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A SPATIAL DISTRIBUTION MODELING OF WEST NILE FEVER VECTORS IN THE GENUS *CULEX* IN THE HORN OF AFRICA

MVSc THESIS

BY

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BISHOFITU, ETHIOPIA

A SPATIAL DISTRIBUTION MODELING OF WEST NILE FEVER VECTORS IN THE GENUS *CULEX* IN THE HORN OF AFRICA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology

By

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Addis Ababa University

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As MVSc research advisors, we certify that we have read and evaluated this thesis prepared under our guidance by **TSEGA BERHE** titled**: A Spatial Distribution Modeling of West Nile Fever Vectors in the Genus *Culex* in the Horn of Africa.**

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DEDICATION

This thesis manuscript is dedicated to my husband, Belayhun Bezabih and my mother, Aster Atara for having with affection and love and for their keen partnership in the success of my life.

STATEMENT OF AUTHOR

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

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ABBREVIATIONS

ANN	Artificial neural networks
CA	Committee averaging
CDC	Centers for Disease Control and Prevention
CTA	Classification tree analysis
CV	Coefficients of variation
Cx	*Culex*
DNA	Deoxyribonucleic acid
ESA	European Space Agency
FDA	Flexible discriminant analysis
GAM	General additive models
GBIF	Global Biodiversity Information Facility
GBM	General boosted models or boosted regression trees
GIS	Geographic information systems
GLM	General linear models
GPS	Global Positioning System
MARS	Multiple adaptive regression splines
MAXENT	Maximum entropy
MODIS	Moderate Resolution Imaging Spectroradiometer
NADHIC	National Animal Health Diagnostic and Investigation Center
NAMA	National Mereology Agency
NVI	National Veterinary Institute
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
RF	Random forests
RT-PCR	Reverse transcriptase-polymerase chain reaction
SDM	Species Distribution Model
SNNPR	Southern Nations Nationalities and Peoples Region
SRE	Surface Range Envelope
TSS	True Skills Statistics

LIST OF ABBREVIATIONS (Continued)

VBD	Vector Born Disease
WHO	World Health Organization
WNF	West Nile Fever
WNV	West Nile Virus
WRBU	Walter Reed Biosystematics Unit

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ABSTRACT

Many species under *Culex* mosquito are common vectors for West Nile Virus (WNV) and distribution of this disease is influenced by biological and physical variations. Spatial modeling of arbovirus mosquito in East African countries becomes influential due to increased frequency outbreaks and emergence. A study on a spatial distribution modeling of the West Nile Fever Vector in Ethiopia was conducted from May 2018 to June 2019. The collected *Culex (Cx)* species were *Cx. pipiens* (38.5%), *Cx. univitattus (29.1%), Cx. antennatus* (11.6%), *Cx. quinquefasciatus* (11%) and other *Culex* species (10.3%), potential vector species for WNV. WNV isolation was performed using Conventional one step reverse transcriptase-PCR (RT-PCR) with specific primer-WNV-F_2 and WNV-Rev_2. Occurrence data was obtained from Global Biodiversity Information Facility (GBIF) website and field sampling was also done in Mid Rift Valley and Southern Ethiopia. The models were created with a set of environmental predictors including climatic data, topographical, land cover, human, and livestock population count. Individual models and ensemble prediction was made using R package Biomod2. With individual models, overall average models agreed in predicting probability *Culex* occurrence highly contributed by soil type (49%) and precipitation (46.5%) but land cover had lowest contribution (13%). In individual models evaluation resulted eight of the total 10 models proved reliable estimations on True skills statistics (TSS≥0.8) with highest value were GLM (TSS=0.932), MARS (TSS=0.925) and RF (TSS=0.919) but result of SRE and MAXENT was found with poor TSS. An ensemble model evaluated using TSS metrics among the 30 models, the ensemble model included TSS ≥0.8. Soil type (37%) and precipitation (31.5%) were with high contribution but solar radiation (5.5%) had lowest contrition. Visualization of predicted probability occurrence of *Culex* showed high probability of occurrence in Oromia followed by SNNP region. Vector distribution varied from location to location depending to on their environmental preferences. Therefore, further investigation was essential on modeling of spatial and temporal situation of both vector and the disease.

Key words: *Culex, Ethiopia, Occurrence, Spatial modeling, West Nile Virus*

1. INTRODUCTION

West Nile Virus (WNV) is one of the most occurring arthropod-borne virus (arboviruses) over extensive geographical range and birds, equine and human working as vertebrate hosts and mosquitoes as vector (Colpitts *et al*., 2012). The virus appears to be maintained in endemic foci in the African continent and is moved yearly to temperate climates (McLean *et al*., 2002).

WNV is principally transmitted by mosquitoes of the genus *Culex* (WHO, 2017). From this genus, *Culex pipiens* complex species are considered as potential carrier which are widely distributed species of mosquito in the world (Amraoui *et al*., 2012). The typical form being found in temperate regions and the subspecies, fatigans, in the tropics (Barr, 1967). According to the proposed system of mosquito vector categorization that included natural infection, vector competence and field vector-host contact; *Cx. univitattus, Cx. antennatus, Cx. tritaeniorhynchus, Cx. modustus, Cx. neavei, Cx. poicilipes, Cx. decens* also accepted as potential vectors for WNV(Couissinier-Paris, 2006; Monini *et al.*, 2010; Olivia *et al*., 2015;Tantely *et al*., 2017).

Culex transmitted WNV is commonly found in Africa, Europe, Middle East, North America and West Asia (WHO, 2017). Historical evidence of WNV in Africa reported around different countries such as Uganda, Sudan, Congo, Kenya and other African countries(WRBU, 2014; Sayed-Ahmed, 2016).

There are different species of *Culex* distributed in Africa; research on experimental transmission about the disease and its vector reported *Cx. pipiens* as major carrier for the disease. The other reported vector for this disease were *Cx. antennatus* and *Cx. univittatus* (Sule *et al*., 2018). From the East and Horn African context, in Kenya mosquito born arbovirus surveillance isolated various *Culex* species that were carrier of WNV (Ochieng *et al*., 2013). Circulation of WNV and its vector *Cx. pipiens* and *Cx. antennatus* reported in different areas in Egypt (Soliman *et al*., 2010). The *Cx.*

quinquefasciatus and other *Culex* species were reported in Tanzania (Thornton *et al*., 2016). In Ethiopia, WNV is one of the listed diseases from the identified zoonotic diseases (Pieracci *et al*., 2016). Seropositive human case was reported and the virus was detected from *Culex* mosquito species (Berger, 2018).

Identification of these mosquito species can be done using different entomological procedures. Initial entomological study is conducted using morphological identification and it is influenced by stage of mosquito and their collection methods. Depending on surveillance plan, *Culex* mosquitos can be collected using various traps such as BG-Sentinel mosquito traps (ECDC and EFSA, 2018), CDC, UV light trap and others (Muturi *et al*., 2007 ; Toma *et al*., 2008; Kline *et al*,. 2018). For further identification at species level different PCR techniques are used at molecular level (Rozo-Lopez and Mengual, 2015; Vogels 2017).

Climate change and global warming are influencing factors in the transmission dynamics of WNV (Kamel, 2015; Lima-Camara, 2016). Rainfall, elevation, temperature, vegetation, land cover and precipitation are major principal factors that affect populations of *Culex* species as well as increased mosquito infestation (Ruizl *et al*., 2010; Mughini-Gras *et al*., 2013; Rechards *et al*., 2015; Yoo *et al*., 2016). Different species under *Culex* are influenced by various geographical ranges. Species distribution marked areas like stagnant water, aquatic, irrigated cropland and deciduous forest, urban environments (Gu *et al*., 2006; Frake, 2014).

A spatial distribution of diseases is influenced by biological and physical variations. Studies can investigate distribution of diseases, causes and significances of spatial variation in disease (Klingseisen, 2010). Spatial data can be studied using geographic information systems (GIS)(Jamison *et al.,* 2015) and growing number of studies have provided evidence indicating the effects of climate variability on vector-borne diseases on spatial model (López *et al*., 2018). The model helps to bring a real situation of arbovirus vectors including mosquitos by developing Species Distribution Models

(SDMs) as numerical tools that combine observations of species occurrence with environmental estimates (Ames, 2016).

There are various spatial modeling studies conducted in different areas of East Africa such as spatial analysis of arboviral disease as well as modeling of spatial distribution of *Culex* mosquito (Grossi-Soyster *et al.,* 2017; Uusitalo, 2017), *Cx. pipiens* and other mosquito species distribution modeling was conducted by focusing on temperate, rainfall and forested habitats (Conley *et al*., 2014). Mapping distributions of *Culex pipiens* complex as well as prediction of environmentally suitable areas were conducted using species occurrence data (Clement *et al*., 2013).

The situation in Ethiopia showed that arboviral disease and its mosquito vector distribution investigated by different researchers. These researchers investigated breeding habitat of *Aedes* and *Culex* mosquitoes as potential vectors for arbovirus (Getachew *et al.,* 2015*;* Lilay *et al.,* 2017; Ferede *et al.,* 2018). WNV was grouped under Ethiopian zoonotic disease lists (Pieracci *et al*., 2016) and occurrence of *Culex* (Berger, 2018) but there is still clear gap as well as lack of research output on spatial modeling of mosquito species and identification of major carrier mosquitos for WNV. Therefore, the following objectives were driven to tackle the described problems and will provide solutions for decision makers.

General Objective

- To develop species distribution model for West Nile Fever virus vectors in the genus *Culex* for the Horn of African countries.

Specific objectives

- To map the distribution of West Nile Fever virus vectors in the genus *Culex* for the Horn of African countries
- To determine the ecological requirement of *Culex* mosquitoes and the influence of environmental factors on the distribution of these mosquitoes.
- To detect WNV from the collected *Culex* mosquitoes.

2. LITERATURE REVIEW

2.1. Arbovirus diseases and their vectors

Arboviruses are arthropod-borne viruses composing a diverse group with similar ecology and maintenance mechanisms (Weaver, 2006). Depending on the vector and virus competence the arboviruses are classified in to three main families of RNA viruses

Table 1: Arboviral infection of human and animals

Arboviral disease	**Virus family**	**References**
WNV	*Flaviviridae*	(OIE, 2009; Clopitts *et al*., 2012).
Dengue virus	*Flaviviridae*	(Clopitts, *et al.*, 2012).
Yellow fever virus	*Flaviviridae*	(BCM, 2018).
Zika virus	*Flaviviridae*	(Braack *et al*., 2018).
Rift Valley fever virus	*Bunyaviridae*	(Shope, 1996; Fontenille *et al.*,1998).
Chikungunya	*Togoviridae*	(Fontenille *et al.*, 1998).

The existence of arboviruses involves competent insect vectors that blood-feed on vertebrate hosts such as humans, livestock, and wild animals. The vector competence and disease transmission is affected by the various environmental variables such as climatic conditions, vegetation and topography which describe the vector and host habitat structure (Klingseisen, 2010). Arboviruses can have significant threat to human and animal health at global level (Mayer *et al*., 2017). There are hundreds of viruses that share a common characteristic of being transmitted by arthropods commonly hematophagous mosquitoes (McFee, 2018). Mosquitos become a carrier of these viruses and the virus can multiply within the tissues to produce in the salivary glands and are then passed on to vertebrate hosts such as humans and animals by the bites (Pfeffer and Dobler, 2010). The most prominent mosquito-borne diseases are transmitted by three genera of mosquitoes such as *Aedes*, *Culex*, and *Anopheles (*Kenney and Brault, 2014; Schorderet-Weber *et al*., 2017*).*

2.2. Mosquito and Mosquito born arbovirus

Mosquitoes are carriers of several diseases. It found in Culicidae family, comprise a monophyletic taxon belonging to order Diptera represent a huge threat for millions of humans and animals worldwide, since they act as vectors for important parasites and pathogens, including malaria, filariasis and threatening arboviruses, such as West Nile, Dangue and Zika virus (Pavela and Benelli, 2016). A total of 3,559 species of *Culicidae* are currently recognized (MTN, 2018) and recorded as large and abundant family that occurs throughout temperate and tropical regions of the world, predominantly in tropic climate. Mosquitos differ from the other biting Diptera in having slender, long-legged insects that are easily recognized by their long proboscis and the presence of scales on most parts of the body (Harbach, 2007).

