Thesis Ref No._____

MOLECULAR DETECTION OF WEST NILE VIRUS IN MOSQUITOES AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES REGARDING MOSQUITO CONTROL AND PREVENTION IN SELECTED RIFT VALLEY AREAS, ETHIOPIA

MVSc THESIS

BY

ODA GIZAW DEWO

ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

June, 2019

Bishoftu, Ethiopia

MOLECULAR DETECTION OF WEST NILE VIRUS IN MOSQUITOES AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES REGARDING MOSQUITO CONTROL AND PREVENTION IN SELECTED RIFT VALLEY AREAS, ETHIOPIA

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 Public Health

By:

Oda Gizaw Dewo

June, 2019

Bishoftu, Ethiopia

SIGNATURE SHEET

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As MVSc research advisors, we here by certify that we have read and evaluated this Thesis prepared under our guidance by Oda Gizaw, entitled: "**MOLECULAR DETECTION OF WEST NILE VIRUS IN MOSQUITOES AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES REGARDING MOSQUITO CONTROL AND PREVENTION IN SELECTED RIFT VALLEY AREAS, ETHIOPIA**"; we recommend that it can be accepted as fulfilling the thesis requirement for the Degree of Master of Veterinary Science in Veterinary Public Health.

Submitted by: Oda Gizaw Dewo _____ _____

Name of Student Signature Date

Approved for submittal to a thesis assessment committee:

1. Dr. Kebede Amenu (PhD, Assoc. Prof.) _____ _____

Major Advisor Signature Date

2. Dr. Haileleul Nigussie (PhD, Assoc. Prof.) _____ _____

Co- Advisor Signature Date

3. Dr. Gezahegh Mammo (PhD, Assoc. Prof.) _____ _____

Department chairperson Signature Date

APPROVAL SHEET

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As member of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the Thesis prepared by: Oda Gizaw, entitled: "**MOLECULAR DETECTION OF WEST NILE VIRUS IN MOSQUITOES AND ASSESSMENT OF KNOWLEDGE, ATTITUDE, AND PRACTICES REGARDING MOSQUITO CONTROL AND PREVENTION IN SELECTED RIFT VALLEY AREAS, ETHIOPIA**"; we recommended that it be accepted as fulfilling the thesis requirement for the degree of Master of Veterinary Science in Veterinary Public Health.

	Signature	Date
Dr Ashenafi Mengistu (PhD, Assoc. Prof.) Chairperson	_____	_____
Dr Adem Hiko (PhD, Assoc. Prof.) External Examiner	_____	_____
Dr Balako Gumi (PhD, Assoc. Prof.) Internal Examiner	_____	_____
Dr. Kebede Amenu (PhD, Assoc. Prof.) Major Advisor	_____	_____
Dr. Haileleul Nigussie (PhD, Assoc. Prof.) Co-Advisor	_____	_____
Dr. Gezahegh Mammo (PhD, Assoc. Prof.) Department Chairperson	_____	_____

DEDICATION

This thesis manuscript is dedicated to ***Bekele Gurmu Gudu*** *(****Abbaa-Gammachiis****) and* ***Elsabet Temesgen Disassa,*** *without whom I may not be a man of today. Thank you for nursing me with affection and love; for your dedicated paternity in the success of my life. I owe more than I could ever repay. It would take me a book to tell of your virtues and parenthood character, but to sum up your life in one statement, it would be:* ***you are a special and unique father and mother ever.***

VIVA !

STATEMENT OF AUTHOR

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

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Name: ODA GIZAW DEWO Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of submission: June 20, 2019

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LIST OF ABBREVIATIONS

bp	Base pair
CDC	Centers for Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
CxFv	*Culex* flavivirus
DENV	Dengue Virus
DNA	Deoxyribonucleic acid
IR	Indoor resting
IVM	Integrated Vector Management
KAP	Knowledge, Attitude and Practice
MBV	Mosquito-borne viruses
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RVF	Rift Valley Fever
Spp	Species
UV	Ultraviolet
VBD	Vector Borne Disease
WHO	World Health Organization
WNV	West Nile Virus
YFV	Yellow fever virus

ABSTRACT

Mosquito borne diseases poses an immense veterinary and public health concern and are major impediments in the path of socio-economic development. West Nile Virus is a widely distributed mosquito-borne *Flavivirus*. Environmental management strategies that reduce or eliminate mosquito breeding sites combined with improved personal prevention strategies can help to significantly reduce transmission of the virus. The main aim of this study was to detect West Nile Virus in mosquitoes collected from different areas in the Rift Valley of Ethiopia and assess the knowledge, attitude and practices of the community living in the study areas. A cross-sectional study was conducted to collect mosquitoes and questionnaire survey in the selected areas. A total of 2,322 mosquitoes were collected, identified to genus level and pooled into 38 groups of approximately 20 to 25 mosquitoes based on their genera, sex and collection site. The mosquito pools were homogenized and the RNA was extracted. WNV genome detection in mosquito pools was conducted using one-step RT-PCR followed by gel electrophoresis visualization. Pretested questionnaire was used for interviewing randomly selected households in which a total of 288 respondents were participated. Descriptive statistical data analysis tests were used. None of the mosquito pools was positive for WNV genome. Majority (94.4%) of the participants know mosquitoes can transmit diseases from humans to humans while only 10.1% know mosquitoes can transmit diseases between human and animals. All of the participants consider mosquitoes as a threat to the community and 93.8% believed controlling mosquitoes can help in prevention of mosquito borne diseases. Ninety three point one percent of the participants were practicing mosquito control measures in the past times. The negative result for WNV genome detection doesn't mean a total absence of the virus in the study area, perhaps due to seasonal, feeding and mosquito abundance factors. The awareness, attitude and practices of the community regarding mosquito control and prevention is satisfactory though there are some gaps.

Keywords: *Mosquito, West Nile Virus, questionnaire, RT-PCR, Ethiopia*

1. INTRODUCTION

Arthropod vectors are responsible for transmitting a number of vector-borne infections and diseases which are mostly viral in origin. Vector borne viruses are recognized as an extremely diverse group that harbors many zoonotic important viruses, which can cause serious disease such as Yellow Fever, Dengue and West Nile Fever in both humans and animals (Pabbaraju *et al*., 2009). For viruses to be transmitted biologically (vertically or horizontally), they must replicate in the arthropod vector prior to transmission (Goddard, 2008; Kuno and Chang, 2005; Weaver and Reisen, 2010). The important vectors transmitting zoonotic diseases are mosquitoes, biting flies, fleas, lice, bugs, ticks, mites, snails and others which actively transmit pathogens from an infected reservoir host animal to another individual (Eldridge, 2005).

Mosquitoes are the best known vector for transmission of a number of viral diseases such as West Nile Virus (WNV), Yellow Fever Virus (YF), Rift Valley Fever (RVF), Dengue Virus (DENV), Japanese Encephalitis (JE) and others (Eldridge, 2005). In tropical countries, *Aedes aegypti* is an important vector of WNV, YF, DENV and other viral diseases (Liu *et al*., 2014). In addition, *Culex* species are important as vectors of arboviral diseases, especially WNV. In some areas they are a considerable nuisance (Pandit *et al*., 2010).

Flaviviruses are single-stranded RNA viruses in the family of *Flaviviridae*, and is the only arbovirus in this group which utilizes mosquitoes (Huang *et al*., 2014a; Singh *et al*., 2015). WNV belongs to the genus *Flavivirus*, family *Flaviviridae*, with single stranded positive-polarity RNA genome of approximately 11kb (Castle *et al*., 1985; Singh *et al*., 2015). A WNV is a mosquito-transmitted flavivirus that belongs to the Japanese encephalitis antigenic complex (Hubalek *et al*., 1999; Petersen and Roehrig, 2001). This virus was isolated in 1937 for the first time in the West Nile Province in Uganda, from the blood of a woman presenting with classical systemic febrile illness (Hubalek and Halouzaka, 1999; Monini *et al*., 2010).

WNV is the causative agent of West Nile Fever and has been spreading globally using migratory birds as dispersal vehicles (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004). The viral strains of WNV have been distributed into two main genetic lineages (lineage 1 and

lineage 2), clearly correlating to the virus geographic distribution (Monini *et al*., 2010; Zeller and Schuffenecker, 2004). West Nile fever outbreaks affecting humans and/or animals have occurred all over the globe, in both tropical and temperate regions (Almeida *et al*., 2008).

The virus is the most wide-spread arbovirus in the world. West Nile Fever was at first considered a minor health concern, as the majority of infections were mild or completely asymptomatic; however, after the major outbreaks that occurred in Europe and North America in 1999, West Nile Fever became a public health priority (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004). Infection by WNV in humans or animals may need a confirmation with the identification of WNV genome, antigen or specific antibodies (Monini *et al*., 2010).

Generally, since arboviruses transmitted by mosquitoes, including WNV, exacerbate poverty by causing illness and disability which prevent people from working and supporting themselves and their family, detecting these viruses in mosquitoes and controlling and prevention of mosquitoes play a central role in poverty reduction and economic development. Many studies have been done on mosquito borne diseases (especially on malaria), extensively in Ethiopia. But, there is a research gap regarding the awareness, attitude and experience on WNV and its vector mosquito control and prevention and in detecting the virus in mosquitoes. Therefore, the objective of this study was intended to fill the information gap in the country on knowledge, attitude and practices of the community regarding control and prevention of mosquitoes and detecting WNV genome in mosquitoes from selected sites of Rift Valley of Ethiopia.

2. LITERATURE REVIEW

2.1. Arboviruses

The term arbovirus originated in the 1940's to describe the viruses transmitted by arthropods (arthropod-borne viruses) (Kuno and Chang, 2005). Arboviruses are recognized as an extremely diverse group that harbors many zoonotic important viruses, which can cause serious disease such as yellow fever, dengue and west nile fever in both humans and animals (Pabbaraju *et al*., 2009). Arboviruses are included mainly into three viral families: *Flaviviridae*, *Togaviridae* and *Bunyaviridae* (Pabbaraju *et al*., 2009). These viruses are, thus, transmitted by arthropod vectors, like mosquitoes, ticks, flies, etc., via a biological process, which can occur horizontally or vertically (Kuno and Chang, 2005; Weaver and Reisen, 2010).

For viruses to be transmitted biologically, they must replicate in the arthropod vector prior to transmission (Goddard, 2008; Kuno and Chang, 2005; Weaver and Reisen, 2010). In vertical transmission, the virus is passed on from the female to offsprings by trans-stadial or trans-ovarial transmission (Kuno and Chang, 2005; Weaver and Reisen, 2010). In turn, horizontal transmission can occur orally or venereally (where the virus is passed on from infected males directly onto females, during mating). This is the most typical arboviral transmission mode, which involves the infection of a susceptible arthropod vector after ingestion of viruses during feeding or, from maternal origin. Viruses are subsequently disseminated within the arthropod, replicate in the salivary glands, ensuring that transmission might occur during the following blood meal, through injection of contaminated saliva in a susceptible host (Kuno and Chang, 2005; Weaver and Reisen, 2010).

Furthermore, not all infected arthropods are capable of pathogen transmission. For it to occur it must be competent for transmission, that is, it must be susceptible to infection by the pathogen, allowing the above mentioned replication and dissemination thus becoming infective, and able to transmit the pathogen via an infective bite when blood- feeding (Goddard, 2008; Weaver and Reisen, 2010). Arboviral transmission can only take place where the three principal elements are present: the virus, the vector and vertebrate hosts (**Figure 1**).

