <- BIOMOD_EnsembleForecasting(EM.output = myBiomodEM,
                                  projection.output = myBiomomodProj)
```

RESULT

```
> setwd("~/")
> library(biomod2)
> Riskmap <- read.csv("C:/Users/user/Desktop/Model data/Riskmap.csv")
> myRespName <- 'Occurrences'
> myResp <- as.numeric(Riskmap[,myRespName])
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+            "C:/Users/user/Desktop/Model data/Livestock.tif",
+            "C:/Users/user/Desktop/Model data/NDVI.tif",
+            "C:/Users/user/Desktop/Model data/prec.tif",
+            "C:/Users/user/Desktop/Model data/tmax.tif",
+            "C:/Users/user/Desktop/Model data/tmin.tif",
+            "C:/Users/user/Desktop/Model data/srad.tif",
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+            "C:/Users/user/Desktop/Model data/Livestock.tif",
+            "C:/Users/user/Desktop/Model data/NDVI.tif",
+            "C:/Users/user/Desktop/Model data/prec.tif",
+            "C:/Users/user/Desktop/Model data/tmax.tif",
+            "C:/Users/user/Desktop/Model data/tmin.tif",
+            "C:/Users/user/Desktop/Model data/srad.tif",
+            "C:/Users/user/Desktop/Model data/vapr.tif",
```

```
+           "C:/Users/user/Desktop/Model data/wind.tif")
> #### checking  for multicollinearity problems using the variance inflation factor
>
> ## checking multicollinearity problems using the variance inflation factor
> ##library(usdm)
> ##vif(myEpl)  # calculates vif for the variables in ExplVar
> ##vifcor(myEpl, th=0.9) # identify collinear variables that should be excluded
> #vifstep(myEpl, th=10) # identify collinear variables that should be excluded
>
> ####################BIOMOD_FormatingData#####
>
> ###2.biommodformating
> myBiomodData <- BIOMOD_FormatingData(resp.var = myResp,
+                            expl.var = myEpl,
+                            resp.xy = myRespXY,
+                            resp.name = myRespName,
+                            PA.nb.absences = 1000,
+                            PA.nb.rep = 1,
+                            PA.strategy = 'sre',
+                            PA.sre.quant = 0.025)
##myBiomodData
> # 3. Defining Mododelling options as default options
> myBiomodOption<-BIOMOD_ModelingOptions()
>
> ## 4. Computing the models
>
>
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+   myBiomodData,
+   models = c('MAXENT.Phillips','RF', 'GAM','GLM', 'GBM',  'CTA', 'ANN', 'SRE', 'FD
A', 'MARS'),
+   models.options = myBiomodOption,
+   NbRunEval=3,
+   DataSplit=80,
+   VarImport=3,
+   models.eval.meth = c('KAPPA','ROC', 'TSS'),
+   SaveObj = TRUE,
+   rescal.all.models = TRUE,
+   do.full.models = FALSE,
+   modeling.id = paste(myRespName,"FirstModeling",sep=""))

> # models scores
> get_evaluations(myBiomodelOut)
> #variable contribution
> get_variables_importance(myBiomodelOut)
##4.Ensemble modeling
```

```
>
> myBiomodEM <- BIOMOD_EnsembleModeling(modeling.output = myBiomodelOut,
+                              chosen.models = 'all',
+                              em.by='all',
+                              eval.metric = c('TSS'),
+                              eval.metric.quality.threshold = c(0.8),
+                              prob.mean = T,
+                              prob.cv = T,
+                              prob.ci = T,
+                              prob.ci.alpha = 0.05,
+                              prob.median = F,
+                              committee.averaging = T,
+                              prob.mean.weight = F,
+                              prob.mean.weight.decay = 'proportional',
+                              VarImport = 3)
> # models scores
> get_evaluations(myBiomodEM)
> get_variables_importance(myBiomodEM)
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+                            new.env = myEpl,
+                            proj.name = 'current',
+                            selected.models = 'all',
+                            binary.meth = 'ROC',
+                            filtered.meth = NULL,
+                            compress = 'xz',
+                            build.clamping.mask = T,
+                            do.stack=F,
+                            output.format = '.img',
+                            on_0_1000=T)

> #Ensemble projections of species over space
> myBiomomodEF <- BIOMOD_EnsembleForecasting(EM.output = myBiomodEM,
+                                  projection.output = myBiomomodProj)
```