There are around 41 genera of mosquitos recorded in the world. Apart from this, *Aedes* and *Culex* are responsible for the transmission of many arboviruses (Kenney and Brault, 2014). The mosquito population dynamics is mainly depends on rainfall, humidity and temperature. Heavy rainfall can lead to high flooding and accessibility of breeding habitats that prompt mass hatching of flood water mosquitoes' eggs (Sang *et al*., 2017).

2.3. *Culex* Mosquitoes and West Nile Virus

There are over 534 viruses are listed under arbovirus disease, above 130 of them linked to human and animal health importance which transmitted principally by mosquitoes (Gubler, 2001). These disease are distributed throughout the world such as epidemic mosquito-borne arboviruses such as yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), chikungunya virus (CHIKV) and Zika virus (ZIKV), have emerged in temperate and tropical during recent centuries. Determinants of these arbovirus emergence and dispersal have an anthropological basis, arthropod vectors and their feeding preferences (Goulda *et al*., 2017). There are various factors can influence the worldwide distribution of these types of arbovirus diseases and their vectors, such as increasing climate change, urban development and human population growth, poor waste

management, increased vector density, and enlarged international travel and trade(Yuill, 1986; Kenney & Brault, 2014).

WNV is mosquito-borne arbovirus and first isolated from a febrile patient from the West Nile district of Northern Uganda in 1937 (Smithburn, *et al*., 1940; Sejvar, 2003). It is caused by flavivirus that affect human, equine, and avian neurological system. The virus is indigenous to Africa, Asia, Europe, and Australia, Russia, and America. Birds are the natural reservoir (amplifying) hosts, and the virus is maintained in nature in a mosquito-bird-mosquito transmission cycle (Campbell *et al*., 2002).

It is estimated that over 150 species of mosquitoes have been known to carry the virus (VDCI, 2013). Beside from this, mosquitoes of the genus *Culex* are generally considered as the principal vectors of WNV (Barr, 1967; Reiter, 2010). *Cx. Pipiens* and *Cx. quinquefasciatus* working as the dominant bridge vectors that are distributed in America, Europe, Africa and Middle east countries (SMS, 2007; Monini *et al.,* 2010; VDCI, 2013). The other investigated culex species; *Cx. univittatus, Cx. antennatus Cx. poicilipes, Cx. neavei, Cx. decens, Cx. modestus, Cx. nigripalpus, Cx. salinarius, Cx. tritaeniorhynchus, and Cx. vishnui* working as potential vectors (Hubálek and Halouzk, 1999; Couissinier-Paris, 2006; Olivia *et al*., 2015; Tantely *et al*., 2017).

Distribution and abundance of mosquitoes are known to be dependent on various biotic and abiotic factors (Beketov *et al.*, 2010). Major *Culex* species of WNV are highly associated with environmental conditions, temperature, precipitation and humid environment reported as main influencing factors for mosquito species abundance (Stilianakis *et al*., 2016). On other hand, major landscape predictors and urban situation, including land-use type, population density elevation stagnant rivers, lake sides, streams are prevalent and artificial containers of water are numerous along with high numbers of catch basins, and these provide habitats for different *Culex* species (Yoo *et al*., 2016; Asigau and Parker, 2018; Moise *et al*., 2018).

2.4. *Culex* mosquito and WNV in East African zone

West Nile Virus (WNV) was first isolated in the West Nile district of Uganda in 1937. It was identified in birds in Nile delta region in 1953 (WHO, 2017). The role of mosquitoes in viral transmission was clearly outlined in Egypt in the 1950s then the disease appeared for the first time in the Western Hemisphere, most likely through the importation of an infected bird(WHO, 2017; OIE, 2018).

The virus evolved into two distinct lineages in sub-Saharan Africa (SSA) that subsequently spread to most continents (Sule *et al.,* 2018). There are various studies about the disease and its vectors were conducted in East African and other African countries. Studies in Kenya and Tanzania identified the potential for emergence of viral diseases, identified high risk areas based on the densities of vectors and with their species on spatial distribution (Lutomiah *et al.,* 2013; Dida *et al.,* 2018).

According to a recent record, in East Africa there are about 45 different culex mosquito species abundant and have ability to carry various diseases (WRBU, 2014). Evidence from different African countries (Figure 1) showed the disease distribution across the East African regions.

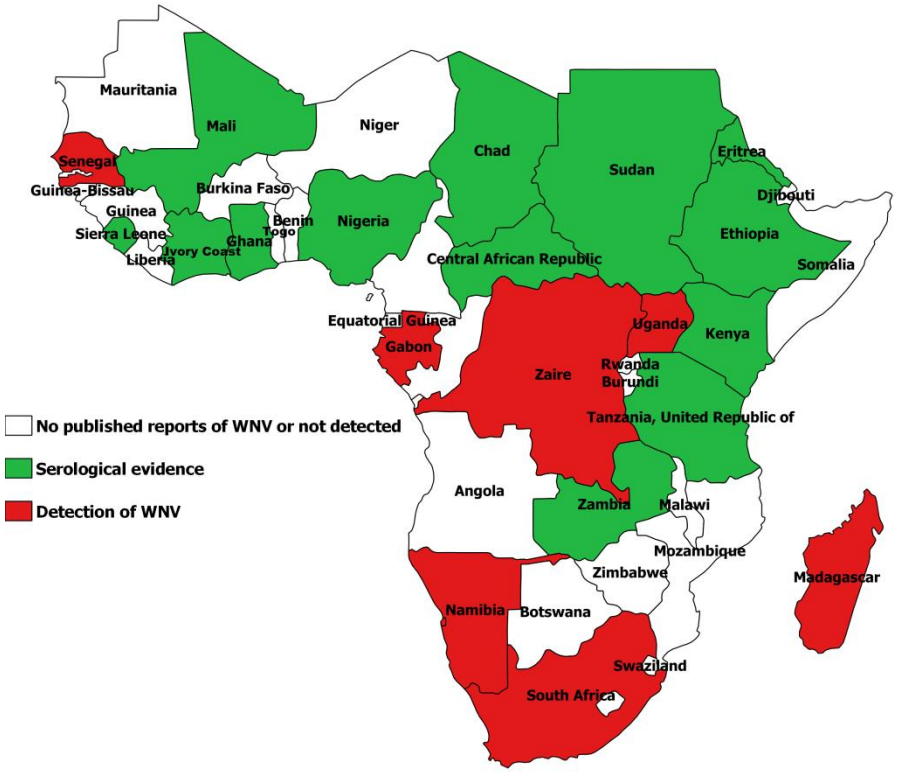

Figure 1: Sub-saharan countries for WNV isolation and serological evidence (Sule *et al.*, 2018)

WNV is currently active in East African countries as evidenced by the detection of antibodies in birds as well as isolated from several mosquito species such as *Cx.pipiens*, *Cx. quinquefasciatus*, *Cx. univittatus*, *Cx. antennatus* (Lutomiah *et al*., 2011; El-Bahnasawy, 2016). Experimental trials in conjunction with field studies were conducted in Egypt and identified *Cx. antennatus*, *Cx. univittatus* and *Cx. pipiens* as potential carrier for *WNV* (Soliman *et al*., 2010). *Culex* occurrence associated with habitat preference such as vegetation cover and water biogeographies. The other reported species include *Cx. duttoni*, *Cx. annulioris*, *Cx. poicilipes*, *Cx. cinereus*, *Cx. tigripes*, *Cx. Trifilatus* (Muturi *et al*., 2007; Ajamma *et al*., 2016). *Cx quinquefasciatus* and *Cx. pipiens* are distributed and reported in Kenya and Tanzania around rivers and streams are often characterized by irrigation-supported agriculture (Mboera *et al., 2000;* Dida *et al.*, 2018*)*. Species of five subgenera of *Culex* that occur in the Sudan recorded and *Cx. pipiens* and *Cx. antennatus* were majorly identified in localities of Sudan and some Sudan species occur in the

western parts of Ethiopian region (Lewis, 1956). Apart from East African context, there were some papers (Table 2) that evidenced the disease status in the Horn of Africa due to restricted availability information about disease and its factors that affect the distribution of disease and vectors.

Table 2: Evidence of WNV in the Horn of Africa

Country	Species	Prevalence	References
Djibouti	Horse	9 % (serological)	(Cabre *et al*., 2006)
	Human	20 % (clinical)	(IAMAT, 2019)
		0.3-60% (serological)	(Eybpoosh *et al*. , 2019)
	Ruminants, horses, dogs	41.35 (serological)	(Marié *et al*., 2016)
Ethiopia	Human	Serological	(Rodhain, *et al*., 1975)
Somalia	Human	Geographical	(Sayed-Ahmed, 2016)
		Serological	(Chancey *et al*., 2016)
Eretria		No evidence	(Chancey *et al*., 2016; Sule *et al.*, 2018)

2.5. Challenges in Ethiopia

Entomological Catalogue of *Culicidae* of the Ethiopian region showed numerous numbers of endemic taxa in genera and sub genera of *Culicidae* occurring in the Ethiopian zoogeographic region (White, 1975; Ardoin, 1976). There are various mosquito species widely distributed in different areas of the country and the vector abundance is highly associated with water surfaces (White, 1975; Getachew *et al.*, 2015). The serological evidence was suggested the presence of WNV in the southern geographical areas and circulation of these viruses could be explained by the presence of *Culex* and other mosquitoes (Rodhain *et al*., 1975; Ferede *et al*., 2018).The presences of water area create favorable condition for the growth and development of *Culex* and its

larvae has seasonal dynamics, which might be associated with the dynamics in rainfall and vegetation. The highest density of mosquito larvae can be observed September, following the long heavy rainy season and lowest in May (Dejenie *et al*., 2011). *Cx. pipiens* complex of subspecies, fatigans, distributed in the tropics and the ancestral form is probably *pipiens*, which arose from a stem from the Ethiopian region (Barr, 1967). The distribution of *Cx. pipiens, Cx. antennatus*, *Cx. univittatus* and other *Culex* species reported in different areas of Ethiopia (WRBU, 2014).

2.6. Spatio-temporal mapping

Developing a strong spatiotemporal model that forecasts mosquito population dynamics is a challenging task because mosquito distribution and density is determined by complex interactions among climatic condition, vegetation, land-use, as well as availability of livestock and human population (Yoo *et al.*, 2016). Climatic changes can be associated with various factors, mainly rising in ambient temperature, urbanization variation in rainfall quantities, have contributed to the outbreak of WNV (Paz, 2013). Nowadays, GIS and other sensing technologies are providing scientists with appropriate tools and data to make clear the geographic associations between the habitats of disease agents, their vectors and hosts (Jacob e*t al.*, 2006; Dhama *et al.*, 2013; Guptill and Moore, 2013).