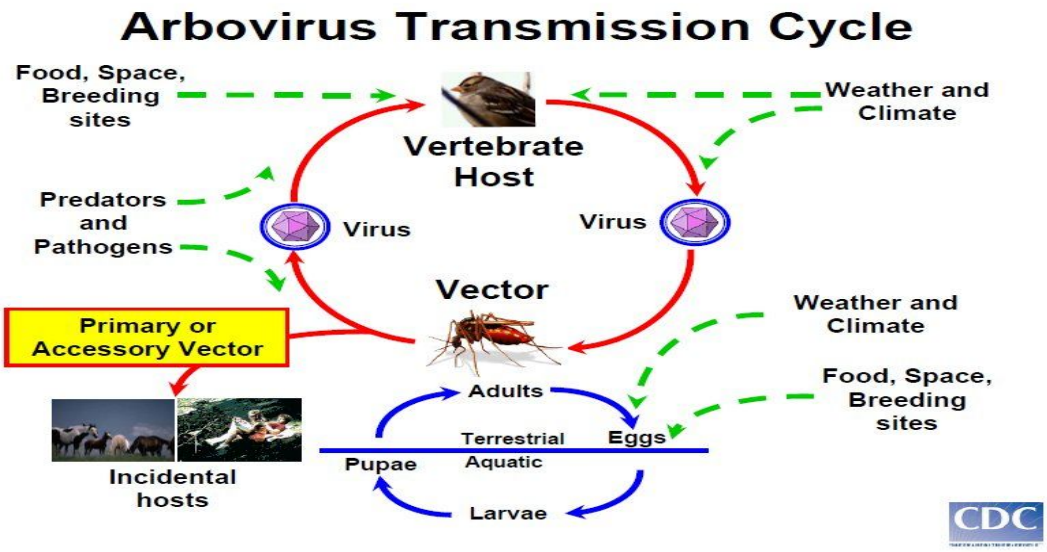


Figure 1: Arbovirus transmission cycle

Transmission of arboviruses appears to be seasonal, depending on vector and reservoir densities, as well as on climatic variables that affect the former (Hollidge *et al*., 2010). For example, despite the fact that in tropical or endemic regions the transmission occurs all year round, there are still seasonal peaks of activity that frequently coincide with the rainy seasons, and leads to high mosquito densities (Hollidge *et al*., 2010).

2.2. Vectors of Veterinary and Public Health Important Flaviviruses

In ancient times, insects were very important in the transmission of communicable diseases. The definition of vector was then related mostly to insects. Later on the term vector has been used more widely to include other non-human animals including snails, dogs and rats (Institute of Medicine, 2008). Alternative definitions are found for vectors, for example, vectors can be defined as: arthropods and other invertebrates which transmit infection by inoculation into or through the skin or mucous membrane by biting or by deposit of infective materials on the skin or on food or other objects (Braks *et al.,* 2011; Institute of Medicine, 2008; Kilpatrick and Randolph, 2012; Upadhyay *et al.,* 2018).

The important vectors transmitting zoonotic diseases are mosquitoes, biting flies, fleas, lice, bugs, ticks, mites, snails, *etc.* which actively transmit pathogens from an infected reservoir host

animal to another individual. Many of these are blood sucker, which ingest disease-producing microorganisms during a blood meal from an infected host (human or animal) and later inject it into a new host during their subsequent blood meal. Mosquitoes are the best known vector for transmission of a number of diseases (Alobuia *et al*., 2015a; Beier *et al*., 2008; Benelli *et al*., 2016; Jaleel *et al*., 2015; Liu *et al*., 2014; Mejia *et al*., 2016a; Pandit *et al*., 2010; Sarwar, 2015; World Health Organization, 2008).

The *Culicidae* family is very important from a human and veterinary medical perspective since it harbors a large number of species, including some of the most important hematophagous arthropods capable of transmitting infectious agents (Eiras, 2004; Eldridge, 2005). This family comprises over 3,500 mosquito species and subspecies, belonging to two public health important subfamilies: the *Anophelinae* and *Culicinae* (Eldridge, 2005). Mosquitoes included in these subfamilies are capable of transmitting arboviruses such as the dengue and yellow fever viruses as is the case of *Aedes aegypti* and *Ae. albopictus*; moreover, *Culex* species mosquitoes that can transmit West Nile virus and Rift Valley fever viruses are also part of the *Culicinae* subfamily (Almeida *et al*., 2008; Eldridge, 2005). Apart from viruses, mosquitoes can also transmit nematode worms and protozoa (Rutledge, 2008).

The mosquito life cycle comprises four distinct stages: the egg, the larvae, pupae and adult (**Figure 2**), as they go through complete metamorphosis (Arijo, 2015). The immature stages of mosquitoes, egg, larvae and pupae, are aquatic while the adult stage is the only that is terrestrial (Rutledge, 2008). Mosquito biology is highly dependent on climatic conditions, particularly temperature variations, and in warmer temperatures, the life cycle may take ten days or less to complete (Eldridge, 2005). Eggs may be deposited in water surface or moist ground, in groups or individually, and hatching occurs either in a day or so or when flooding occurs (Eldridge, 2005; Rutledge, 2008). A larva must undergo four molts to become a pupa; though their time of development has no direct relation to water temperatures (Eldridge, 2005). Adult emergence usually happens 1-3 days after pupa formation(Eldridge, 2005; Rutledge, 2008). Mosquito distribution is determined by the climatic conditions, hence their permanent existence in tropical, warm and humid climates, temperate countries and in cooler climate countries. Therefore, changes in these climatic conditions may forcibly

change their bioecology (Rutledge, 2008).

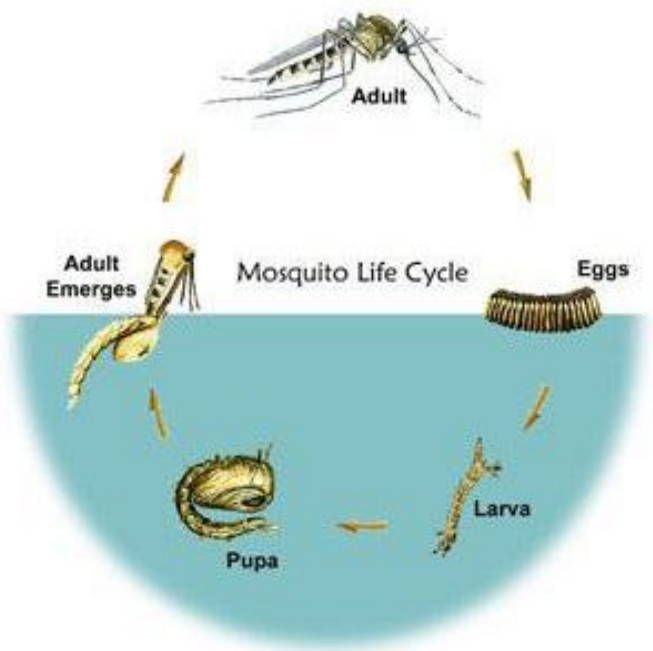


Figure 2: Mosquito life cycle

The behavior of mosquitoes determines whether they are important as nuisance insects or vectors of disease, and governs the selection of control methods. Species that prefer to feed on animals are usually not very effective in transmitting diseases from person to person. Those that bite in the early evening may be more difficult to avoid than species that feed at night. Mosquitoes that rest indoors are the easiest to control (Giordani *et al*., 2014).

2.2.1. Aedes mosquitoes

Aedes mosquitoes occur around the world and they can cause a serious biting nuisance to people and animals, both in the tropics and in cooler climates. In tropical countries *Aedes aegypti* is an important vector of dengue, yellow fever, west nile fever and other viral diseases. A closely related species, *Aedes albopictus*, can also transmit dengue. In some areas *Aedes* species transmit filariasis (Liu *et al*., 2014).

Aedes aegypti mainly breeds in the domestic environment: its preferred habitats are water storage tanks and jars inside and outside houses, and roof gutters, leaf axils, bamboo stumps and

temporary containers such as jars, drums, used car tyres, tin cans, bottles and plant pots. All these habitats typically contain relatively clean water(Sharma *et al*., 2017a). *Aedes* mosquitoes bite mainly in the morning or evening. Most species bite and rest outdoors but in tropical towns *Aedes aegypti* breeds, feeds and rests in and around houses(Jaleel *et al*., 2015).

2.2.2. Culex mosquitoes

About 550 species of *Culex* have been described, most of them from tropical and subtropical regions. Some species are important as vectors of arboviral diseases. In some areas they are a considerable nuisance(Pandit *et al*., 2010). *Culex* mosquitoes breed in a large variety of still waters, ranging from artificial containers and catchment basins of drainage systems to large bodies of permanent water. The adult females bite people and animals throughout the night, indoors and outdoors. During the day they are inactive and are often found resting in dark corners of rooms, shelters and culverts. They also rest outdoors on vegetation and in holes in trees in forested areas(Khan, 2015).

2.3. Control and Prevention of Mosquitoes

To achieve progress and stability in animal and human health, vectors have to be controlled effectively (Hurwitz *et al*., 2011). In olden days, people traditionally practiced various tips to control vectors including mosquitoes. They are grouped into two main headings: Lifestyle oriented practices and Agriculture oriented practices (Gubler, 1998; Hurwitz *et al*., 2011).

Additionally, as many vector control methods are effective against multiple diseases, they can be integrated together to combat multiple diseases at once(Hurwitz *et al*., 2011). The World Health Organization therefore recommends "Integrated Vector Management" as the process for developing and implementing strategies for vector control. It (IVM) focuses on utilizing preventative methods to control or eliminate vector populations (Benelli *et al.,* 2016; Braks *et al.,* 2011; Chanda *et al.,* 2013; Upadhyay *et al.,* 2018; WHO, 2014). Common vector control methods according to IVM measures are:

2.3.1. Habitat and environmental control

Removing or reducing areas where mosquitoes can easily breed can help limit their growth. For example, stagnant water removal, destruction of old tires and cans which serve as mosquito breeding environments, and good management of used water can reduce areas of excessive vector incidence. Further examples for environmental control are by reducing the prevalence of open defecation or improving the designs and maintenance of pit latrines. This can reduce the incidence of mosquitoes acting as vectors to spread diseases (WHO, 2012).

Reducing contact

Limiting exposure to mosquitoes or animals that are known disease vectors can reduce infection risks significantly. For example, bed nets, window screens on homes, or protective clothing can help reduce the likelihood of contact with mosquitoes. To be effective this requires education and promotion of methods among the population to raise the awareness of vector threats (WHO, 2012).

Chemical control

Insecticides, larvicides, rodenticides, lethal ovitraps and repellents can be used to control mosquitoes. For example, larvicides can be used in mosquito breeding zones; insecticides can be applied to house walls or bed nets, and use of personal repellents can reduce incidence of insect bites and thus infection. The use of pesticides for vector control is promoted by the World Health Organization (WHO) and has proven to be highly effective (Chanda *et al.,* 2013).

Biological control

The use of natural vector predators, such as bacterial toxins or botanical compounds, can help control mosquito populations. Using fish that eat mosquito larvae or reducing breeding rates by introducing sterilized male tsetse flies have been shown to control vector populations and reduce infection risks (Benelli *et al.,* 2016).

2.4. Mosquito-Borne Flaviviruses of Veterinary and Public Health Importance

Several arboviruses have become important human and veterinary pathogens causing febrile illnesses and encephalitis (Liang *et al.*, 2015). *Flaviviruses* are single-stranded RNA viruses in *Flaviviridae* family, and is the only arbovirus in this group which utilizes mosquitoes and ticks as primary vectors. Most of the arboviruses are zoonotic, transmissible from animals to humans (Huang *et al*., 2014a; Singh *et al*., 2015).

Although more than 70 *flaviviruses* have been identified, approximately half of which can cause disease in humans, only a few are of major importance. The viruses can be divided according to their vector, geographical area, or the clinical syndrome with which they most commonly present (Datta *et al*., 2015; Fang *et al*., 2018). *Flavivirus* infection is initiated with the bite of a carrier mosquito followed by local replication of the virus in Langerhans cells and continued replication in regional lymph nodes and other tissues, leading to hematogenous spread of the virus to target organs (Huang *et al*., 2014a; Morales-Betoulle *et al*., 2008).

After infection with a *flavivirus*, the host animal develops lifelong immunity to that virus. *Flaviviruses* thus need a constant supply of new non-immune hosts if they are to flourish. For this reason most *flaviviruses* are endemic, using birds or small mammals as the natural hosts (Hoyos-Lopez *et al*., 2016). These animals have high reproductive rates, providing a ready supply of immunologically naive hosts. For the endemic *flaviviruses* humans become infected accidentally because they come into close proximity with the natural cycle. Humans do not produce high viraemias, and therefore subsequent vector bites do not result in transmission, making them 'dead end' hosts (Gould and Solomon, 2008; Solomon and Mallewa, 2001).

Mosquito-borne *flaviviruses* are transmitted in nature in one or more distinct or overlapping cycles that include a mosquito vector, generally *Aedes* spp. mosquitoes for yellow fever (YFV) and dengue (DENV) and *Culex* spp. mosquitoes for West Nile (WNV), and a mammalian or avian host (Singh *et al*., 2015). Transmission between mosquitoes and vertebrate hosts is termed horizontal transmissions and causes disease in vertebrates. In contrast to horizontal transmission, mosquito-borne *flaviviruses* can be maintained in the environment through vertical, *i.e.*, transgenerational, transmissions which allow the spread of *flaviviruses* solely in mosquitoes (Gould and Solomon, 2008).

Transmission by mosquitoes between people has been reported for dengue virus, yellow fever virus, and West Nile virus that cause diseases in animals and humans in which human beings are usually dead-end hosts (Pierre *et al*., 1994; Solomon and Mallewa, 2001). *Flaviviruses* are usually recovered from entomological or biological samples by intracerebral inoculation of suckling mice and by infection of susceptible mosquito and monkey cell lines. *Flaviviruses* generally do not have a direct pathogenic effect on vectors, and the virus can be isolated seven days after the infectious blood meal (Pierre *et al*., 1994).

There are no antiviral drugs against *flaviviruses*, and vaccines exist for only a few. Rapid virus identification and quantification are crucial for accurate diagnosis of ongoing infections, as well as for selection and timely introduction of control measures in outbreaks scenarios (Sanchez-Seco *et al*., 2005).

2.5. West Nile Virus

WNV belongs to the genus *Flavivirus*, family *Flaviviridae*, with single stranded positive-polarity RNA genome of approximately 11 kb, containing 10 genes flanked by 5' and 3' non-coding regions (NCR) with no polyadenylation tail at the 3' end (Castle *et al*., 1985; Hoyos-Lopez *et al*., 2016; Speight *et al*., 1988). The viral genome encodes a single polyprotein that can be co- and post-translationally cleaved into 3 structural proteins (i.e. Capsid (C); Pre-M/Membrane (prM/M); and Envelope (E)) (Gonzalez-Reiche *et al*., 2010).

It also has 7 nonstructural (NS) proteins: NS1; NS2A; NS2B; NS3; NS4A; NS4B; and NS5 (Speight *et al*., 1988) that has a multifunctional, with a critical role in viral RNA synthesis and/or assembly (Gonzalez-Reiche *et al*., 2010). These NS proteins can also modulate cell signaling and immune responses. WNV NS1 protein particularly antagonizes the host's antiviral defenses through inhibition of TLR3 signal transduction and STAT1/STAT2 activation (Wilson *et al*., 2008). Moreover, cell surface-associated NS1 represents a major target for host antibodies which contributes to clearance of WNV-infected cells through F_c-gamma receptor-mediated phagocytosis (Kyung *et al*., 2007).

WNV virion has ~50nm in diameter and has icosahedral particle surrounded by a lipid bilayer. The nucleocapsid is composed of C protein and associated with the RNA genome that mediates

viral assembly (Markoff *et al*., 1997). prM and E protein has a heterodimers that become a part of lipid bilayer of the virus during assembly and are exposed on the virion surface. The prMprotein is associated with the protection of premature fusion prior to viral budding from cell surface. This is done by blocking the fusion loop of E which is cleaved off during the viral maturation process (Zhang *et al*., 2003). The other protein is E protein which mediates the binding of the receptor on the cell surface for viral entry and fusion with the membrane of the host cell (Smit *et al*., 2011).

2.5.1. Epidemiology

West Nile virus (WNV) is a mosquito-transmitted flavivirus that belongs to the Japanese encephalitis antigenic complex (Hubalek and Halouzaka, 1999; Petersen *et al*., 2013; Petersen and Hayes, 2008). This virus was isolated in 1937 for the first time in the West Nile Province in Uganda, from the blood of a woman presenting with classical systemic febrile illness (Monini *et al*., 2010). The virus was later recognized and isolated from mosquitoes, birds and humans in the 1950's, in Egypt, and also in France (1962-63). During the 1970s-1990s WNV caused isolated outbreaks in countries such as South Africa (1974), India (1980-81) and Romania (1996) (Hubalek and Halouzaka, 1999; Monini *et al*., 2010; Zeller and Schuffenecker, 2004). Since its first isolation, WNV has been considered one of the most widely spread flaviviruses thus becoming an increasing public health and veterinarian problem all over the globe (Zeller and Schuffenecker, 2004).

The virus is thought to have entered the United States through viraemic migratory birds or imported domestic birds, however the exact means of entry are unclear (Epstein and Defilippo, 2001; Solomon and Mallewa, 2001). WNV is the causative agent of West Nile fever and has been spreading globally (**Figure 3**) using migratory birds as dispersal vehicles since it was first documented in Africa in the 1930's (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004).

Most known viral strains have been distributed into two main genetic lineages, clearly correlating to the virus geographic distribution (Monini *et al*., 2010; Zeller and Schuffenecker, 2004). Viruses in lineage 1 are classified as more virulent and broadly

distributed through Africa, Australia, India, Asia and Europe, mainly in Mediterranean countries (Monini *et al*., 2010).

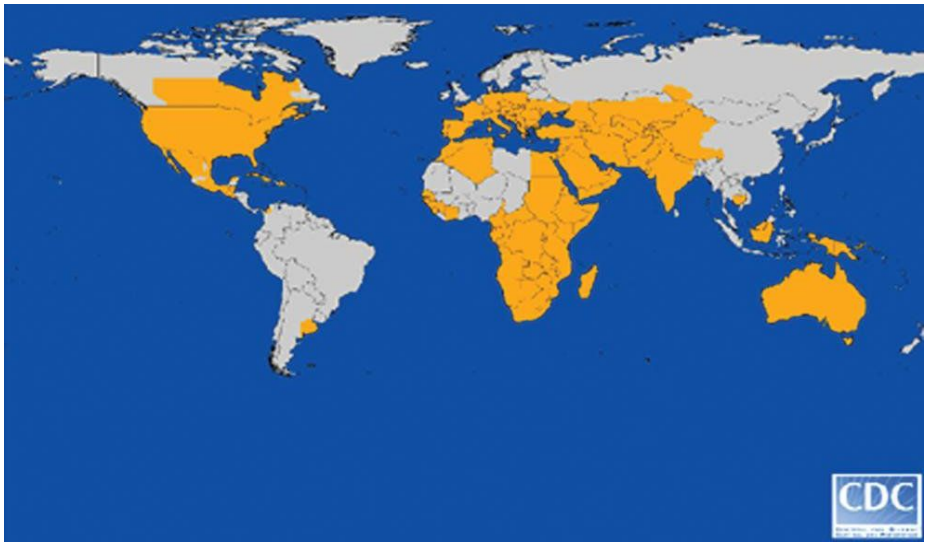


Figure 3: Approximate West Nile virus distribution map, 2006 (Gubler, 2007)

In the other hand, WNV strains composing lineage 2 have kept a more strict distribution in Sub-Saharan Africa (Petersen and Marfin, 2005; Petersen and Roerhig, 2001; Weaver and Reisen, 2010; Zeller and Schuffenecker, 2004). However, they have recently been found circulating in Hungary and Greece (Chaskopouluo *et al*., 2011; Zeller and Schuffenecker, 2004). Until very recently, only lineage 1 virus strains had been associated with severe human disease, including cases of clinical human encephalitis (Petersen and Marfin, 2005; Petersen and Roerhig, 2001).

West Nile fever outbreaks affecting humans and/or animals have occurred all over the globe, in both tropical and temperate regions (Almeida *et al*., 2008). In 1996-1997, Romania had the largest outbreak of arboviral disease seen in Europe, where more than 600 people presented neurological complications of which nearly 10% died (Hubalek and Halouzaka, 1999; Solomon and Mallewa, 2001).

Other outbreaks have occurred in Israel in the 1950's and in 2000, South Africa in the 1970's, Algeria in 1994, Morocco in 1996, Tunisia in 1997, Italy in 1998, Russia in 1999, France in 2000 (Monini *et al*., 2010). By 1999, the virus had spread to New York causing 60 clinical cases

of encephalitis leading to 7 human deaths with a dramatically higher equine and bird mortality rate (Gould and Solomon, 2008; Hubalek and Halouzaka, 1999; Zeller and Schuffenecker, 2004).

2.5.2. Global distribution of WNV

WNV is the most widely distributed *arbovirus* occurring in all continents except Antarctica (figure 3). After its isolation from Uganda, it subsequently appeared as a sporadic case and as an outbreak in Africa, Eurasia, Australia, and the Middle East. Further serosurveys of humans and equines together with entomological studies gives more global understanding for the ecology of the virus as well as the vectors (Kanagarajan *et al*., 2003).

Mild febrile illness of human cases appeared infrequently in different parts of the world, mainly Israel and Africa till early 1990s. However, after this, new viral strains with likely African origin increased human disease incidence in parts of Russia and southern and eastern Europe, with large outbreaks having increased clinical severity (May *et al*., 2011). In the western hemisphere, WNV spread from its 1999 appearance in Americas and circulates in most of the countries with human and animal cases (Petersen and Hayes, 2008).

2.5.3. Transmission cycle of WNV

WNV is mainly transmitted by mosquito species belonging to the *Culex* genus via two possible modes of transmission, the rural and the urban cycles (Hubalek and Halouzaka, 1999; Monini *et al*., 2010). In a rural setting, the primary transmission cycle occurs involving competent ornitophilic *Culex* species mosquitoes and migratory and local bird species that act as reservoirs as well as amplifying hosts whilst humans and horses represent accidental "dead-end" hosts (**Figure 4**), since they do not reach the level of viraemia needed for mosquito infection (Monini *et al*., 2010; Petersen and Marfin, 2005; Solomon and Mallewa, 2001; Weaver and Reisen, 2010).

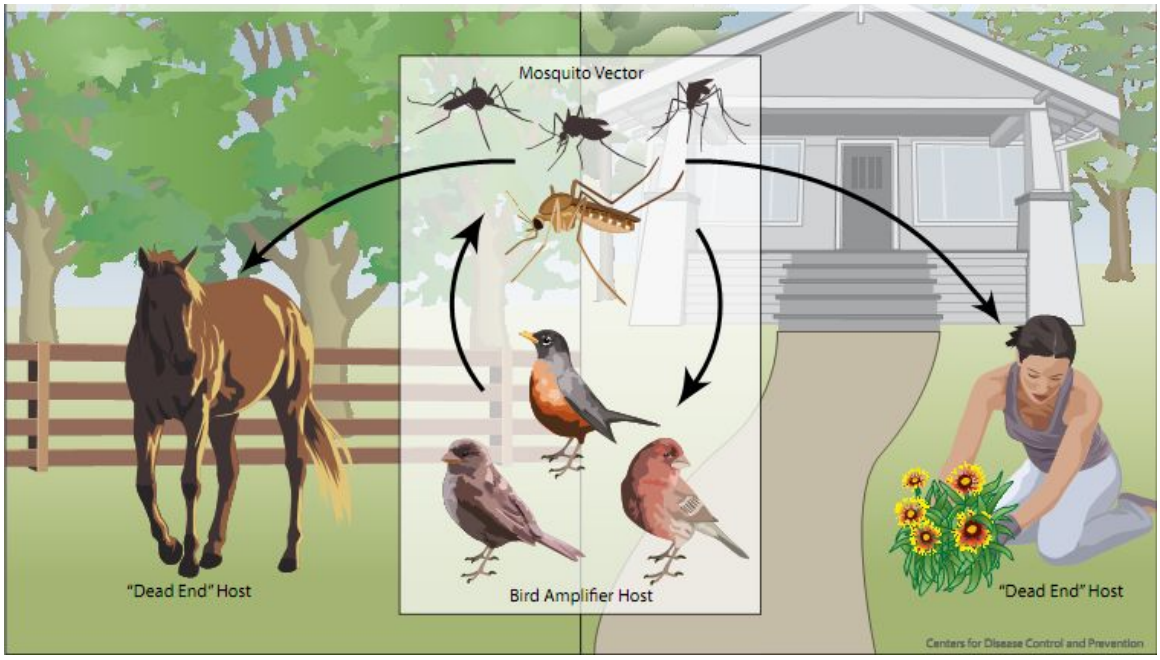


Figure 4: WNV transmission cycle: Source: CDC

The urban cycle occurs in a similar way, however it does involve different vector and bird species; for example, in this transmission mode, the principal vectors are *Culex pipiens* and *Cx. molestus* seen as both species feed on synanthropic, domestic birds and humans (Hubalek and Halouzaka, 1999).