Visualization of spatial and temporal patterns allows detecting when and where outbreaks occur as well as understanding which factors govern the spatial pattern and rate of spread of diseases. The application of GIS involve development of model and predict species or pathogen invasion of new and unknown geographical regions or new suitable habitats or climates (Tami *et al*., 2016). Predicting areas at high risk for disease transmission requires an accurate model of vector distribution (Diuk-Wasser e*t al.*, 2006). Environmental predictors are most informative measures and widely they show appropriate information to determine suitable mosquito habitat from unsuitable one. The spatial model helps to identity of the most significant vector-habitat relationship (Conley *et al*., 2014). Building environmental situation compose areas of intensive the land covered by physical infra structures and different climatic variables (Jacob e*t al.*, 2006). Environmental and presence data can be used to identify mosquito habitats and predict

species distribution and abundance across a landscape. The effects of landscape heterogeneity on the abundance of the WNV determined by associating environmental data at multiple spatial extents and abundance *Culex* (Diuk-Wasser *et al*., 2006; Schurich e*t al.*, 2014).

The spatial associations between vector-host contact and environmental layers has been researched by using MaxEnt as well as BIOMOD2 for species distribution model (Georges & Thuiller, 2013a; Sallam *et al*., 2017). This SDM is becoming a widely used framework for studying distribution and risk of vector-borne diseases. It is a numerical tool that combines observations of species occurrence with environmental estimates. These models are commonly used to predict distributions of species across a landscape, often extrapolating across time and space (Ames, 2016).

2.7. Mosquito surveillance

Currently, various procedures are available for mosquito species identification at different level. Mosquitoes surveillance has ability to provide valuable information for integration of spatial dynamics and environmental factors affecting WNV transmission (Gu *et al.,* 2006). Field sampling methods for mosquitoes may include methods for collecting flying adults, resting adults, larvae and eggs. The combined use of these methods depends on the objective of the surveillance or monitoring campaign, the target species, the environmental conditions at the selected sampling sites, the availability of resources and further identification (ECDC & EFSA, 2018). Identifying members of the *Culex* populations through morphological methods are difficult, time consuming, and often limited to adult males. The accurate identification of these mosquitoes can be conducted through molecular characterization after conducting a morphological identification (Kamel, 2015).

3. MATERIAL AND METHODS

3.1. Study area

Study area was located in the Horn of Africa of greater region containing the countries of Ethiopia, Djibouti, Eritrea and Somalia (Augustyn, 2019). These countries are crossed and touched with great rift valley features (FAO, 1997; New World Encyclopedia contributors, 2017). Regarding Ethiopia, the rift valley cross various regions and the country has diverse agro-ecological regions. The central plateau, lie between 1,800 m and 2,500 m above sea level, covers the large part of the country (EthioVisit, 2019), covered with a total area of 1,104,300 km^2, geographically located between 3°-14.8 ° latitude, 33°-48° longitude in the Eastern part of Africa laying between the Equator and the Tropic (CIA, 2019; Teklehaimanot, 2019). The typical climate is very mild, with temperatures on the most of the highlands averaging below 27°C. The average minimum during the coldest season is 5.5°C, while the average maximum rarely exceeds 27°C. Heavy rainfall occurs in most of the regions during June, July, and August. Directly after the rains the highlands are wonderfully green, covered with wildflowers (BTG 2014; EthioVisit, 2019). The human population is estimated about 108,386,391(CIA, 2019). Crop production systems as well as different livestock rearing practices are the main livelihoods for households (FAO, 2019). The sampling sites represent Mid Rift Valley areas (Figure 2) and these areas were recruited in to the study after developing preliminary habitat suitability map.

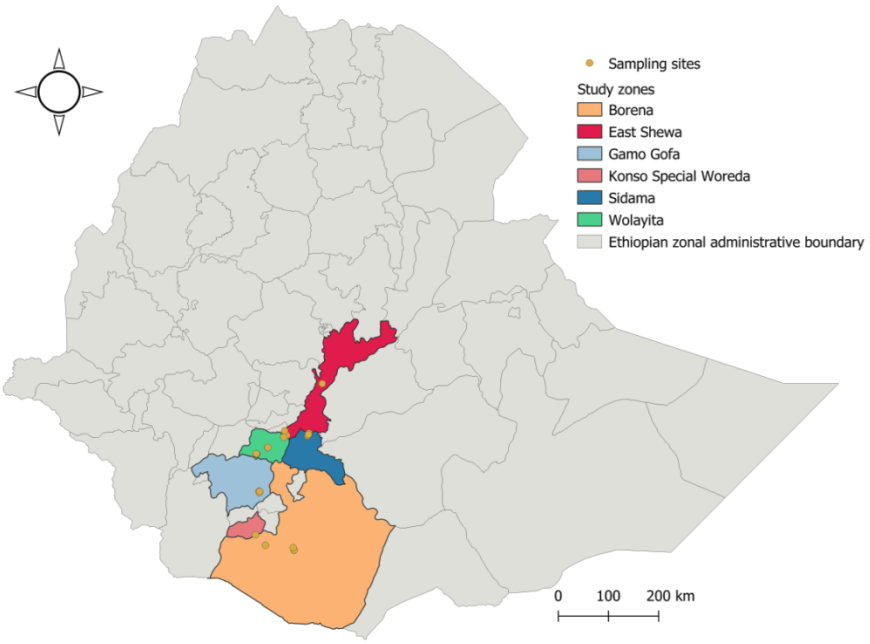

Figure 2. Map of sampling site of the study area

3.2. Study design

A cross sectional study was conducted from May 2018 to June 2019. Prior to sample site selection a preliminary habitat suitability map was developed for different species of *Culex* mosquitoes. The sampling site selection was determined with the help of the preliminary map.

3.3. Data layers

3.3.1. Environmental data

Explanatory variables of various environmental layers were recruited from global websites for the study. The global level environmental data was clipped to the Horn Africa's extent at 28 to 54 longitude and 26 to -8 latitude. The layers of temperature, precipitation, wind, water vapor pressure and solar radiation indices were obtained from the worldclim version 2 (Table 3) (Fickand Hijmans, 2017) databases.

Soil type and elevation data

Altitude layers were accessed from USGS websites. This site has a Shuttle Radar Topography Mission (SRTM) data which is worldwide data available to the geospatial data. It provide more complete digital elevation model (DEM) with a resolution of 3 arc-seconds (~ 90 meters) for global coverage (SRTM, 2000). QGIS was used to derive altitude at 2000 m extents using the DEM.

Table 3: Selected environmental layers

	Variables	**variable name**	**Data source**	**Units**
1	Tmax	Mean annual maximum temperature	Worldclim	°C
2	Tmin	Mean annual minimum temperature	Worldclim	°C
3	prec	Annual Precipitation	Worldclim	mm
4	Vapr	Water vapor Pressure	Wordclim	kPa
5	Srad	Solar radiation	Worldclim	kJ m^{-2} day^{-1}
6	Wind	Wind speed	Worldclim	m s^{-1}
7	Landcover	Land cover	ESA	
8	Altitude	Elevation in meter	USGS-SRTM	m
9	Livestock	Livestock 5km	FAO	
10	Human population	Human population count of 2015	SEDAC	
11	Soil	Soil type	USGS	
12	Tavg	Mean annual average temperature	Worldclim	°C

Land cover data

Land cover data was included for present study and accessed from European Space Agency GlobCover Portal (ESA, 2010). The downloaded dataset had 2.3 version v2009 (Global Land Cover Map) and it was released on 21st December 2010. The incorporated dataset had very high resolution of 205 m.

Livestock and human population data

From livestock perspective, equine and other livestock populated areas were recorded as suitable habitats for *Culex* abundance (Lysyk, 2010; Mughini-Gras *et al.*, 2013; Liu, 2018). In order to show the influence host population for blood meal of *Culex*, the 2010 year of Gridded Livestock of the world data was taken. This data includes global distributions of cattle, buffaloes, sheep, goats, horses, pigs, chickens and ducks at a spatial resolution of 5 minutes of arc (FAO, 2019). The association between livestock population density at Horn Africa and *Culex* species estimated with 5 Km radius, buffer zones centered at mosquito presence site.

Pervious research showed the positive association between *Cx. pipiens* population abundance with human population density and housing unit density (Trawinski & Mackay, 2010). So, the gridded population of the Horn Africa from the world data was added for the present study. Human population estimates accessed with counts by age and sex of the year 2010, consistent with national censuses and population registers, as raster data (CIESIN, 2013).

3.3.2. *Culex* occurrence data

The occurrence data was taken from field and GBIF database. *Cx. pipiens, Cx. antennatus, Cx. univittatus and Cx. quinquefasciatus* species occurrence data was collected from Oromia and SNNP regions using CDC and UV light mosquito traps (Appendexix 1).

On addition, these species data at Horn Africa's extent was collected from the Global Biodiversity Information Facility (GBIF) webpage (GBIF, 2019). The global Website extracted occurrence data was compiled with trapped *Culex* data. This updated dataset was created in order to ensemble species distribution modeling of the current study. The four species of major WNV unique presence points were run in BIOMOD2 package in R software. Pseudo-absence (background absence points that are artificially selected by the model) (VanDerWal *et al*., 2009; Ames, 2016).

Mosquito sampling data

The adult mosquito data collection was conducted in Oromia and SNNP regions using CDC and UV light mosquito traps (Appendix 1). The traps were positioned using GPS points and close to resting areas of mosquitoes including outdoors or in natural shelters as well as indoor conditions(ECDC & EFSA, 2018). Collection was conducted during the night time when there is no stormy weather or strong wind, or wind mixed rain since mosquitoes tend to hide. Traps were placed in outdoor, indoor habitats, different hosts, river and lakesides were the potential resting sites and collected those mosquitoes. The traps hanged at 18:00 hours in the evening and retrieved at 06:00 hours in the morning because the mosquitos hide in the dark places (ICIPE, 2018). At the end, indoor and outdoor collected mosquitos were placed with deep frozen and transferred to laboratory for morphological identification.

3.4. Morphological identification and virus detection

Morphological identification

All collected mosquitoes were sorted into tubes, deep frozen and transferred to NADHIC laboratory for morphological identification using stereomicroscope. Morphological identification of the collected mosquitoes was conducted at genera and species level for *Culex* based on the Walter Reed Biosystematics Unit (WRBU, 2014). The identified *Culex* species were categorized and worked as occurrence data by complying with GBIF data as presence data for spatial distribution modeling.

Virus detection

Virus extraction and PCR amplification was conducted on cooperation with the NADHIC and National veterinary Institute (NVI). Morphologically identified *Culex* mosquitoes collected from South Ethiopia (Borena and Konso) were submitted to virology laboratory for virus detection. Mosquitoes for virus detection were placed in individual 0.5 ml tubes containing RNA-*later* and crushed with a toothpick. 9 Pools of 208 mosquitoes from Yabelo, Moyale, Eleweye and Konso districts were crushed in each 1.5 ml eppendorf

tubes containing RNA-*later* and then stored at -20ºC. The specimens in RNA- *later* required a continuous freezing chain, so were stored at -20ºC initially; and then stored at -70 C° before further processing. The specimens was maintained under the above cold chain conditions to preserve virus viability in the samples, pooled specimens according to date, trap location and species (Rizzo, 2014; Patsoula *et al.*, 2016). Then, RNA extracted from mosquito pools based on the protocol (QIAGEN, 2011) of RNeasy Plus Mini Kit. Because of unavailability of positive control, the assay was done without positive control reference. The PCR assay was performed using Conventional One step RT- PCR with specific probe sets for WNV such as Primer- WNV-F_2 Fow-5pm/ µl(5'GTGCTGGTAAAACAAGGAGG-3') and Primer-WNV-Rev_2-5pm/µL 5('TGTAT CCTCTAGCCGCGATG-3'). The master mix preparation, PCR reaction and agarose gel preparation procedures were attached on the appendix 4. The amplification procedures were cDNA synthesis, denaturation, annealing and elongation run with their temperature, time and cycle. The result was read using UV–light (Frost *et al*., 2012; Rizzo *et al*., 2017).