Moreover, the main WNV vectors vary according to their geographic distribution, for example, the predominant vector species in Europe are *Culex pipiens*, *Cx. molestus* and *Coquillettidia richiardii* (Hubalek and Halouzaka, 1999). In Africa and The Middle East, the most important vector is *Culex univittatus*, whereas in Asia, *Culex quinquefasciatus*, *Cx. tritaeniorhyncus* and *Cx. vishnui* are the main vectors (Hubalek and Halouzaka, 1999).

Although the rural cycle is of utmost importance for WNV transmission, this flavivirus can also be transmitted via organ transplants, blood transfusions and via infectious maternal milk (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004). Additionally, reports show that transovarial transmission occurs in some species, namely *Cx. univittatus*, *Cx. tritaeniorhyncus*, *Aedes aegypti* and *Ae. Albopictus* (Hubalek and Halouzaka, 1999).

2.5.4. Arthropod vectors of WNV in Africa

WNV is primarily spread via mosquito that fed on birds that develop high levels of the vireamia. Those mosquitoes transmit the virus to susceptible vertebrates, including humans (Hayes *et al*., 2005). The abundance and feeding patterns of infected mosquitoes, influence the likelihood of WNV transmission (Dauphin *et al*., 2004; Hayes, 1989; McIntosh *et al*., 1976). As a result of the abundance of human biting mosquitoes in sub-saharan Africa (SSA), compared to the low incidence reported during epidemics in Europe and North America, up to 55% of human populations appeared to be seropositive during epidemics in Africa (Dauphin *et al*., 2004; Hayes, 1989).

Experimental trials in conjunction with field studies demonstrated that mosquitoes are the vectors of WNV. Isolates of the virus was obtained from different species of *Culex* (*Cx*) mosquito (*Cx. antennatus*, *Cx. univittatus* and *Cx. pipiens*) (Becker *et al*., 2003; Benelli *et al*., 2016; Bisimwa *et al*., 2016). *Cx. univittatus* has been implicated as the primary WNV vector in Egypt and South Africa, based on field isolation rates. In Egypt, WNV isolates were obtained from mosquitoes and febrile children (Hayes, 1989; Hayes *et al*., 2005; Huang *et al*., 2014b). In South Africa the isolates were obtained from *Cx. theileri*, *Cx. pipiens*, *Cx. neavei*, *Ae. caballus*, *Ae. circumluteolus* and *Coquillettidia* spp. WNV was also isolated from *Cx. poicilipes* in Senegal and *Ae. Albocephalus* in Madagascar (Hubalek and Halouzaka, 1999). Miller *et al*. (2000) reported the isolation of WNV from a male *Cx. univittatus* mosquito trapped in northwestern Kenya. This isolation suggests the transovarian transmission of WNV in vector population.

The other vectors that are suspected for WNV transmissions are ticks, although their importance is unclear. WNV was isolated from soft ticks like *Argas hermanni* from Egypt (Hubalek and Halouzaka, 1999). Additionally, isolates was also recovered from varies hard tick genera such as *Hyalomma*, *Rhipicephalus*, *Amblyomma* and *Dermacentor* from Africa (Lwande *et al*., 2013). There is no report related with WNV detection in Ethiopia yet.

2.5.5. Clinical features

West Nile disease was at first considered a minor health concern, as the majority of infections were mild or completely asymptomatic; however, after the major outbreaks that

occurred in Europe and especially in North America from 1999, WN disease became a public health priority (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004).

After an incubation period of 3 to 14 days, between 15-20% of humans present with a mild febrile illness accompanied by flu-like symptoms, a rapid onset of moderate to high fever, headache, malaise, myalgia, anorexia, nausea, backache and retro-orbital pain (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004). Other disease manifestations include lymphadenopathy, conjunctivitis, maculopapular or roseolar rash, which is normally observed in 50% of patients (Gould and Solomon, 2008; Hubalek and Halouzaka, 1999). Around 1% of all patients tend to develop neurological signs such as acute aseptic meningitis, encephalitis or myelitis (Zeller and Schuffenecker, 2004). Additionally, severe infections may also provoke hepato- and splenomegaly, hepatitis, pancreatitis and myocarditis (Gould and Solomon, 2008; Zeller and Schuffenecker, 2004). A mortality rate of 5 to 10% usually results from all patients presenting with neurological symptoms (Gould and Solomon, 2008).

2.5.6. WNV detection methods

Clinical signs of WN viral infections are non-specific which mimics those caused by other pathogens and toxins. So, infection in humans or animals may need a confirmation with the identification of WNV genome, antigen or specific antibodies. Many tests developed like WNV isolation in Vero or mosquito cell lines, or isolation in infected suckling mice brain tissue followed by serologic identification (Beasley, 2005; Cho and Diamond, 2012; Dauphin *et al*., 2004; Hayes *et al*., 2005; Kanagarajan *et al*., 2003). WNV-specific antibodies as well as antigen can be detected using IgM antibody-capture ELISA (MAC-ELISA), HI antibody detection, complement fixation test (CFT), immunofluorescence assay (IFA), microsphere immunoassay and plaque reduction neutralization test (PRNT) (Beasley, 2005).

Serologically, WNV is a member of the Japanese encephalitis serocomplex. This complex includes Japanese encephalitis virus and an endemic North American flavivirus, St Louis encephalitis virus. WNV grouped in to 5 phylogenetic lineages while only lineages 1 and 2 WNV were mentioned in causing human disease outbreak (May *et al*., 2011). WNV usually known in causing brain pathology with its nature of neurovirulence; however, critical

requirements for this nature in human rely on the virus capacity to enter in to the central nervous system (CNS) (ie, neuroinvasiveness) (Petersen *et al*., 2013).

Suggestion for WNV neuroinvasive mechanisms could be with direct viral crossing of the barrier (blood-brain barrier/BBB) due to cytokine-mediated increased vascular permeability; passing via the endothelium of the BBB; by means of a 'Trojan horse' mechanism in which the infected tissue macrophages are trafficked across the BBB; and retrograde axonal transport of the virus to the CNS through infection of olfactory or peripheral neurons (Cho and Diamond, 2012).

In serological assay, confirmation with neutralization test (NT) is crucial due to cross-reactions between flaviviruses. In Africa, many of those methods have been used in surveillance for WNV. The surveillance was done in camels, goats, cattle and sheep in Nigeria by heamagglutination inhibition (HI) test for WNV antibodies (Olalye *et al*., 1990). Furthermore, enzyme linked immune-sorbent assay (ELISA) and plaque reduction neutralization tests (PRNT) have been used for WNV sero-surveillance in one of the study conducted in Nigeria (Baba *et al*., 2013). More methods was used as described by (Venter *et al*., 2017) to diagnose WN disease in horses using a combination of post-mortem histopathologic examination of brain samples and WNV-NS5-specific nested real-time RT-PCR in South Africa.

Although virus isolation is considered a "gold standard" method for detection and identification of viruses, it is time consuming, relatively expensive, and requires specialized laboratories with tissue culture and biosafety level 3 conditions. An alternative to virus isolation is to use molecular techniques to detect viral genomic RNA. Molecular based assays are highly sensitive and specific and are readily standardized with good reproducibility within and between laboratories. As a result, nucleic acid detection assays have become acceptable alternatives to virus isolation and important tools for diagnosis and research in virology (Chao *et al*., 2007; Mackay *et al*., 2002). As common alternatives, the use of reverse transcriptase polymerase chain reaction (RT-PCR) is a common method for the detection of viral RNA from field-collected samples. A method for WNV RNA genome detection in viral vector (i.e. mosquitoes) is used for viral surveillance in tropical regions endemic for other *flaviviruses*. RT-PCR assays, standard and real time PCR, are important to detect WNV and other flaviviruses (Gonzalez-Reiche *et al*., 2010).

RT-PCR is that technology by which RNA molecules are converted into their complementary DNA (cDNA) sequences by reverse transcriptase, which is followed by the amplification of the newly synthesized cDNA by standard PCR procedures. This approach to study gene expression is universally known as RT-PCR, because of the role of reverse transcriptase (RT) in the synthesis of first-strand cDNA. In doing RT-PCR, sample normalization is very important, and the efficiency of first-strand cDNA synthesis is one of the most important determinants in the success or failure of this method. For this reason, it is strategically better to make a large cDNA pool from which aliquots may be drawn for individual applications rather than repeating the same cDNA synthesis reaction over and over. Furthermore, primer designing promote a proper balance between template specificity, thermodynamic stability when base-paired to the template, and capacity of one primer to function with the other(s) to support RT-PCR (Beasley, 2005; Bisimwa *et al*., 2016; Gonzalez-Reiche *et al*., 2010; Hoyos-Lopez *et al*., 2016).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in selected areas of the Ethiopian Rift Valley areas such as Batu, Hawassa, Wolayta Sodo, and Arba-Minch. The areas were selected based on their ecological significance for maintenance of mosquitoes and mosquito borne diseases and have permanent water bodies (Lake Ziway, Lake Hawassa, Lake Chamo and Lake Abaya) that may favor breeding of mosquitoes all year round. Also suspected Yellow Fever outbreak in October 2018 was the other criteria for site selection.