3.5. Statistical analysis and modeling

Statistical analysis was conducted using different analytical methods. In our case, occurrence data set as response variable and selected environmental data as explanatory variables. Before proceeding to data analysis both response and explanatory variables organized into statistically meaningful format.

Spatial thinning of the four *Culex* species occurrence was done using a randomization approach in the spThin R package (appendix 2). This georeferenced occurrence records were spatially thinned using a thinning distance of 5 km. These datasets were saved as comma separated values (csv) files containing the columns: species name, latitude, and longitude (Aiello-Lammens *et al*., 2015).

The environmental layers were arranged on QGIS (ver 2.18) and these data were rescaled to 2.5 arcminute (~5 km^2) resolution. Then statistical analysis was conducted using the biomod2 package in R (3.4.4.) statistical computing software. The four *Culex* species

occurrence data set as response variable and environmental layers as explanatory variables. Multicollinearity among explanatory variables was checked using the variance inflation factor (VIF) analysis, "vifcor"& "*vifstep"* command in R using "*usdm"* package (Naimi, 2017). The selection of explanatory variables should also be based on selection of scientifically meaningful environmental variables. Thus, scientifically meaningful environmental variables namely temperature minimum and temperature maximum were kept in the model even if they were collinear (Franklin & Miller, 2010; Bucklin, 2017).

Statistical analysis was enabled a more detail assessment between selected factors and the two species distribution. The three main modeling steps were conducted includes formatting the data, computing the models and making the projections. Presence-absence data of species observations were used in the biomod2 package and it is an ensemble species distribution model. This model requires two sets of data inputs such as presence points and environmental data related to the selected species. The *Culex* species presence data was explained as response variable and environmental data as explanatory variable. The pseudo-absence data was generated using the Surface Range Envelope (SRE) model (Thuiller *et al*., 2016). The modeling procedure within biomod2 included all 10 different algorithms such as general linear models (GLM), general boosted models or boosted regression trees (GBM), general additive models (GAM), classification tree analysis (CTA), artificial neural networks (ANN), SRE, flexible discriminant analysis (FDA), multiple adaptive regression splines (MARS), random forests (RF), and maximum entropy (MAXENT) (Thuiller *et al*., 2009). These all methods were incorporated to estimate the species distribution in this study.

The data was evaluated by dividing into two portions; calibrated the model as training data, and another portion was validated the predictions as testing data. The model predictive score was evaluated by 80% as training data and 20% as testing data. The area under the relative operating characteristics (ROC), Cohen's kappa statistics (Kappa) and true skills statistics (TSS) were used to evaluate the performance of the models (Thuiller *et al*., 2012). The three evaluation metrics were run giving 30 models (10 models x 3 folds), thus the average value of ROC, Kappa, and TSS of the cross validation was made.

TSS scores range from -1 to 1, where +1 differentiate actual suitable and unsuitable habitat and values of zero or less indicate a performance no better than random. The model performance evaluation was performed in each run for species distribution; as a result averages of the runs were taken as report. For the ensemble modeling of the species distribution, only those models with TSS score greater than 0.8 were considered (Georges & Thuiller, 2013b). After running all steps in the model development, the model projected and ensemble for the potential distribution of the species. An ensemble model of *Culex* distribution was combining all excellent models or mixed algorisms which help to overcome uncertainty in model selection. The models' mean, coefficient of variation (CV) and committee averaging (CA) evaluated then high-accuracy predictive distribution models were combined to build ensemble model for species distribution (Georges & Thuiller, 2013a; Wu-Jun *et al.*, 2018)

3.6. Ethical clearance

The ethical clearance certificate was taken at project level. Before starting data collection the sample collection protocol was approved by Addis Ababa University College of Veterinary Medicine and Agriculture animal research ethical committee with reference number VM/ERC/02/06/10/2018 (Appendix 5). The approval was focusing on geo-statistical and biological approval in investigation of vector-born disease of veterinary and public health importance**.**

4. RESULTS

The morphological identification was conducted from the collected mosquito samples and four major WNV vectors from *Culex* genera were identified. The modeling was conducted and responded on the spatial mapping of the identified *Culex* species. The developed model was reliably estimated the distribution of *Culex* mosquitoes. As presence points about four species of *Culex* were collected from the Oromia, and SNNP regions but the collected *Culex* species data was not sufficient number of observations for modeling. Therefore, these field and GBIF data extent from the horns of Africa were merged and created presence only data in order to model the species distribution.

4.1. Observed major *Culex* species for WNV and their distribution in Ethiopia

The entomological study conducted starting from collecting different mosquitoes using CDC, UV light and modified traps. Regional and zonal level results were summarized with morphologically identified *Culex* mosquitoes at species level (Table 4). A total of 543 *Culex* of four major *Culex* species were collected from Oromia (3 districts) and SNNP (6 districts) regions of Ethiopia during the study. These species were *Cx. pipiens*, *Cx. univittatus*, *Cx. antennatus*, *Cx. quinquefasciatus*, they are potential vectors for WNV. The highest numbers of collections were of *Cx. pipiens* with 209 from 543 *Culex* mosquitoes followed by 158 *Cx. univittatus* collection. The highest distribution was found in Oromia region and about zonal distribution Borena had resulted with high number of *Culex* followed by Gamo Gofa zone.

Table 4: Frequency and percentage of *Culex* species based on region and Zonal distribution in Ethiopia

	Cx.pipiens	***Cx.antennatus***	***Cx.univitattus***	***Cx. quinquefasciatus***	***Cx. theileri***	**Total**
Region						
SNNP	90(27.6)	35(10.7)	158(48.5)	9(2.8)	34(10.4)	326(60)
Oromia	119(54.8)	28(12.9)	0(0)	51(23.5)	19(8.8)	217(40)
	209(38.5)	63(11.6)	158(29.1)	60(11.0)	53(9.8)	543(100)
Zone						
Borena	119(54.8)	28(12.9)	0(0.0)	51(23.5)	19(8.8)	217(40.0)
Sidama	0(0.0)	35(24.6)	73(51.4)	0(0.0)	34(23.9)	142(26.2)
Gamo	81(48.8)	0(0.0)	85(51.2)	0(0.0)	0(0.0)	166(30.6)
Gofa						
Woliata	1(50.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	2(0.4)
Konso	8(50.0)	0(0.0)	0(0.0)	8(50.0)	0(0.0)	16(2.9)
Total	209(38.5)	63(11.6)	158(29.1)	60(11.0)	53(9.8)	543

These mosquitoes were collected from lake, river side, animal pen, indoor and outdoor habitats (Figure 3). Also, these mosquitoes were collected from dairy, crocodile farm as well as aquatic environment. The collection was conducted by considering GPS points and different elevation meters and the altitude ranged from 850 to 1700m above sea level.

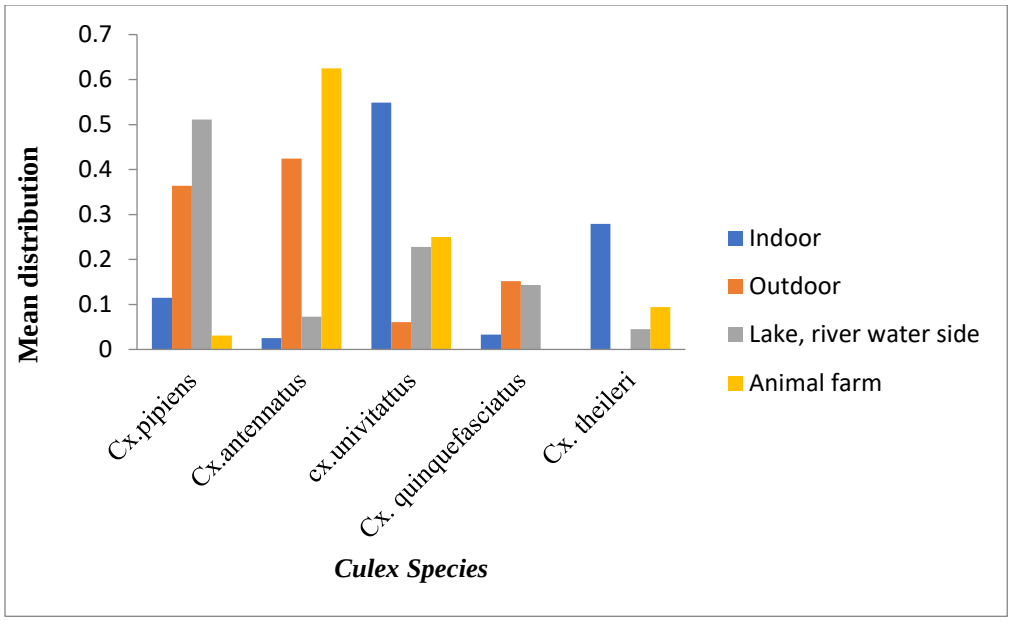

Figure 3: Mean distribution of *Culex* species based on their habitat in Ethiopia

4.2. Isolation WNV from *Culex* mosquitoes

Forward and reverse primers -WNV-F_2 and WNV-Rev_2 were used to amplify RNA from mosquito samples and a representative gel is shown (Figure 4). A total of 9 mosquito pools had been screened from Borena and Konso were result of virus isolation from *Culex.* No any bands read from gel electrophoresis which indicates negative result but not the absence of WNV.

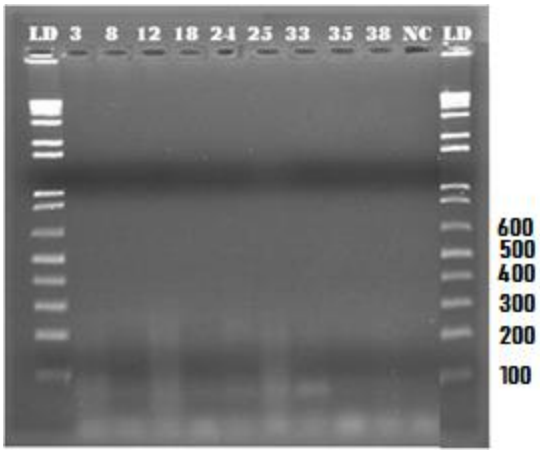

Figure 4. RT-PCR protocol from 9 independent mosquito RNA samples pools

4.3. Model performance evaluation on estimation of *Culex* distributions

The individual model was evaluated to respond for best individual model on estimation of *Culex* presence. The performance of 30 models was evaluated using the TSS metrics and KAPPA, ROC and TSS values were reported on Table 5 in *Culex* estimations, the statistical models with highest TSS-value were GLM (TSS=0.932), MARS (TSS=0.925), RF (TSS=0.919) and GBM (TSS=0.914). As a result, eight of the total 10 models proved reliable estimations (TSS≥0.8). The evaluation result of SRE and MAXENT was found with poor TSS values (TSS< 0.8) but they were better on evaluation using (ROC= 0.738) and (ROC=0.806) respectively. A result from average of the three evaluation metrics, RF (KAPPA=0.793, ROC=0.978, and TSS=0.919) has better model evaluation performance followed by GBM (KAPPA=0.754, ROC=0.987 and TSS=0.914) model and SRE (KAPPA=0.429, ROC=0.738 and TSS=0.475) scored lowest in all evaluation metrics. The individual model projection of the total models were attached on appendix 6 and showed the occurrence of *Culex.*

Table 5 : Performance Evaluation of the model on estimation of *Culex* distributions

Model	KAPPPA	ROC	TSS
RF	0.793	0.978	0.919
GAM	0.667	0.931	0.862
GLM	0.439	0.969	0.932
GBM	0.754	0.987	0.914
CTA	0.396	0.854	0.708
ANN	0.294	0.903	0.750
SRE	0.429	0.738	0.475
FDA	0.684	0.940	0.816
MARS	0.578	0.976	0.925
MAXENT	0.669	0.806	0.613

4.4. Variable contributions on estimations of *Culex* distributions

The variable contributions of 30 models were resulted taking the average of the runs. The result from table (Table 6) focused on eleven environmental variables studying the individual model contributed by these variables. The contribution of each variable in all ten models was stable for variables that were accepted as good predictors. The overall reviews of variable contributions were described in each model.