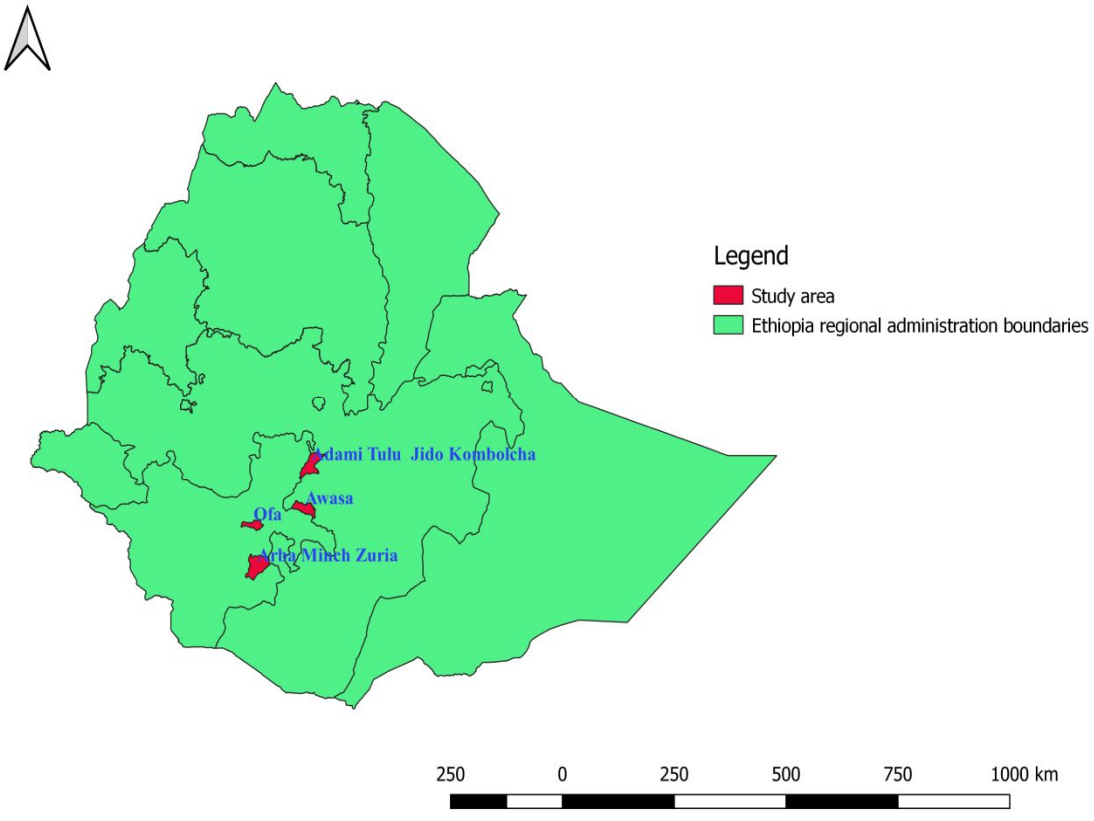
Batu is located in Adami Tulu Jido Kombolcha district which is one of the districts in Eastern Shoa zone of Oromia region in the mid Rift Valley of Ethiopia. The main town of district is Batu (also called Ziway) which is found on the western shore of the Lake taking same name. Batu is located on the road connecting Addis Ababa to Hawassa to Nairobi in the East Shewa Zone of the Oromia Regional state of Ethiopia. Batu has a latitude and longitude of 7°56′N 38°43′E with an elevation of 1643 meters above sea level. Lake Batu is one of the lakes in the rift valley used for multiple purposes like irrigation, fishing, domestic water supply, transportation, recreation, supply of fresh water and horticulture farming (CSA, 2013).

Hawassa is the largest city in the Great Rift Valley of Ethiopia on the shores of Lake Hawassa. Hawassa is found in Southern Nations Nationalities and Peoples Regional State (SNNPR), situated 275 km south of Addis Ababa (the capital of Ethiopia) at a latitude of 7°04'N and a longitude 38°31'E on the escarpment of the Great Rift Valley. The altitude ranges from 1650 to 1700m above sea level. The mean annual rainfall and temperature are 900-1100 mm and 27°C, respectively. The total livestock population of Sidama zone is estimated to constitute, 1,721,341 cattle, 228,941 goats, 457,465 sheep, 204,460 equines, 725,540 poultry and 44,492 bee hives (Admasu, 2015; CSA, 2013).

Wolayta Sodo is located in the Southern Nations Nationalities and Peoples Regional State (SNNPR) about 383 km from Addis Ababa. Its altitude ranges from 1650 to 2980 meters above sea level, it receives an annual rain fall of 100 – 1200 mm and an annual temperature of

25 – 35°C. The altitude of the area is a mid altitude which is below 1600 and livestock population found in the Wolayta Sodo town comprises about 128,919 cattle, 29191 sheep, 4606 equines and 55278 poultry (CSA, 2013).

Arba-Minch is a city again situated in SNNPR, Gamo Goffa Zone. Arba Minch is in the western side of the Great Rift Valley, on the shores of two major lakes, Lake Chamo to the south and Lake Abaya to the north. It is surrounded by mountainous high lands from South-West directions and the rest covered with jungle of natural forest. It is located 505 kms far from Addis Ababa to the south at an elevation of 1285 meters above sea level. The town is one of the low lands in the SNNPR having a hot climate with average temperature of 29^0C and annual mean rain fall of 900mm. The town has 2 subdivisions; Siecha and Sikela, each 5kms apart (CSA, 2013; Feki, 2014).



3.2. Study Design and Study Population

Between December 2018 and April 2019, a cross-sectional study was conducted to collect mosquitoes and undertake questionnaire survey in the selected areas. This study included survey for different mosquitoes with the genus *Aedes*, *Culex, Anopheles* and *Mansoni* for viral detection while livestock owners, local farmers, and other communities were assessed for their knowledge, attitude and practices (KAP) regarding control and prevention of mosquitoes. The participants in KAP assessment were eligible if they were residents of each specific locality, age over 18 years, a husband/wife in the selected households and volunteer to be interviewed.

3.3. Sample Collection and Processing

3.3.1. Mosquito collection and identification

Mosquito collection in the selected sites was carried out using CDC light traps for a period of at least 12 hours (in order to include sunset and sunrise) thus covering mosquito activity peaks. CDC light traps were placed mainly inside animal shelters or in their close proximity and also in the houses of animal owners since their animal pass the night time in the house with the family members. Field-collected mosquitoes were kept in liquid nitrogen containers for transportation to the laboratory. Collected specimens were morphologically identified to the genus level using stereomicroscope, according to the morphological identification keys of Walter Reed BioSystemics Unit WRBU; Potter, (2016); Becker *et al*. (2003); Ribeiro *et al*. (1999). Mosquitoes were then pooled into groups of approximately 20-25 mosquitoes based on their genus and collection site, assigned numbers and placed into 1.5ml vacutainer tubes, and stored at -80ºC until further processing.

3.3.2. Mosquito processing

The mosquito pools were homogenized in a biosafety level 2 laboratories at the National Veterinary Institute of Ethiopia. The pools of mosquitoes were grounded using sterile mortar and pestle by adding 2 ml of sterile phosphate-buffered saline (PBS) containing antibiotic. The supernatant was then harvested by centrifuged at 12,000 rpm for 10 minutes and stored in a 1.5 ml cryovial at -20°C for further testing.

3.3.3. Viral RNA extraction

Viral RNA was extracted using the QIAamp Viral RNA Mini Kit for nucleic acid extraction (QIAGEN, AMBION, Inc., Austin, Texas, USA) accordance with the manufacturer's instructions. Briefly, 560 µl of lysis buffer containing carrier RNA and 140 µl of homogenized mosquito supernatant were added to a 1.5ml micro centrifuge tube. The contents were pulse vortexed for 15 second and afterwards incubated at room temperature for 10 min to ensure complete viral particle and cellular lysis. Protein precipitation was conducted by adding 560 µl of absolute ethanol followed by pulse vortexing for 15 second. Precipitates were settled by centrifugation at 13,000 g for 5 min. The supernatant was carefully withdrawn and passed through a silica-gel column, followed by washing of the column twice with 500 µl of each of the washing buffers AW1 and AW2, respectively. Finally, RNA was eluted by addition of 50 µl of buffer AVE equilibrated at room temperature. The viral RNA extracted was immediately stored at -20^{o}C until amplification (**Appendix I**).

3.3.4. Reverse transcription PCR

West Nile Virus genome detection in mosquito pools was conducted using reverse transcription PCR (RT-PCR). RT-PCR amplification was performed using specific primers (forward primers: 5'- GTGCTGGTAAAACAAGGAGG -3' and reverse primers: 5'-TGTATCCTCTAGCCGCGATG-3') targeting non-structural protein (NS3) to amplify a region of 292bp according to Frost *et al*. (2012). The NS3 is the conserved region of the viral genome and plays an important role in the replication of the virus. RT-PCR was performed by using one-step RT-PCR kit (Invitrogen, USA) and the master mixes were made according to manufacturer's instructions. Briefly, a total of 25 µl of master mix was prepared, containing 5µl of template RNA, 2µl of each forward and reverse primers, 5 µl of 5x one-step conventional gel based-PCR buffer, 5µl of 5x Q solution, 1µl of 10mM each deoxynucleoside triphosphate (dNTP) mix, 1µl one-step enzyme mix, and 4 µl of RNase free water. The region of interest was amplified with the following conditions: 50°C for 30 min, followed by 94°C for 15 min, then 35 cycles of denaturation at 94°C for 30 sec, primers annealing temperature at 50°C for 30 sec, and extension at 68°C for 45 sec. The reaction was then subjected to final extension at 68^{o}C for 10 min.

3.3.5. Visualization of RT-PCR products

The RT-PCR products were visualized by electrophoresis in a 1.5% agarose gel in 0.5 x Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer (SERVA, Heidelberg, Germany) stained with GelRed nucleic acid stain. Each well was loaded with 5 µl of the PCR product and 5 µl of 6 x DNA loading dye (Promega, Madison, USA). Samples were separated along with DNA ladder (Promega, Madison, USA) at 120V for 80min. The PCR products were visualized using gel documentation system and scoring was done based on the size of the PCR products (**Appendix II**).

3.3.6. Questionnaire

The kebeles surrounding each city were selected to represent different direction in the cities and based on their accessibility for transportation. The households were again selected by transect-walking through each kebele and stopping at regular interval (often every 3-4 households). The demography of selected individuals was shown in **Table 1**. Semi-structured questionnaire was developed in English, based on information from available literatures regarding community knowledge, attitudes and practices about vectors from previous studies elsewhere. The questionnaire was pre-tested for clarity and acceptability in the study areas by non-participatory individuals in the study. During pre-testing, additional information was gathered and some of the questions have been modified. The participants were interviewed about the veterinary and public health importance, control methods, and preventive methods of mosquitoes in their own local language. Each interview was made by a house-to-house visit. Information on the socio-demographic characteristics of the participants was also included in the questionnaire (**Appendix III**).

Out of a total of 288 persons participated in the study, majority (39.2%) was selected from Batu and 71.9% were male. Among the study participants, higher number of respondents 165(57.3%) were having age ranges between 31-45 years and relatively few number of people were age greater than 60 years. It was only a little bit higher than a quarter of the total respondents were without formal education and majority of them were educated at different levels. Nearly 60% of the study participants' primary occupation was non-farming. All of the farming households

included in the study were keeping cattle and majority also owning goats, equids and chicken. Most of the households had number of family numbers ranging from 6-8 persons (**Table 1**).

Table 1: Socio-demographic characteristics of the study participants (n=288)

Factors	Categories	n	%
Sites	Batu	113	39.2
	Arbaminch	44	15.3
	Hawassa	70	24.3
	Wolaita Sodo	61	21.2
Gender	Male	207	71.9
	Female	81	28.1
Age (years)	18-30	34	11.8
	31-45	165	57.3
	46-60	76	26.4
	> 60	13	4.5
Level of education	No formal education	76	26.4
	Primary	78	27.1
	Secondary	52	18.1
	TVET/Diploma	41	14.2
	First Degree	41	14.2
Primary occupation	Farming	116	40.3
	Non-farming	172	59.7
Domestic animal ownership (species)	Cattle	117	40.6
	Goats	71	24.7
	Sheep	15	5.2
	Equids	77	26.7
	Chicken	70	24.3
Family size (number)	<=5	80	27.8
	6-8	161	55.9
	9-12	47	16.3

3.4. Data Management and Analysis

The data generated from the study was arranged, coded and entered to Excel spread sheet (Microsoft® offices excel 2007) and the statistical software RStudio, Version 1.1.383.0 was used for the data analysis. Descriptive statistical data analysis tests were used and the score of knowledge, attitude and practice of the community regarding control and prevention of vectors was generated and summed-up. P-values below 5% were considered as indicators of statistical significance.

3.5. Ethical Clearance

The study protocol for the project was approved by research ethical review committee of Addis Ababa University College of Veterinary Medicine and Agriculture (**Appendix IV**). The aim of the study was explained to each participant on KAP assessment and verbal consent was obtained. Verbal informed consent was deemed appropriate due to the expectation of relatively low literacy levels among participants. Each participant was interviewed independently and the collected information was kept confidential.

4. RESULTS

4.1. Viral Detection

In this study, a total of 2,322 mosquitoes were collected from all study areas. Based on morphological identification, these mosquitoes were belonging to four genera: *Culex* (n = 460; 20%), *Aedes* (n = 418; 18%), *Mansoni* (n = 225; 9.7%) and *Anopheles* (n = 120; 4.7%) as shown in **Table 2**. For WNV genome detection, a total of 38 pools of mosquitoes containing approximately 20-25/pool were examined using RT-PCR, of which none of the mosquito pools was positive for WNV genome as illustrated in **Figure 5 – 7**.