Regarding with variable contribution, soil type (49%) and precipitation (46.5%) were highly important variables explaining the *Culex* distribution in each model. It was followed by altitude and minimum temperature, but all variable importance was varied in each model. On other hand, wind (16%), solar radiation (15%) and land cover (5%) were grouped as least influential on variable contributions. The contribution of soil type and precipitation varied from (2-84%) and (15-75%) in the models, respectively. Solar radiation (0-58%) and wind (0-74%) had lower effect resulted with low contributions models. Land cover had lowest contribution with 0-13% from the overall models.

Table 6: contributions of the environmental variables to the selected models

	% variable contribution on each model										
Variables	**RF**	**GAM**	**GLM**	**GBM**	**CTA**	**ANN**	**SRE**	**FDA**	**MARS**	**MAXENT**	**Total**
Maximum Temperature	5.9	45.0	59.5	0.7	4.6	6.7	29.3	22.3	43.3	1.7	**21.9**
Minimum Temperature	11.6	70.3	54.5	3.0	23.8	0.2	34.8	36.2	62.6	20.1	**31.7**
Precipitation	35.2	68.2	22.3	73.3	82.7	15.8	14.8	19.0	58.3	75.4	**46.5**
Livestock	8.2	45.5	7.6	7.4	3.5	29.3	26.8	10.2	11.3	52.8	**20.3**
Land Cover	6.4	9.9	8.0	0.0	0.0	9.3	0.0	3.1	0.0	13.2	**5**
Solar radiation	3.3	57.7	18.3	0.5	1.1	21.5	23.8	0.0	22.7	0.0	**14.9**

Table 6. "Continued"

Vapor pressure	7.9	48.5	58.3	0.9	22.8	0.4	51.3	40.0	0.0	24.3	**25.4**
Wind	4.9	73.6	36.7	1.6	3.8	0.1	9.7	8.9	18.1	4.6	**16.2**
Soil type	1.7	83.6	74.6	11.5	29.6	70.3	83.7	20.3	70.5	47.4	**49.3**
Altitude	17.4	83.6	55.0	23.7	19.3	22.8	7.5	45.1	22.0	46.8	**34.3**
Population	16.9	25.1	11.9	6.2	17.3	60.6	12.3	31.8	26.4	51.1	**26**
Total	**10.9**	**55.5**	**37**	**11.7**	**19**	**21.5**	**26.7**	**21.5**	**30.5**	**30.7**	**26.5**

The result of overall variable contributions were reported on each model, predictors were better influential when modeling *Culex* by GAM (55.5%) which ranged from 9.9 to 83.6 % and followed by GLM (37%) ranged from 8-74.6%. Soil had strong contribution on estimating *Culex* presence by GAM and SRE. Except precipitation, most of the variables had least contribution when estimating *Culex* presence by GBM

4.5. Ensemble distribution modeling of *Culex*

Among all ensemble model algorithms performances, and predictor sets, most SDMs performed well, with overall mean probability of the selected model resulted TSS value (TSS=0.989), (KAPP=0.961) and (ROC=1); with low evaluation metrics of the best-performing algorithm through committee averaging resulted (TSS=0.988), (KAPP=0.896) and (ROC=0.999).

An ensemble model of variable contribution showed various result from the mean analysis of the selected models. Soil condition (37%) and precipitation (31.5%) were highly contributing variables on developing *Culex* distribution. Altitude (29%) and minimum temperature (25%) also had better contribution on estimating the *Culex* presence. Apart from this, Land cover (0.9%), livestock population on 5 km (4.7%), solar radiation (5.5%) and human population (8%) had low importance on prediction of *Culex* presence.

4.6. The predictive maps on distributions of WNV vector *Culex*

Through incorporating the above ensemble models a predictive map was developed. The predictive maps estimated the mean probability (Figure 5) of Culex occurrence along the horn of Africa indicating the study area. The vector estimated to occur in different parts of the country and the predicted occurrence of *Culex* varied from region to region. The model showed that the high probability of predictive occurrence in Oromia region observed in most parts of Borena followed by Bale areas. The intermediate probability of predictive occurrence visualized in East Harerghe, West Harerghe, Arsi and Shewa. Apart from this, low occurrence visualized in West Wellega, West Shewa, Jimma and Illubabor areas. In SNNP region the high probability of occurrence located Konso, Burji, Amaro, Derashe, South Omo, Sidama, Woliata, Gamo Gofa, Hadiya and kamabata Tembaro areas. Visualization of low probability of occurrence observed in some parts of south Omo, Dawuro, Basketo, Konta and the reaminig areas found with least occurrences. In Somali region high probability of occurrence pictured in Liben near Borena border. In Afar, it was occurred in boarder with Oromia. The remaining regions were visualized with low mean probability of occurrence. Visualization of maps showed the occurrence of *Culex* covers the rift valley areas and follows somewhat the water lines such as lakes and rivers.

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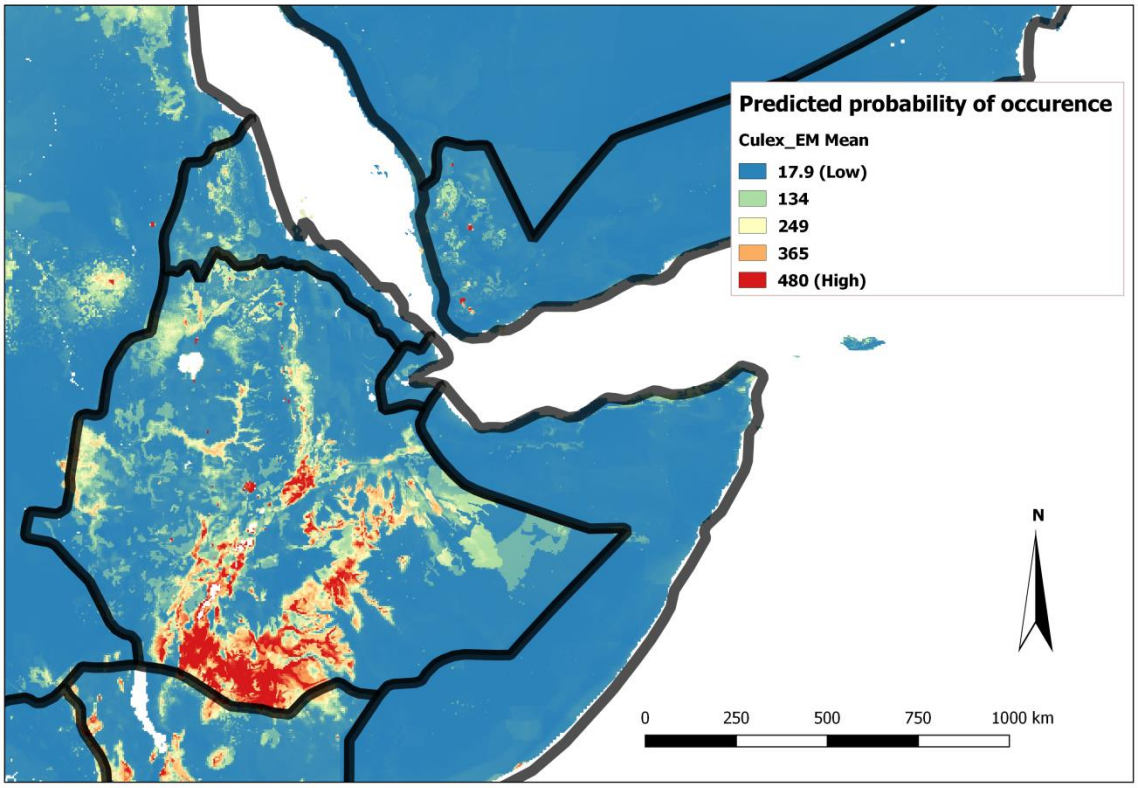
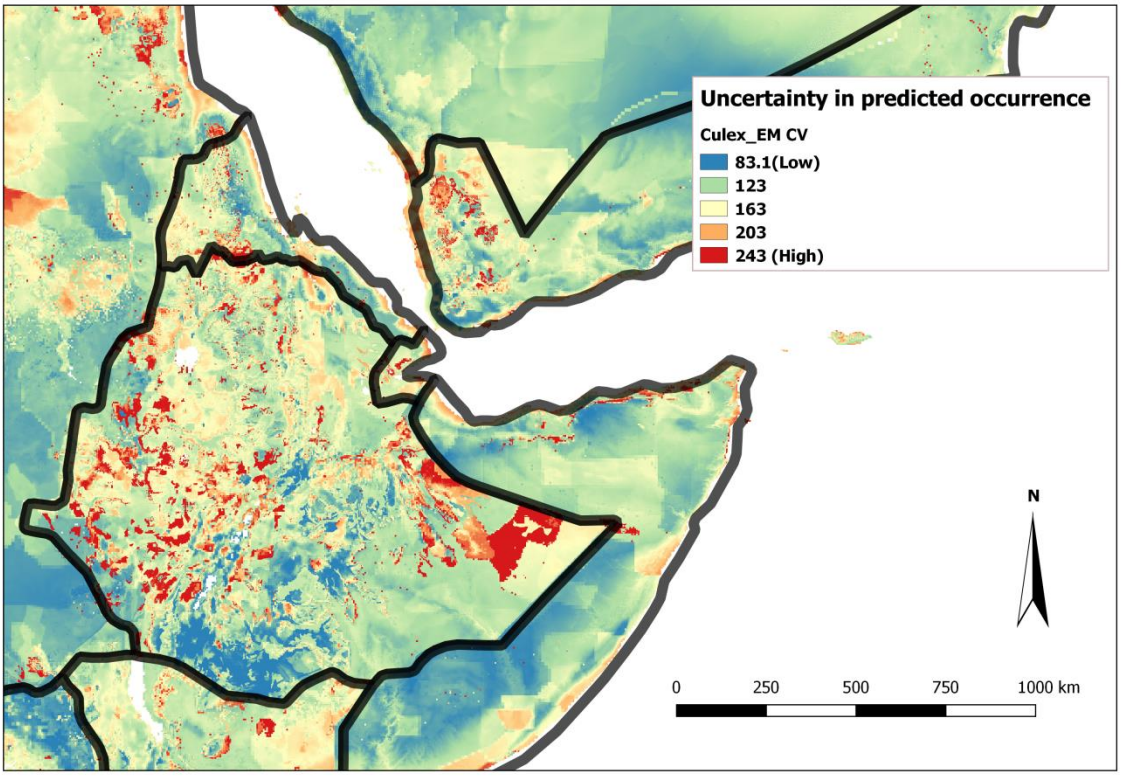


EM mean: Ensemble models mean

Figure 5. Ensemble model prediction on presence of *Culex* in Ethiopia along the Horns of Africa

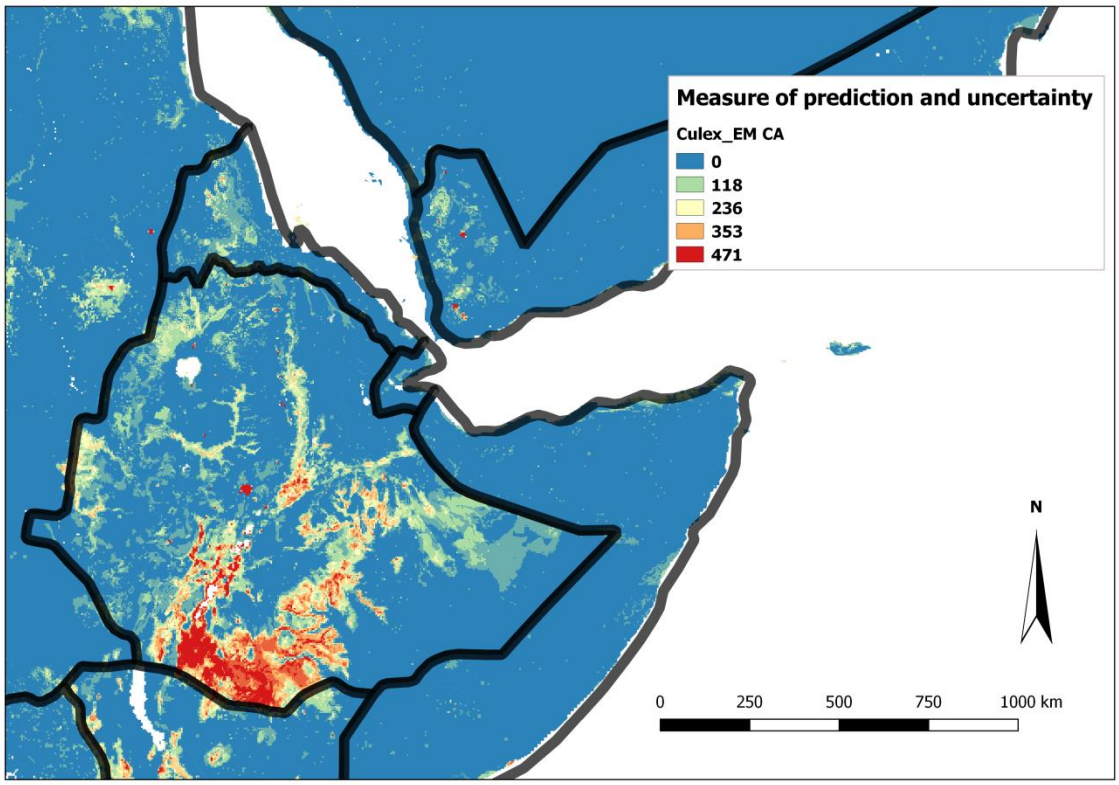
The next uncertainty model (Figure 6) was showed how predictive occurrence deviate from of the mean probability of occurrence. This map was the complement of mean probability of *Culex* presence in the area. High uncertainty was observed in Jimma, Wollega, Metekel and West Shewa areas from Oromia region, most parts of Southern region, Eastern Tigray, North Gonder and Jijiga, Dege Habur and Welwel & warder zones in Somali. From the model low uncertainty was viewed around Borena and Bale areas in Oromia region, South parts of Ethiopia.



EM CV: Ensemble models Coefficients of Variation

Figure 6. Ensemble model uncertainty in the presence of *Culex* in Ethiopia along the Horn of Africa

The other model was committee averaging and the map presented in Figure 7. In this model, the presence and absence areas for *Culex* mosquitoes selected from each model. It was developed by measuring both a prediction of *Culex* occurrence and uncertainty on *Culex* observation.



EM CA: Ensemble models Committee Averaging

Figure. 7 Ensemble model estimated prediction and uncertainty in the presence of *Culex* in Ethiopia along the Horns of Africa

5. **DISCUSSION**

Geospatial modeling techniques are essential in developing predictive distribution maps of mosquito as well as locating the potential arbovirus disease areas. In this study, our model was developed on the spatial mapping of the *Culex* species distribution which appears as a potential vector of WNV (Noori, 2015; WHO, 2017). Modeling mosquito distributions in Ethiopia helps to understand the environmental preferences of *Culex* and identification of the risk areas for potential distribution. Our study focused on modeling the distribution of *Culex* in Ethiopia and this vector was taken into our study considering as potential vector for West Nile Virus.

The entomological result showed a high number of Culex detected in Oromia region mainly in Borena area and SNNP in Gamo Gofa and Sidama areas. Our report showed a high number of *Cx. pipiens* and *Cx. univitattus* collection. Studies in Kenya reported *Cx. quinquefasciatus* and *Cx. pipiens* as dominant species (Mwangangi *et al*., 2011; Dida *et al*., 2018). Different habitats were taken into account and water associated areas were investigated with an increased number of Culex collection. Habitat selection for *Culex* depends on their developmental stage and breed in various types of stagnant water. Preferred oviposition habitats such as rainwater barrels, catch basins, storm drains, and septic tanks are rich in organic material (Noori, 2015; Valent Bioscience, 2019). Related studies were made on habitat preference of different mosquitoes for breeding (Bashar *et al*., 2016), other finding investigated (Lutomiah *et al*., 2013) *Culex* mosquitoes found in Rift Valley areas around the lake shores.

Our individual model results showed that topographical variables such as soil type and altitude contribute better in the modeling of *Culex* distribution. A similar result was reported for *Culex* and other mosquitoes (Naeem *et al*., 2016). Other finding reported elevation as an important factor even though; high and low altitudes had different predictive suitability(Liu *et al*., 2018).

Our investigation showed that precipitation and temperature were the influential drivers in mosquito presence and absence, this finding was consistent with (Uusitalo, 2017; Asigau & Parker, 2018) report from Kenya. This finding was in agreement with the finding in East Africa (Mweya *et al*., 2013) where focused on *Cx .pipiens* complex. In addition, precipitation was highly important variables explaining the *Culex* distribution in each model. Our result is similar to the findings by (Naeem *et al*., 2016; Uusitalo, 2017), in which they found that minimum temperature as the influential variables. The other investigation was dealing with the proportion of *Cx. pipiens* complex members exhibited by distinct seasonal variation. Low and high-temperature months can have an effect on the distribution of different *Culex* species (Gao e*t al*., 2016).

A human population variable was one of the variables that included in the modeling of *Culex* occurrence. Meanwhile the relationship between mosquito population density and human hosts is important in determining the infective biting rate and transmission risk of arboviruses (Sallam *et al*., 2017). Based on our finding human population was also intermediate predictor in *Culex* distribution models. A similar investigation was made (Conley *et al*., 2014) but reported as a strong predictor. Land cover is the least important factors contributed to the modeling of this vector. Other findings were differently reported about land cover contribution (Naeem *et al.*, 2016; Sallam *et al*., 2017).

The results from each variable contribution were different in all 10 models which varied from 11 to 55.5%. From all models, GAM and GLM found with the highest proportion of overall variables. Similarly, an investigator from Kenya estimated 8 models for variable contribution (Uusitalo, 2017). Apart from this, other studies estimated variable importance using only MaxEnt model which produces predictions of habitat suitability by comparing the conditional density of predictors at presence sites with the marginal density of predictors across the study area (Conley *et al*., 2014; Sallam, 2017). Regarding model evaluation, each model has its own performance. Normally, eight of the total 10 models proved reliable estimations and from this, RF and GBM were best SRE and MAXENT was found with poor performance evaluation points. RF showed highest prediction power for mosquito occurrence. Correspondingly, the other report showed that

the RF was the best-suited model for defining environmental conditions for several mosquito species. Documents on RF described this model has several advantages over other statistical methods, such as high classification accuracy, an efficient method of determining variable importance, and the ability to model complex interactions among predictor variables (Kwon *et al.*, 2015).

An ensemble modeling combines individual model and makes ensemble prediction. In our case, the TSS evaluation metric was used among the 30 models and the ensemble model included models only with TSS value was greater or equal to 0.8 (Georges & Thuiller, 2013a). The result showed that soil properties (Larson *et al*., 2010) and precipitation (Kwon *et al*., 2015) were the most influential variables in the ensemble model. Related studies developed ecological niche modeling using Maxent and GARP model and showed variable contribution varies between species under *Culex.* For example, The ecological niche modeling for *Cx. tarsalis* revealed that a combination of layers including higher average temperatures combined with various other layers including distance to rivers, soil properties, and grassland cover was likely responsible. Models created for the distribution *Cx. pipiens* is considered an urban species (Larson *et al*., 2010). Precipitation and temperature had influence on future mosquito population dynamics (Morin, *et al*., 2013; Mughini-Gras *et al*., 2013). Other study conducted ensemble model development using multimode inference for WNV infection rates among mosquito vectors using meteorological and hydrological conditions (Little *et al*., 2016). Our result showed land cover, livestock population, solar radiation and human population variables had low prediction power on *Culex* presence. A similar report was documented (Mughini-Gras *et al*., 2013) on these variables contribution but in contrary, investigation in the Middle East and North Africa showed contribution of environmental parameters such as human population and vegetation indices to species distribution models (Conley *et al*., 2014).

Visual inspection of the ensemble model showed a high probability of predictive occurrence in Oromia region around Borena and Bale areas. It was located in SNNP region around Omo and Gamo Gofa areas as well as around Somali border with Oromia.

These occurrence points were associated with various environmental conditions. Other studies in Egypt agree with models in predicting a moderate to a high probability of *Cx. pipiens* presence along the banks of the Nile and throughout the Nile Delta (Conley *et al*., 2014). In addition, ecological modeling *Culex* was conducted in Kenya by including similar environmental variables (Uusitalo, 2017).

Regarding with virus detection, our results demonstrated a conventional, one-step RT-PCR method for screening pools of mosquitoes from *Culex* species for WNV (Kauffman, 2003; Hoffmann *et al*., 2004). Negative results come up with our assay on detection of WNV from collected *Culex* mosquitoes. Our result had absence of positive control and failed as to report these samples as negative for WNV. Similar study was conducted (Medina *et al*., 2008) on detection of WNV from *Culex* using RT-PCR and confirmed negative results. Other study was conducted on isolation WNV from *Culex* and other mosquitoes and efficiently confirmed virulent strain (Frost *et al*., 2012).

6. CONCLUSIONS AND RECOMMENDATIONS

In this study, the Entomological study shows the abundance of various species of mosquito in the genus *Culex* in Ethiopia, predominantly *Cx. pipiens* and *Cx. univittatus*. Species distribution modeling for *Culex* indicates vector soil type, precipitation and temperature are the most dominant predictors as major environmental preferences. The model displays the occurrence of *Culex* in the rift valley areas and increased distribution established in water bodies such as lakes and river sides. The species distribution model shows the intensity of *Culex* varied from region to region and high probability of occurrence is found in Oromia region mainly in Borena zone, low lands of the SNNP and Somali lined with Oromia region. The occurrence of these *Culex* species has ability to create the WNF disease emergence in different parts of Ethiopia. This predicted distribution model helps in understanding the risk of introduction and distribution of WNV of these vectors and the disease for application of nationwide vector and disease control programs.

Based on the above conclusion the following recommendations are forwarded

- Further investigation is essential on ecological modeling targeting seasonal dynamics of vector and disease
- Additional investigation needed for isolation and confirmation of WNV from suspected mosquito species
- Further research is required to model more WNV vectors in other areas with records of disease epidemics

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8. LIST OF APPENDIX

Appendix 1: Mosquito sampling procedure from field

Materials and equipment

Paper cups, nets, rubber band, hand torch, dry cell batteries, wooden box, towel, CDC traps, UV-light trap, chloroform, binocular, stereomicroscope, Eppendorf tube, silica gel, plastic box

Culex mosquito collection and storage

Culex mosquitoes are nocturnal (night biters). Therefore, a collection was done during the night time and the collection sites were indoor and outdoor, animal farms, lake, river, other water sides as well aquatic areas.