Table 2: Summary of mosquito genera collected and tested for WNV

	Batu	Hawassa	Wo/Sodo	Arba-Minch	Total
Culex	161	142	28	129	460(20%)
Aedes	147	137	22	112	418(18%)
Mansoni	89	63	25	48	225(9.7)
Anopheles	44	51	14	11	120(4.7)

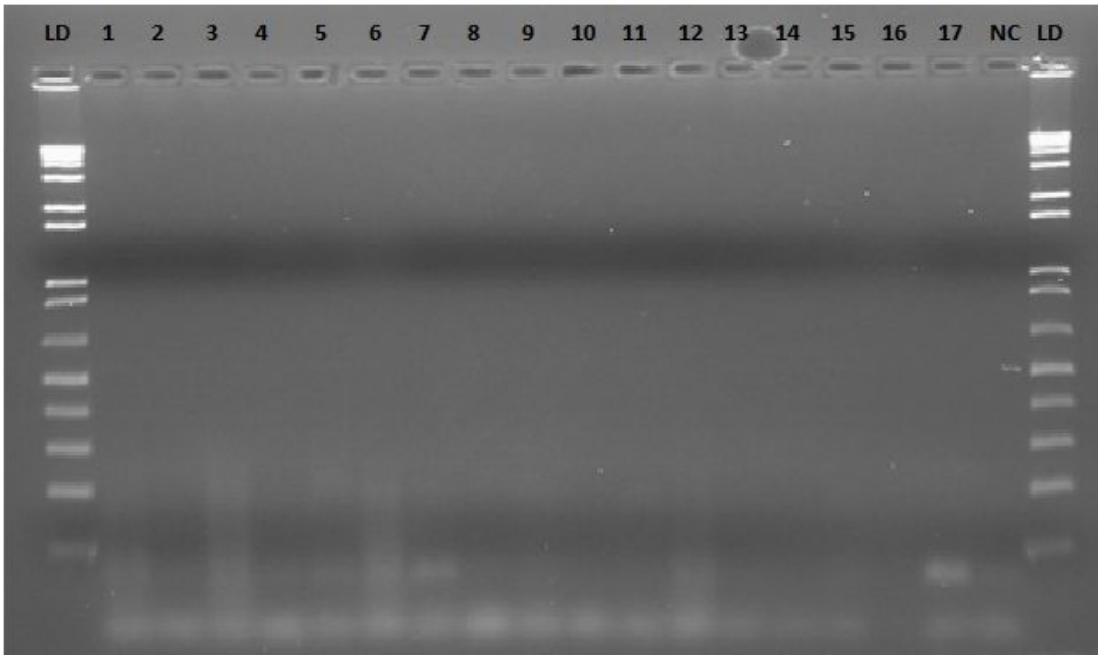


Figure 5: Illustrate WNV genome detection from mosquito pools collected during study periods. Amplification of WNV genome using RT-PCR targeting the NS3 region to amplify 292 bp. NC: negative control; LD: ladder. Lane numbers indicate the pool identity from 1 – 17.

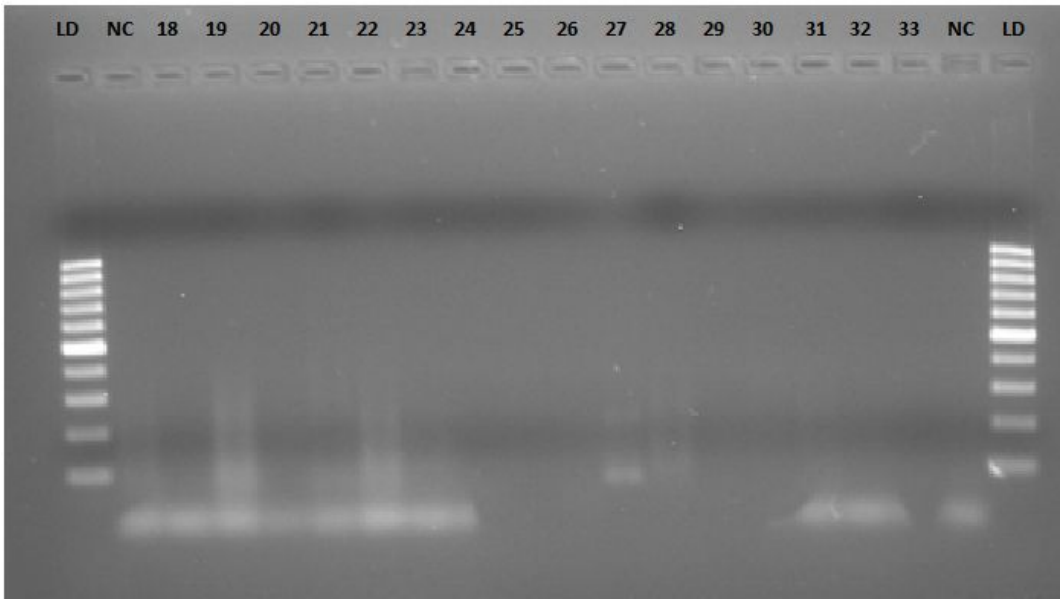


Figure 6: Illustrate WNV genome detection from mosquito pools collected during study periods. Amplification of WNV genome using RT-PCR targeting the NS3 region to amplify 292 bp. NC: negative control; LD: ladder. Lane numbers indicate the pool identity from 18 – 33.

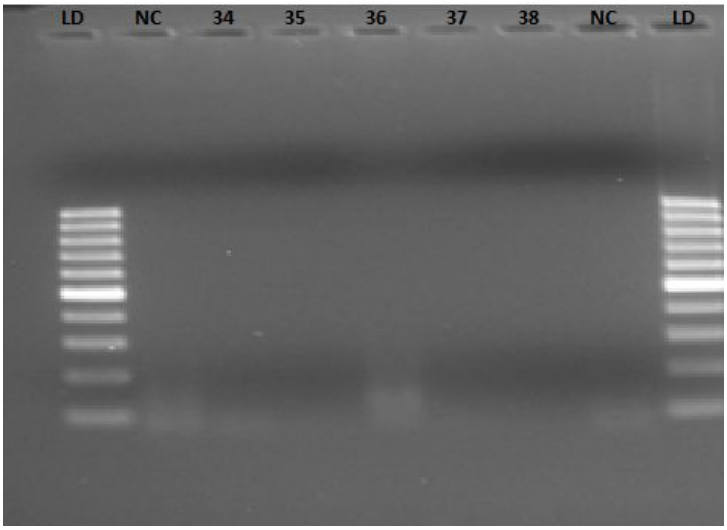


Figure 7: Illustrate WNV genome detection from mosquito pools collected during study periods. Amplification of WNV genome using RT-PCR targeting the NS3 region to amplify 292 bp. NC: negative control; LD: ladder. Lane numbers indicate the pool identity from 34 – 38.

4.2. Knowledge, Attitude and Practices with Regard to Arthropod Vectors (Emphasis on Mosquitoes)

4.2.1. Community knowledge about mosquitoes

Even though almost all of the participants 282(97.9%) were knew that mosquitoes bite humans, only 182(63.2%) respondents were aware that mosquitoes bite both humans and animals. However, (32.3%) of the respondents were knew that mosquitoes bite animals. Participants of the survey had different level of awareness about other insect vectors those bite humans, animals and both (73(25.3%) and 85(29.5%) of the participants were knew that flea and tsetse flies bite both humans and animals. **Table 3** depicts the frequency of people listing insects which can bite and cause negative impacts on human or animal health or both.

Table 3: Frequency of people listing insects which can bite and cause negative impacts on human or animal health (n=288)

	*Biting effect	
Insects	Humans	Animals
Mosquitoes	97.9	32.3
Flea	42.2	27.8
Lice	25.0	4.5
Tsetse	30.2	63.5
Ticks	-	44.4
Bedbug	71.5	-

*Percentage of responded "yes"

Among the participants, 138(47.9%) were aware that marshy areas were a breeding source for mosquitoes and 195(67.7%) were knew mosquitoes breed on dirty places around the house and irrigation farms of tomatoes and other vegetables. Slightly higher than the half of the participants 160(55.6%) were knew bushes were favorable breeding sites for mosquitoes while the majority of the participants 220(76.4%) responded they knew mosquitoes rear on water collections. Higher number of participants 272(94.4%) were aware that wet season is a season of high mosquitoes population and except one participant (0.3%), all of the respondents 287(99.7%) answered that mosquito borne diseases transmitted by mosquito bite during night time.

Community knowledge about vector activities of mosquitoes and diseases associated with the vectors were shown in Table 4. Almost all of the respondents 286(99.3%) were mentioned biting is the major negative impact of mosquitoes even if nuisance 227(78.8%) and itching 141(49%) were also the other negative impacts. In the free listing question regarding diseases transmitted by mosquitoes, 279(96.9%) interviewees mentioned malaria, and two-third of the respondents mentioned yellow fever. Of interviewed, 89(30.9%) were thought mosquitoes can transmit thyphus.

Table 4: Community knowledge about vectoral activities of mosquitoes and diseases associated with the vectors

Factors	Categories	n	%
Mosquito breeding sites	Marshy areas	138	47.9
	Dirty areas	195	67.7
	Water bodies	220	76.4
	Bushes	160	55.6
Mosquito biting time	Day	1	0.3
	Night	287	99.7
When high mosquito population is observed	Wet season	272	94.4
	Dry season	16	5.6
Diseases transmitted by mosquitoes	Malaria	279	96.9
	Yellow fever	208	72.2
	Typhus	89	30.9
	Typhoid	2	0.7
Do mosquitoes transmit diseases between human and animals?	Yes*	29	10.1
Do mosquitoes transmit diseases from humans to humans?	Yes*	272	94.4
Is that possible to control mosquitoes?	Yes*	283	98.3
Source of information	Media	49	17.0
	Society	123	42.7
	Professionals	259	89.9

*Number and percentage responded "Yes".

In the present study 272(94.4%) of the participants were aware that mosquitoes can transmit diseases from humans to humans, however, only few participants 29(10.1%) were knew that mosquitoes can transmit diseases between animals and humans. Health professionals 259(89.9%) were the major sources of information to the community regarding mosquitoes potential to transmit diseases from human to human with additional awareness contributed from the community 123(42.7%) indigenously.

4.2.2. Community attitude about mosquitoes

All of the participants 288(100%) and 285(99%) considered mosquitoes are a threat to the community in their area and they are at risk of getting diseases transmitted by mosquitoes respectively. Very high proportion of the respondents 270 (93.8%) believed that controlling mosquitoes can help in prevention of mosquito borne diseases. The majority 226(78.5%) responded that it is the sole responsibility of government on mosquito management while 149(51.7%) and 168(58.3%) of the respondents suggested that professionals (vets and health professional) and every individual, respectively, has responsibility to control mosquitoes. Most 273(94.85) of the respondents believed they can protect themselves from mosquitoes. About two third of the community were mentioned they have responsibility individually 222(77.1%) and also the community is responsible 215(74.7%) in mosquito control measures. **Table 5** shows the attitude of participants about mosquitoes.

Table 5: Attitude of participants about mosquito vectors and their control

Statements	n	%
Mosquitoes are threat to the community	288	100.0
Are you at risk of diseases transmitted by mosquitoes?	285	99.0
Do controlling mosquitoes can help in prevention of diseases?	270	93.8
Who is responsible to control mosquitoes?		
Government	226	78.5
Vets and health professionals	149	51.7
Any individual	168	58.3

4.2.3. Community practices on mosquito control

Among the participants in the current study, 268(93.1%) were practiced mosquito control measures in the past times. A slightly higher than half of the respondents 165(57.3%) were using insecticide chemicals on regular basis (82(28.5%) yearly, 61(21.2%) six months and 22(7.6%) three months intervals); and more than three fourth 225(78.1%) of the participants reported they were using bed nets daily (**Table 6**).