- CDC and UV-light traps were placed in the selected mosquito collection sites
- Collections were best conducted in days when there is no stormy weather or strong wind, or wind mixed rain since mosquitoes tend to hide in such days.
- The collected mosquitoes were transferred to sampling tube labelled with date, trap number, location.
- The collected mosquitos were transferred to the field laboratory and morphological identification was conducted for each mosquito at genus level for *Culex*
- Standard storage in the eppendorf tube
    - Small beads of silica gel was added at the base of the eppendorf tube
    - Small piece of cotton on top of silica gel was used and put one mosquito on top of cotton with forceps
    - Another layer of cotton was added on top of the mosquito and tube was sealed carefully and labeled with date, place, resting site, method of collection.

Appendix 2: *Culex* presence only data thinning using R

```
culex <- read.csv("C:/Users/user/Documents/tsega/culex.csv")
attach(culex)
library(spThin)
```{r}
data(culex )
head(culex )
```
```{r}
thinned_culex5km_dataset_02_16_2019 <-
  thin(loc.data = culex,
       lat.col = "latitude", long.col = "longitude",
       spec.col = "culex",
       thin.par = 5, reps = 100,
       locs.thinned.list.return = TRUE,
       write.files = TRUE,
       max.files = 5,
       out.dir = "culex_thinned5km_02_16_2019/", out.base = "culex_thinned5km_02_16_2019",
       write.log.file = TRUE,
       log.file = "culex_thinned_02_16_2019_log_file5km.txt" )
```
```

Appendix 3: Conventional One step RT- PCR for West Nile Virus Isolation procedure

1-Master mix preparation

Ser. no	Type of reagent	For one reaction	Total reaction	Remark
1	RNase free water	4 µl		
2	Primer- WNV-F_2 Fow-5pm/ µl 5'GTGCTGGTAAAACAAGGAGG-3'	2 µl		
3	Primer-WNV-Rev $_2$-5pm/ µL 5'TGTATCCTCTAGCCGCGATG-3'	2µl		
4	5X PCR buffer	5 µl		
5	5X Q- solution	5 µl		
6	10mM dNTPs mix	1 µl		
7	One step RT-PCR enzyme mix	1 µl		
8	Add Template (DN A)	5 µl		
	T0tal volume	25 µl		

2-Run PCR Reaction

	Temperature	Time	Cycle	Remark
cDNA synthsis	50 ^{o}c	30 mints	1-Cycle	
Initial Denaturation	95^{o}c	15 mints	1-Cycle	
Denaturation	95oc	30 Sec	35 Cycles	
Annealing	55^{o}c	1 Mint		
Elongation	68^{o}c	45 sec		
Final Elongation	68^{o}c	10 mints	1-Cycle	
Put at	4$_o$c	Until machine off		

3-Agrose gel preparation

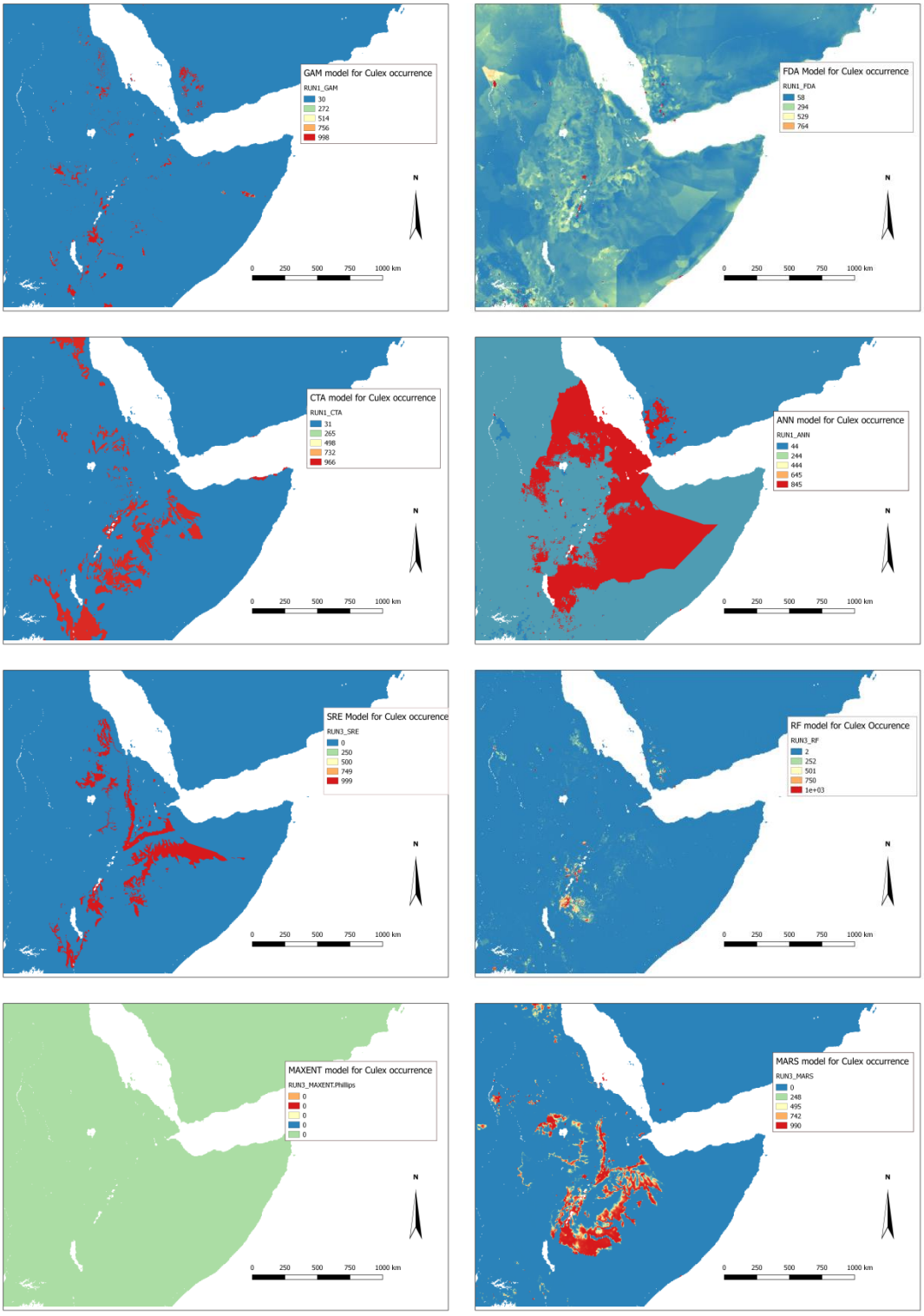
-Prepare 1. 5% Agarose gel

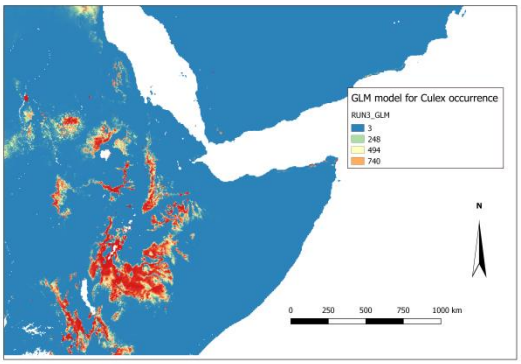
-Add 4µ Gel red with Loading dye, 10PCR product and 10 µl markers (Ladder)

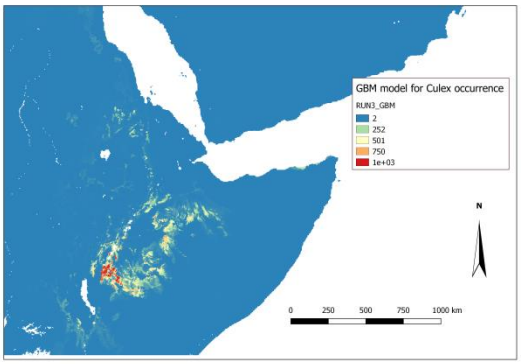
-Run Electrophoresis for 1:20hour at 120V

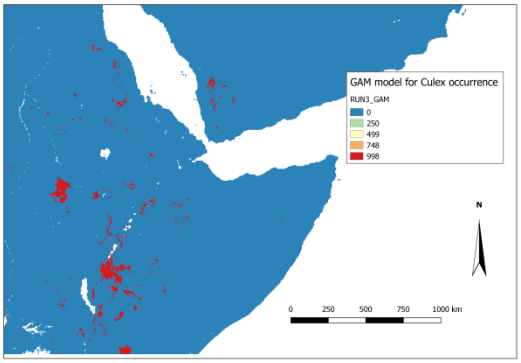
-Read the result by using UV –light

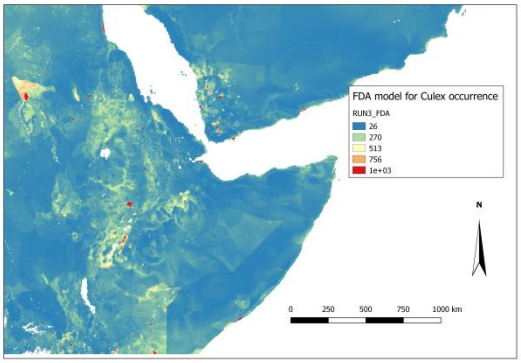
Appendix 4: Individual model projection of the 10 models by three runs

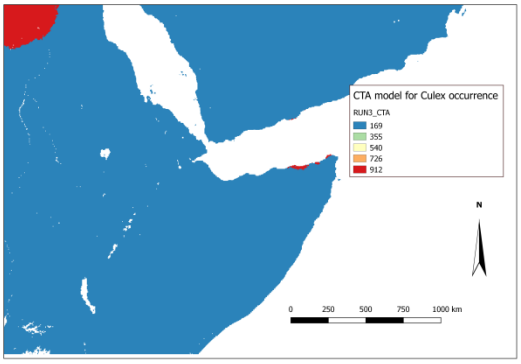


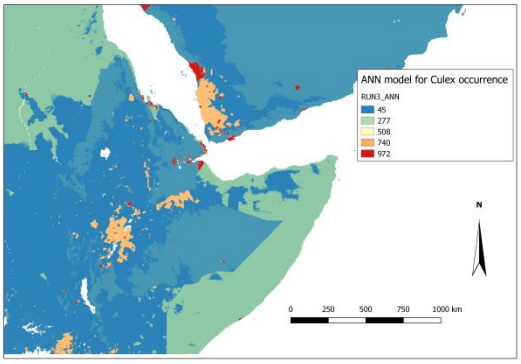

GLM model for Culex occurrence
RUN3_GLM
3
248
494
740
N
0 250 500 750 1000 km

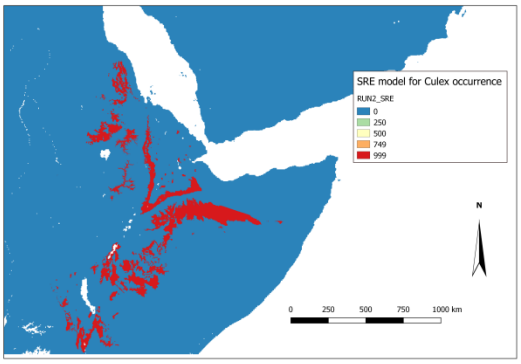

GBM model for Culex occurrence
RUN3_GBM
2
252
501
750
1e+03
N
0 250 500 750 1000 km

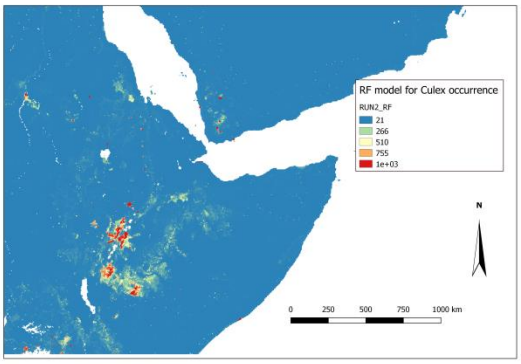

GAM model for Culex occurrence
RUN3_GAM
0
250
499
748
998
N
0 250 500 750 1000 km

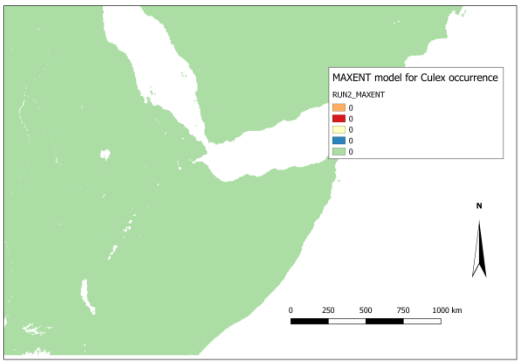

FDA model for Culex occurrence
RUN3_FDA
26
270
513
756
1e+03
N
0 250 500 750 1000 km

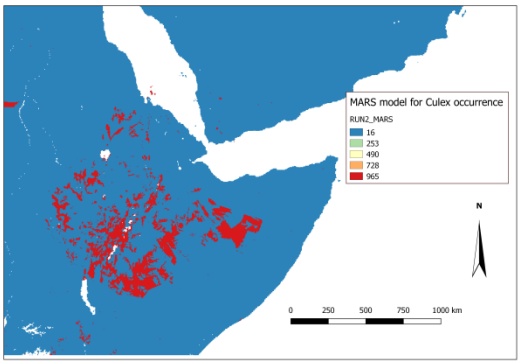

CTA model for Culex occurrence
RUN3_CTA
169
355
540
726
912
N
0 250 500 750 1000 km

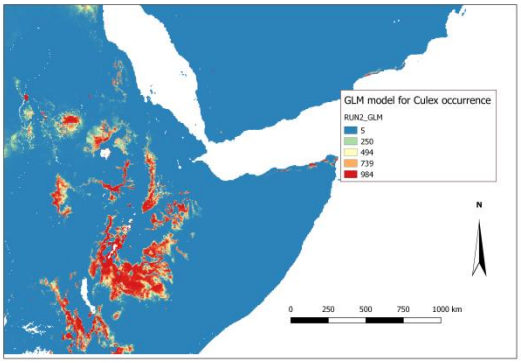

ANN model for Culex occurrence
RUN3_ANN
45
277
508
740
972
N
0 250 500 750 1000 km

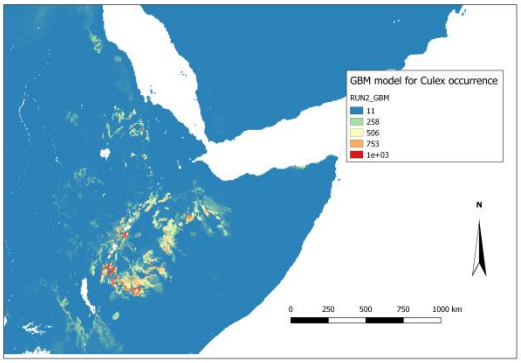

SRE model for Culex occurrence
RUN2_SRE
0
250
500
749
999
N
0 250 500 750 1000 km

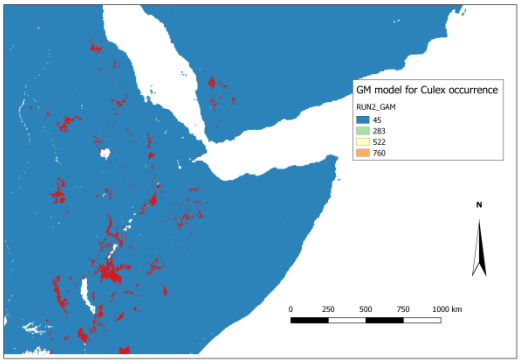

RF model for Culex occurrence
RUN2_RF
21
266
510
755
1e+03
N
0 250 500 750 1000 km

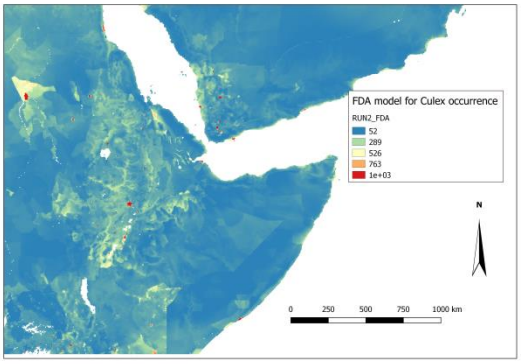

MAXENT model for Culex occurrence
RUN2_MAXENT
0
0
0
0
0
N
0 250 500 750 1000 km

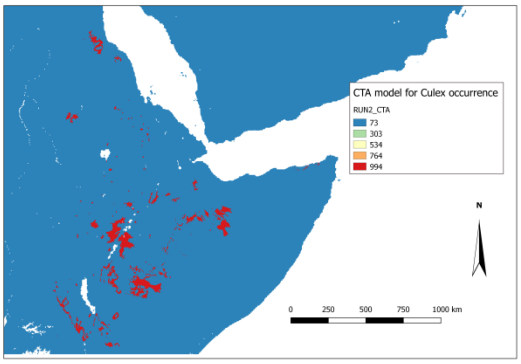

MARS model for Culex occurrence
RUN2_MARS
16
253
490
728
965
N
0 250 500 750 1000 km

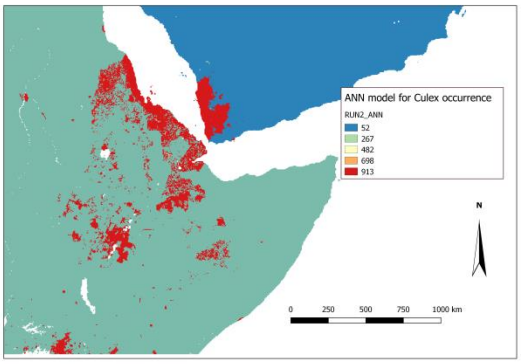

GLM model for Culex occurrence
RUN2_GLM
5
250
494
739
984
N
0 250 500 750 1000 km

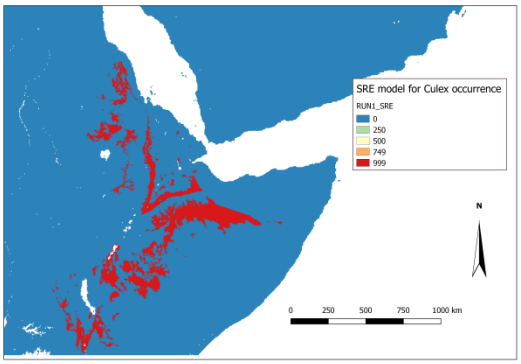

GBM model for Culex occurrence
RUN2_GBM
11
258
506
753
1e+03
N
0 250 500 750 1000 km

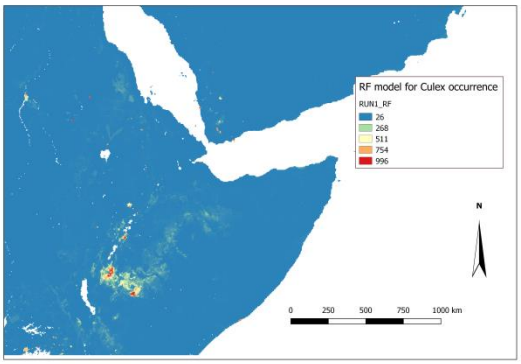

GM model for Culex occurrence
RUN2_GAM
45
283
522
760
N
0 250 500 750 1000 km

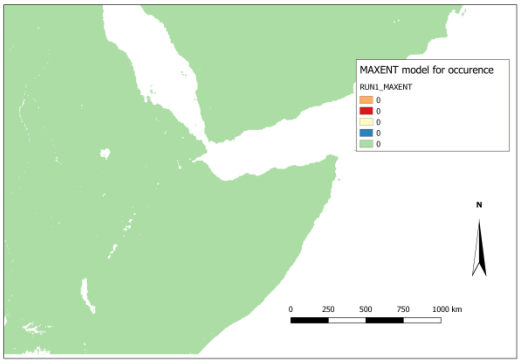

FDA model for Culex occurrence
RUN2_FDA
52
289
526
763
1e+03
N
0 250 500 750 1000 km

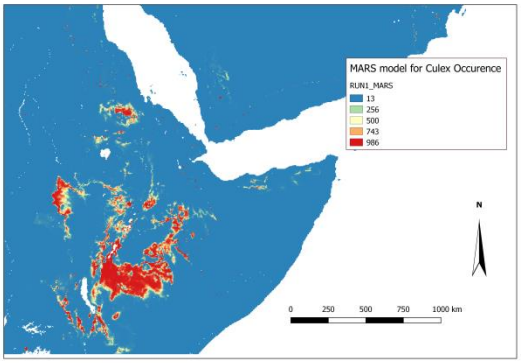

CTA model for Culex occurrence
RUN2_CTA
73
303
534
764
994
N
0 250 500 750 1000 km

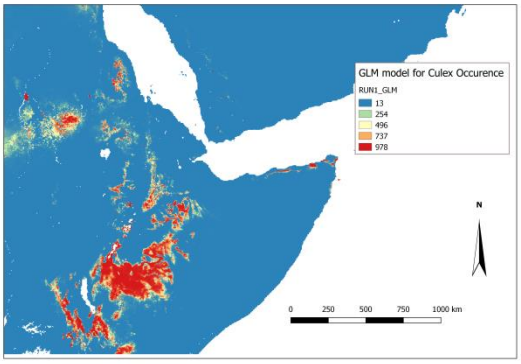

ANN model for Culex occurrence
RUN2_ANN
52
267
482
698
913
N
0 250 500 750 1000 km

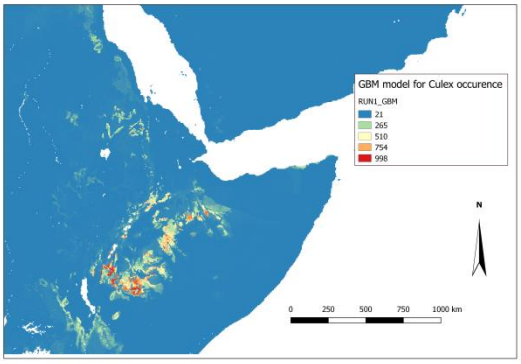

SRE model for Culex occurrence
RUN1_SRE
0
250
500
749
999
N
0 250 500 750 1000 km


RF model for Culex occurrence
RUN1_RF
26
268
511
754
996
N
0 250 500 750 1000 km


MAXENT model for occurence
RUN1_MAXENT
0
0
0
0
0
N
0 250 500 750 1000 km


MARS model for Culex Occurence
RUN1_MARS
13
256
500
743
986
N
0 250 500 750 1000 km


GLM model for Culex Occurence
RUN1_GLM
13
254
496
737
978
N
0 250 500 750 1000 km


GBM model for Culex occurence
RUN1_GBM
21
265
510
754
998
N
0 250 500 750 1000 km

Appendix 5: Ethical clearance certificate

አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ/ደብረ ዘይት

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

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

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