Table 6: Practices of the participants on mosquito control (n=288)

Practices	n	%
1. What practices do you follow to control mosquitoes?		
Use insecticide chemicals	165	57.3
Use bed nets	225	78.1
Eliminate standing water around the house	259	89.9
Cut down bushes in the yard	252	87.5
Prevent water stagnation	267	92.7
Cleaning of garbage/trash	265	92.0
Disposing water holding containers	267	92.7
Smoking different plants	251	87.2
Burning farms and pasture	38	13.2
2. How often do you practice mosquito control measures?		
Daily (bed nets)	224	77.8
Three months (insecticides)	22	7.6
Six months (insecticides)	61	21.2
Yearly (insecticides)	82	28.5
3. What do you do in case you get these diseases		
Consult physician	287	99.7
Traditional healers	2	0.7
Home remedy	54	18.8

About 259(89.9%) of the respondents reported that they eliminate standing water around the house. The majority of the respondents 252(87.5%), 267(92.7%) and 265(92.0%) were reported that they regularly cut down bushes in the yard, prevent water stagnation and clean garbages, respectively. Of the total respondents, 267(92.7%) were reported they properly dispose water holding containers such as tires, parts of automobiles, plastic bottles, cracked pots etc. to prevent water storage. Even though only few 38(13.2%) participants were reported regular burning of their farms and grazing lands, 251(87.2%) respondents were reported daily smoking of different aromatic plants in and around their house. When the participants were asked what their course of

action would be if they noticed they had mosquito borne diseases, all of them 288(100%) reported they would consult a physician and few 54(18.8%) added that they would use other home remedies.

5. DISCUSSION

The present study was conducted to detect West Nile Viruses in mosquitoes using molecular techniques and to assess the knowledge, attitude and practices of the community regarding the control and prevention of mosquitoes in selected areas of Rift Valley of Ethiopia. Although many studies have been done on mosquito borne diseases (MBDs), especially on malaria, extensively in Ethiopia, prior to the present study, no information was available concerning WNV in mosquitoes and KAP assessment on mosquito control and prevention methods. This baseline information should help to fill this gap and in the development and improvement of targeted mosquito control and prevention strategies and measures in the future.

In the present study, WNV was not detected in any of the mosquito pools. Similar with this study, a study conducted in selected areas in Kenya also did not detect any human-related arboviruses (WNV, YF, RVF, DENV and others) by reverse transcription-polymerase chain reaction (RT-PCR) (Iwashita *et al*., 2018). Even though the result directly depicts the absence of the virus circulating in mosquito vectors in the study areas, there are also some other factors that can determine the results. The first factor may be the season in which the vectors were collected, which might affect the feeding pattern, abundance and movement of mosquitoes that can limit them from accessing blood meal (Huang *et al*., 2014). The other factor may be the density of pools with Culex mosquitoes comprises only 20% of the total pool since Culex species are implicated as the primary WNV vector in sub-saharan Africa, based on field isolation rates (Hubalek and Halouzaka, 1999; Iwashita *et al*., 2018).

In the present study, almost all of the participants (97.9%) were aware that mosquitoes can bite humans and only two-third of the respondents knew mosquitoes can bite both humans and animals. Similar to the current study in which 94.4% of the participants knew mosquitoes can transmit diseases, a study by Dhaduk et al., (2013) showed high awareness level of people about mosquito. If every person residing in an endemic area is aware about this fact it would lead to full-fledged mosquito control measures among residents in that area. Health professionals are contributing much higher as the major sources (89.9%) of information for the community

regarding this awareness. This result is in line with a study conducted by Astatkie (2010) in Arba-minch town and Arba-Minch zuria districts, Southern Ethiopia.

Except one participant (0.3%), all of the respondents (99.7%) replied that mosquitoes bother or bite the most at night during sleeping and this is in contrary with the report of Sharma et al. (2017) (26.5%). However, 83.8% night biting habit of mosquitoes was reported by Abate et al. (2013) in Shewa-Robit town, north-eastern Ethiopia. This correct perception among respondents of the present study is encouraging to take appropriate preventive measures and proper use of mosquito nets.

About 283 (98.3%) respondents thought mosquitoes can be prevented and 93.8% believed MBDs are preventable for which all respondents would seek medical help while 18.8% would prefer treatment at home additionally in case they get MBDs. Studies found by Alobuia et al. (2015) in western Jamaica (95.3%) and, Kumar and Gururaj (2005) (95.7%), Malhota et al. (2014) (94.13%) and Sharma et al. (2017) (90.06%) in India are consistent with the current study.

Higher number of respondents (94.4%) aware that wet season was a season of high mosquito population. This is because of the rain and due to lack of proper drainage systems in the villages/towns and stagnant rain water and mud is found here and there throughout the country in rainy/wet season. A study conducted by Astatkie (2010) in Arba-minch town and Arba-Minch zuria districts, Southern Ethiopia, revealed similar finding(~96%).

With respect to type of diseases transmitted by mosquitoes, Malaria (96.9%) is most commonly known mosquito borne disease followed by yellow fever (72.2%) which is in line with Sharma et al. (2017) (94.51%). Dhaduk et al. (2013) also reported 90.67% in Jamnagar, India. In the present study, 30.9% of respondents thought mosquitoes can transmit typhus while Kumar and Gururaj (2005) reported 23.78% transmission of typhoid through mosquitoes.

The knowledge of the community, as seen in the study, regarding the breeding places of the mosquitoes was limited. Among the participants, less than half (47.9%) aware that marshy areas are a breeding source for mosquitoes which is similar with report of Legesse et al. (2018) in South Omo area, Southern Ethiopia (47.9%). Two-third of the respondents (67.7%) knew mosquitoes breed on dirty places around the house and irrigation farms of tomatoes and other

vegetables, and slightly higher than half of the participants 55.6% know bushes are favorable breeding sites for mosquitoes. This is by far higher than report of Mehta et al. (2015) in an Urban Area of Bhavnagar (13.5%) and Dhaduk et al. (2013) in Jamnagar district, India (5.33%).

Three-fourth of the participants (76.4%) aware that mosquitoes rear on water collections and this finding is lower than that of Abate et al. (2013) in Shewa-Robit town, north-eastern Ethiopia (91.6%) and Legesse et al. (2018) in South Omo area, Southern Ethiopia (94.5%) even if it is higher than Mehta et al., (2015) in an Urban Area of Bhavnagar (27.7%). None of the respondents mentioned about indoor breeding of mosquitoes. Therefore, vector-borne disease control program should stress the importance of source reduction as a method for control of indoor mosquito breeding for prevention activities. A sound knowledge-base about vector-borne diseases and methods of vector control must be built among the community. Almost all of the respondents (99.3%) perceived biting is the major negative impact of mosquitoes followed by nuisance (78.8%) and itching (49%). This is in line with a report from Karnataka state, India by Kumar and Gururaj (2005).

Regarding community attitude, all of the participants considered mosquitoes as a threat to the community in their area and they are at risk of getting diseases transmitted by mosquitoes. Very high proportion of the respondents (93.8%) believed that controlling mosquitoes can help in prevention of mosquito borne diseases and this is in consistent with report of Mejia et al. (2016) in a Salvadoran urban community (96.4%). Even though 78.5% of the participants in the study believe that it is the sole responsibility of the government, it is interesting that more than half (58.3%) of respondents believe that it is every individual's responsibility to control mosquitoes. Half of the participants anticipate it from professionals (vets and health professional). Contrary to the present study, in the study conducted by Mejia et al. (2016) in a Salvadoran urban community, majority (93.2%) of the respondents believed that it is their own responsibility while only 25.5% suggested it is governments responsibility. Alobuia et al. (2015) reported 23.2% suggested it is their responsibility while 20.4% pointed toward the government in western Jamaica.

Among the participants in the current study, 93.1% are practiced mosquito control measures in the past times. Mejia et al. (2016) reported implementation of practices toward the prevention of

mosquito reproduction (58.5%) and a poor implementation to prevent mosquito bites (38.3%) in a Salvadoran urban community which much less than the present study. Also, all of them reported they consult a physician in case they noticed they had mosquito borne diseases and few (18.8%) added that they would use other home remedies additionally. The current study is in line with the study conducted by Sharma et al. (2017) (96%) and Legesse et al. (2018) in South Omo area, Southern Ethiopia (93.3%). Mehta et al. (2015) reported 17.3% of the community in an Urban Area of Bhavnagar practice home remedies to treat MBDs which is in line with the current study.

A slightly higher than half of the respondents (57.3%) use insecticide chemicals. This is lower than a report by Abate et al. (2013) in Shewa-Robit town, north-eastern Ethiopia (78.9%), but higher than the report in Jamaica (34.2%) by Alobuia et al. (2015) and (13.87%) by Sharma et al. (2017). More than three fourth (78.1%) of the participants reported they use bed nets daily and this is lower than a report in other parts of Ethiopia (Abate *et al*., 2013; Astatkie, 2010; Tomass *et al*., 2011).

The majority of the respondents reported that they regularly cut down bushes in the yard, prevent water stagnation and clean garbage, and also, of the total respondents, 92.7% reported they properly dispose water holding containers such as tires, parts of automobiles, plastic bottles, cracked pots etc. to prevent water storage. The current study is by far higher than the report recorded by Kebede et al. (2017) in Areka town, southern Ethiopia and Kumar and Gururaj (2005) from Karnataka state, India. 87.2% of respondents reported smoking of different aromatic plants in and around their house which is also reported by Abate et al. (2013) in Shewa-Robit town, north-eastern Ethiopia as fumigation.

The absence of dry ice, attractant for mosquitoes, during mosquito collection and inefficiency of the traps were the limitations of the current study; as a result, this study inadvertently fails to show much about abundance patterns of mosquito *spp.* It is also important to note that lack of positive control for detection of WNV genome by RT-PCR is another limitation that would hinder the result from realistically describe total absence of WNV in mosquito vectors in the study areas.

6. CONCLUSION AND RECOMMENDATIONS

The current study focused on detection of WNV in mosquitoes and assessing the knowledge, attitude and practices of the community regarding mosquito control and prevention. One step RT-PCR was conducted to detect WNV in different genera of mosquitoes (*Culex, Aedes, Mansoni and Anopheles*) collected from mid rift valley areas of Ethiopia. Regardless of season of collection, vector abundance, feeding pattern of the vectors and any other factors, the result from the study showed that mosquitoes in the study areas are free from WNV infection. The result does not mean the community and animals in the study areas are totally free from WNV.

The KAP assessment show that the community in the study areas had awareness about the importance of mosquitoes in disease transmission, the breeding season and biting time even if there are limitations on the knowledge about mosquitoes public health importance in transmitting diseases between humans and animals, breeding sites and diseases transmitted by mosquitoes. In the present study, the community in the study areas has interesting thoughts regarding the risk, control and prevention of mosquitoes. The assessment shows that, more than half of the total community believes it is individuals' responsibility to control and prevent mosquitoes side by side with government actions. Majority of the participants were keen in mosquito prevention and control measures in and around their home by using bednets, insecticide chemicals, cut down bushes, proper disposal of water holding containers, fumigation and etc. But, there is a large setback on taking action on common water sources, ponds, marshy and muddy areas and etc found in the village to prevent mosquitoes rearing on it.

In line with the present findings and available information, the following recommendations were forwarded:

- Further investigation on the virus should be conducted in animals (birds and horses) and humans in addition to mosquitoes in abundant season to find out the presence of circulating WNV in the country.
- Annual and/or seasonal based investigations on WNV were recommended.

- Continuous awareness should be created to the community concerning the breading sites of mosquitoes, diseases transmitted by mosquitoes and public health importance of mosquitoes by the concerning bodies.
- Since risk diseases are still prevalent, continuous effort should be put specially regarding the ability of mosquitoes to transmit diseases between human and animals to minimize risk of mosquito borne diseases.
- Practice of cleaning a village/town and common usage areas that are favorable for mosquito reproduction should be implemented.
- There should be promotions and rewards to motivate people on the control and prevention of mosquitoes from themselves and their community too.

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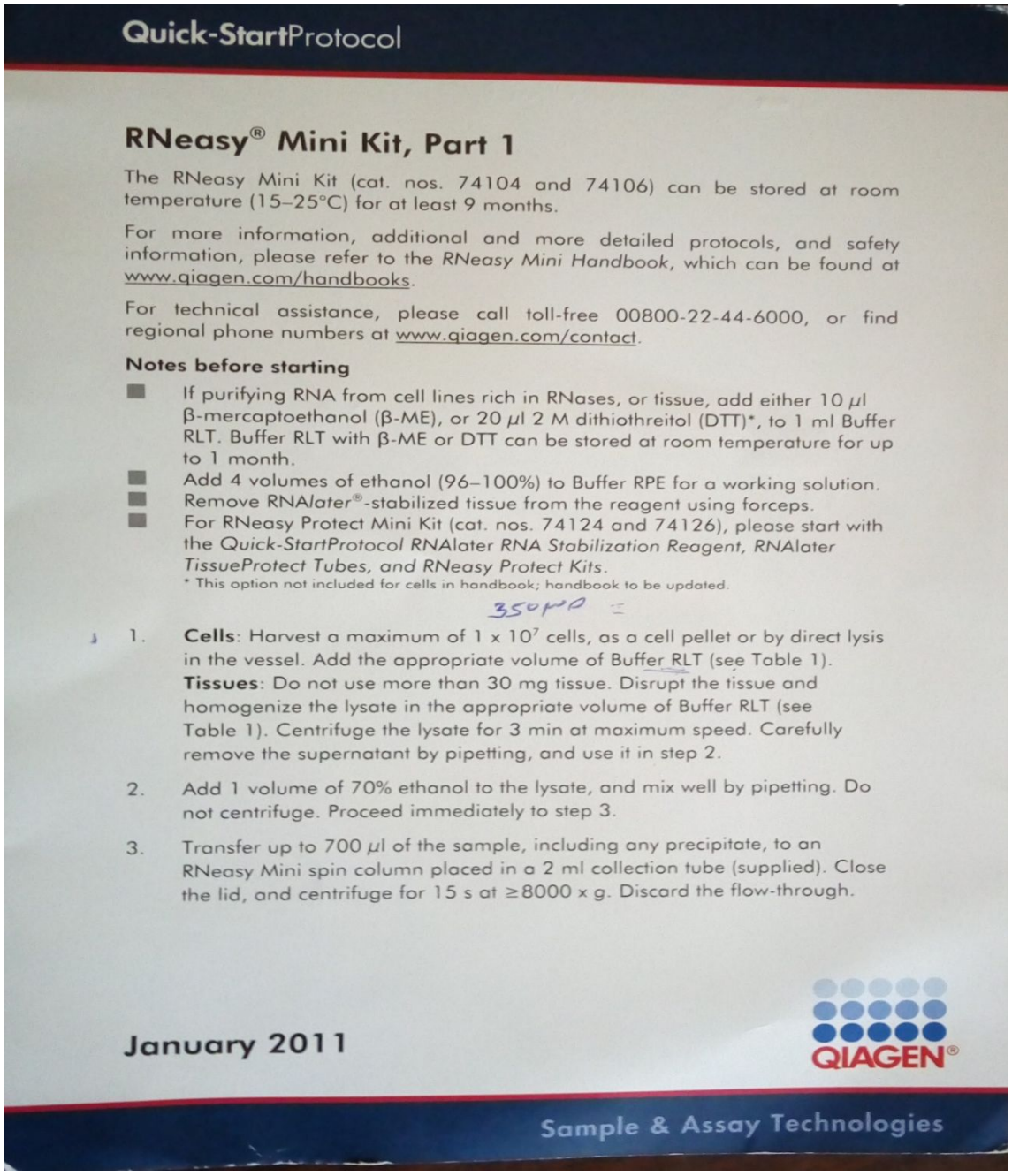
7. APPENDICES

Appendix I: Viral RNA extraction protocol (RNeasy®Mini Kit Part 1)



Quick-StartProtocol

RNeasy® Mini Kit, Part 1

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

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

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

Notes before starting

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

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

350µl

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

January 2011

QIAGEN®

Sample & Assay Technologies

RNeasy® mini kit

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

4. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
5. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x g. Discard the flow-through.
6. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x g.

   Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to dry the membrane.
7. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x g to elute the RNA.
8. If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µl of RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.

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

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

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

Appendex II : Conventional One step RT- PCR for West Nile Virus Isolation procedure

	NATIONAL VETERINARY INSTITUTE	Document No. NVI -QMS - QF - 41		
Title - Master mix preparation and PCR work sheet		Effective Date 20/11/2016	Issue No. 1	Page No. 1of 1

Date:-29/05/2019

Reference no MB 105/19

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

1-Master mix preparation

S.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 °c	30 mints	1-Cycle	
Initial Denaturation	94°c	15 mints	1-Cycle	
Denaturation	95oc	30 Sec	35 Cycles	
Annealing	55°c	1 Mint		
Elongation	68°c	45 sec		
Final Elongation	68°c	10 mints	1-Cycle	
Put at	4_oc	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

-It is around 292 bp positive results.

Appendex III: Questionnaire

Addis Ababa University

College of Veterinary Medicine and Agriculture

People's perceptions and practices regarding the occurrence, health impacts, control and prevention of mosquitoes in the mid Rift Valley of Ethiopia

Questionnaire format

Instruction for the interviewer/enumerator:

- ✓ Choose the target respondent based on the following criteria. The respondent is preferably a father or mother of a household. If both father and mother are absent, interview an adult member of the family (at least more than 18 years old). Select the household by transect walking through a specific village going to be selected in four directions. Select the households systematically (take the first house in direction of your walk or drive and then again take every fifth household until you get required number of respondents in the quarter of the specific village).
- ✓ Read and explain for the study participant(s) the objective of the study using Afan Oromo or Amharic or any other language, as appropriate
- ✓ Ask for verbal consent of the study participant to be involved in the study
- ✓ If she/he agrees, kindly request the respondent to find a place where he/she feels comfortable to answer the questions without much distraction.
- ✓ If the respondent will not agree to participate in the study for any reason, thank him/her and go to the next person/household. Kindly take note of the number of households
- ✓ **Throughout the interviews:**
  - ➢ get organized and do not put yourself and the respondent in hurry; give enough time for the respondent to answer
  - ➢ don't give your opinion about the answer of any question; only be attentive with neutral expressions
  - ➢ in case the respondent asks you about your opinion on the topic, tell her/him to wait until the end of the questions

INTRODUCTION

Good morning/good afternoon!

We are researchers from the College of Veterinary Medicine and Agriculture of Addis Ababa University and we would like to ask you questions regarding biting insects potentially affecting humans and/or animals. The questions will focus on: about your socio-demographic characteristics (as respondent) and what you know about biting insects. We would very much appreciate your participation in this survey and the information going to be collected is highly crucial for our works. Throughout the interview, there is no right and wrong answers. All the questions are asked to assess the existing situations and your opinions. We assure you that your answer will remain confidential and any of the information collected about your personal identity and the identity of your household we will not be shared with third party. Participation in this survey is voluntary and you can choose not to answer any individual question. However, as indicated above your participation and answering all of the questions in this survey is very important for us.

Respondent agreement:

I have understood above statements and

- ☐ Agree to participate.
- ☐ Not agree to participate.

[For interviewer]:

- ✓ If no agreement, pass to the next respondent

Name of the data collector_____ Signature_____

Start time of the interview (hour: minute): _____

Date_____

SOCIO-DEMOGRAPHIC INFORMATION

1. Address of the Respondent

   Region_____ Zone_____

   Woreda_____ Kebele _____

2. What is your marital status? [If mother or father, mark married without asking]

   a) Single b) Married c) Other(Specify)_____

3. Gender of Respondent:

   a) Male b) Female

4. How old are you? (age in years) [Circle the interval]

   a) 18-30 b) 31-45 c) 46-60 d) >60

5. What is your role in the family?

   a) Father b) Mother c) Child(male) d)Child (female)

6. How many people live in your household including yourself?

   a) 1-4 b) 5-8 c) 9-12 d) >12

7. What is the highest level of education you have completed? (choose only one)

   a) None b) Primary c) Secondary d) TVET (Diploma)

   e) First Degree f) Other (specify) _____

8. What is your occupation? (multiple options possible)

   a) Student b) Gov't employee c) Farmer d) Homemaker

   e) Causal Laborer f) NGO employee g)Other (Specify) _____

9. Which domestic animals you or your household/family own?

   a) Cattle b) Goats c)Sheep d) Camels e) Equids

   f)Chicken g)others (specify) _____

KNOWLEDGE REGARDING BITING INSECT VECTORS

10. Which insects (vectors) do you know which bite humans? Free listing (up to the number one can list)

11. Which insects (vectors) do you know that bite animals? Free listing (up to the number one can list)

12. Which insects (vectors) do you know that bite both humans and animals? Free listing (up to the number one can list)

13. What are the breeding sites for mosquitoes?

14. Is there seasonality in the occurrence mosquitoes in your area?

15. At which time of the day (24 hours time) do mosquitoes are active?

16. What are the negative impacts of the mosquitoes to you or your family?

17. Which diseases transmitted by mosquitoes do you know?

18. Do you know that mosquitoes can transmit diseases to humans?

a) Yes b) No

19. What is your source of information for vectors of veterinary and public health importance?

a) Media b) Family c) Neighbors d)Professionals

e)Other(specify) _____

20. Are mosquitoes can be controlled and/or prevented?

a) Yes b) No c) I don't know

21. Can mosquitoes transmit disease between animals and humans?

a) Yes b) No c) I don't know

ATTITUDE REGARDING BITING INSECTS (VECTORS)

22. Are mosquitoes a threat to wellbeing of the community?
    a) Yes b) No c) I don't know

23. Are you at risk of diseases transmitted by mosquitoes?
    a) Yes b) No c) I don't know

24. What do you do in case you get diseases transmitted by mosquitoes?
    a) Consult physicians b) consult traditional healers
    b) Home remedy (specify)_____ d) I don't know

25. Is that possible to control and/or prevent mosquitoes?
    a) Yes b) No c) I don't know

26. Do you think controlling mosquitoes can help in prevention of diseases?
    a) Yes b) No c) I am not sure

27. Who is responsible for mosquito control as you think?
    a) Government b) Veterinarian and physicians c)Individuals
    c) Others (specify) _____

28. Do you think you can prevent yourself from mosquitoes?
    a) Yes b) No c) I am not sure

29. Are you responsible to control mosquitoes?
    a) Yes b) No c) I don't know

30. Do you think communities should actively participate in controlling mosquitoes?
    a) Yes b) No c) I am not sure

PRACTICES REGARDING BITING INSECTS AND POTENTIAL DISEASES TRANSMITTED

31. Do you have experiences regarding mosquito control?

a) Yes b) No

32. What practices do you follow to control mosquitoes?

a) Use insecticide sprays Yes____ No____
b) Use bednets (*Agober*) Yes____ No____
c) Eliminate standing water around the house Yes____ No____
d) Cut down bushes in the yard Yes____ No____
e) Prevent water stagnation Yes____ No____
f) Cleaning of garbage/trash Yes____ No____
g) Disposing water holding containers such as tires, parts of automobiles, plastic bottles, crack pots etc. Yes____ No____
h) Smoking different plants (traditionally) Yes____ No____
i) Burning farms and pasture Yes____ No____
j) Other (specify)_____

33. How often do you practice mosquito control measures?

a) Bednets_____
b) Insecticides_____
c) Cleaning_____

Appendix IV: Ethical clearance from AAU-CVMA

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

Animal Research Ethics Review Committee

Ethical clearance certificate

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

Name of Applicant: Samson Leta (DVM, MVSc)

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

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

Date of application: **18/05/2018**

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

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

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

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

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

Dr Getachew Tereffa
Chairman

Signature

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Please quote Our Ref. No. When replying

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Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34

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Bishoftu/Debre Zeit, Ethiopia